Effects of Caffeine at Different Temperatures on Contractile Properties of Slow-Twitch and Fast-Twitch Rat Muscles

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Summary

The slow-twitch soleus muscle (SOL) exhibits decreased twitch tension (cold depression) in response to a decreased temperature, whereas the fast-twitch extensor digitorum longus (EDL) muscle shows enhanced twitch tension (cold potentiation). On the other hand, the slow-twitch SOL muscle is more sensitive to twitch potentiation and contractures evoked by caffeine than the fast-twitch EDL muscle. In order to reveal the effects of these counteracting conditions (temperature and caffeine), we have studied the combined effects of temperature changes on the potentiation effects of caffeine in modulating muscle contractions and contractures in both muscles. Isolated muscles, bathed in a Tyrode solution containing 0.1-60 mM caffeine, were stimulated directly and isometric single twitches, fused tetanic contractions and contractures were recorded at 35 °C and 20 °C. Our results showed that twitches and tetani of both SOL and EDL were potentiated and prolonged in the presence of 0.3-10 mM caffeine. Despite the cold depression, the extent of potentiation of the twitch tension by caffeine in the SOL muscle at 20 °C was by 10-15 % higher than that at 35 °C, while no significant difference was noted in the EDL muscle between both temperatures. Since the increase of twitch tension was significantly higher than potentiation of tetani in both muscles, the twitch-tetanus ratio was enhanced. Higher concentrations of caffeine induced contractures in both muscles; the contracture threshold was, however, lower in the SOL than in the EDL muscle at both temperatures. Furthermore, the maximal tension was achieved at lower caffeine concentrations in the SOL muscle at both 35 °C and 20 °C compared to the EDL muscle. These effects of caffeine were rapidly and completely reversed in both muscles when the test solution was replaced by the Tyrode solution. The results have indicated that the potentiation effect of caffeine is both time- and temperaturedependent process that is more pronounced in the slow-twitch SOL than in the fast-twitch EDL muscles.

Key words

Rat • Slow and fast muscles • Contractile properties • Caffeine • Temperature dependence • Calcium transients

Introduction

Caffeine (1,3,7 trimethylxanthine), a well-known

extract of coffee beans, is being used as a constant tool in muscle research, especially for its interaction with excitation-contraction (E-C) coupling and for its ability to

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ISSN 0862-8408 Fax +420 241 062 164 http://www.biomed.cas.cz/physiolres induce complete, but reversible emptying of Ca^{2+} stores in the sarcoplasmic reticulum (SR). It is now evident that caffeine acts at the activation site of ryanodine receptors (RyRs) in the SR Ca^{2+} release channel by increasing the affinity for Ca^{2+} (for review see Hermann-Frank *et al.* 1999, Hille 2001). Caffeine effects on the E-C coupling process are manifested at lower concentrations (0.1-10 mM, depending on muscle type), whereas at higher concentrations (above 10-20 mM) caffeine has a direct and reversible effect on the contractile apparatus causing muscle contracture.

It was found that slow- and fast-twitch muscles differ in their response to a lower temperature (Close and Hoh 1968a, Buller et al. 1968, 1984, Ranatunga 1984, Ranatunga and Wylie 1989, Asmussen and Gaunitz 1989, Barnes 1993), lyotropic (Br⁻, NO₃⁻, and I⁻) anions (Klemm et al. 1998, Gong et al. 2002, Wondmikun et al. 2003) or caffeine (e.g. Weber and Herz 1969, Anwyl et al. 1984, Fryer and Neering 1989, Pagala and Taylor 1998, Choisy et al. 2000, Hleihel et al. 2001). In muscles composed mainly of slow-twitch (type I) fibers, a decrease in muscle temperature reduces the twitch tension (cold depression), whereas in muscles mainly containing fast-twitch (type IIA, IID/X or IIB) fibers, the twitch tension is enhanced (cold potentiation) (e.g. Wondmikun et al. 2003). The maximum tetanic force progressively diminishes with decreasing temperature in all muscles regardless of their fiber type composition (Rall and Woledge 1990). Consequently, cooling increases the twitch-tetanus ratio in the fast-twitch and decreases it in the slow-twitch muscles. Therefore, investigation of the temperature effect can be used for classifying a given muscle (Asmussen and Gaunitz 1989). Furthermore, we have shown that compared to normal Cl⁻ Tyrode solution, Br⁻, NO₃⁻ and I⁻ profoundly alter the contractile behavior of the slow-twitch soleus (SOL) and fast-twitch extensor digitorum longus (EDL) muscles as they prolong the contraction and half-relaxation times, albeit to a different extent, and enhance the twitch force (Wondmikun et al. 2003). SOL and EDL muscles also differ in their response to caffeine, as the SOL muscle is more sensitive to twitch potentiation and contractures evoked by caffeine than the EDL muscle (Gutmann and Hanzlíková 1968, Isaacson et al. 1970, Singh and Dryden 1989, Adnet et al. 1993, Pagala and Taylor 1998, Lamb et al. 2001, Journaa and Léoty 2002).

The aim of our study was to compare the combined effects of temperature and caffeine changes in modulating muscle contractions and contractures in the slow-twitch SOL and the fast-twitch EDL muscles. Our results demonstrate that the effects of caffeine are temperature- and time-dependent processes and that they are more marked in the slow-twitch SOL than in the fast-twitch EDL muscles.

Methods

The SOL and EDL muscles of 23 Wistar rats of either sex (age 21-30 days, body mass 90-120 g) were used for in vitro experiments. The young rats were used, because their muscles are smaller, their diffusion conditions are better and their muscles survive longer in vitro (Close and Hoh 1968b). The contractile properties measured are therefore not identical, but they are quite comparable to those of adult rats (Close 1972). The 3- to 4-week-old rats have in the SOL muscle a slow fiber proportion of about 50-60 % and a contraction time of about 35 ms, while 4- to 6-month-old rats have a slow fiber content of about 90 % and contraction time of about 40 ms. There are no significant differences in the contraction times of the EDL between younger and older rats, because the fiber type composition does not change significantly after the second month (e.g. Zachařová et al. 2005). The experimental animals were anesthetized for several hours with urethane (1.5 g/kg body mass i.p.) and the muscles were successively excised during the experiments.

Anesthesia with urethane lasts for up to 15 hours (so called non-return narcosis). It has no influence on neuromuscular transmission (Lüllmann/Mainz, personal communication). The animal is in deep anesthesia for the whole experimental period and when measurements of one muscle are terminated, the experimentator can prepare a "fresh" muscle with an intact blood supply. After finishing the experimental analysis of the last muscle, the animals are sacrificed with an overdose of the anesthetic.

The Ethical Principles and Guidelines for Scientific Experiments on Animals were respected throughout this study. The maintenance and handling of experimental animals followed the EU Council Directive (86/609 EEC) and the animals were treated in accordance with principles of the Care and Use of Animals.

Tyrode solution and tension recording

The isolated muscles were fixed vertically in a thermo-stabilized plexiglass chamber (volume 30 ml). A continuous flow (5 ml/min) of thermo-stabilized Tyrode

solution bubbled with a mixture of 95 % O₂ and 5 % CO₂ was maintained. The small volume of the chamber enabled a fast exchange of the solution, e.g. during a switch from 20 °C to 35 °C and back or between different caffeine concentrations. A complete exchange of solutions lasted less than 5 s. The Tyrode solution had the following composition [mM]: NaCl 137, KCl 5, CaCl₂ 2, MgCl₂ 1, NaH₂PO₄ 1, NaHCO₃ 12, glucose 11. pH was adjusted to 7.4, and the temperature, measured by an electronic thermometer in the muscle chamber, was kept constant both at 35 °C and 20 °C (maximum fluctuation of \pm 0.3 °C).

Mechanical responses recorded were isometrically using a modified mechanoelectric transducer (51 D 17, Disa Electronic Copenhagen, Denmark) with a compliance of 0.24 mm/mN and a linear $(\pm 3 \%)$ characteristic in the range of 5-1000 mN. The undamped resonance frequency was 950 Hz. The amplified output signal of the transducer was displayed on an oscilloscope, recorded photographically and registered (basic tension) by a compensation recorder (response time < 0.2 s). The length of the muscle was extended until twitch tension (Pt) was maximal (optimal muscle length, L₀).

Stimulation

Neuromuscular transmission was blocked by exposing the muscles to a Tyrode solution containing 1.5 x 10^{-5} g/ml d-tubocurarine (Curarin-Asta) for 20-30 min before starting the experiments. The direct stimulation was carried out by a transverse field of two smooth platinum electrodes 1 cm apart from each other, set on either side of the muscle and extending beyond its length and breadth ("massive" stimulation, Mostofsky and Sandow 1951). Because the mechanical responses depend on intensity and duration of the stimulus (Close and Hoh 1968b), a supramaximal (≥ 0.9 A/cm²) stimulus intensity was chosen with duration of 0.1 ms.

Parameters measured

During the experiments of 2-4 h duration, the amplitudes of single twitches and tetanic contractions remained nearly unchanged. Only experiments in which the amplitude of a single twitch at the end of the experiment was more than 90 % of that at the beginning were analyzed. The following contractile parameters were determined at optimal muscle length (L_0): the maximum isometric tension (P_t) of a single twitch [mN]; the contraction time [CT, ms] = time from the onset of

tension development of a single twitch to its peak (P_t); the half-relaxation time [HRT, ms] = decay time of a single twitch measured from the peak (P_t) to 50 % of the tension developed; the maximum isometric tension (T₀) of a fused tetanic contraction [mN]; stimulation frequency at 35 °C: EDL = 200 Hz, SOL = 80 Hz; at 25 °C: EDL = 100 Hz, SOL = 40 Hz; stimulation duration: 500 ms; the twitch-tetanus ratio = P_t/T₀.

Experimental protocols

For the temperature studies, the excised muscles were set up for recording at either 37 °C or 15 °C and isometric twitches were subsequently recorded at centigrade intervals.

In the caffeine studies, records were obtained from each muscle for about 10 min in a Tyrode solution at either 35 °C or 20 °C. 35 °C served as control for subsequent measurements, whereas 20 °C was chosen because the twitch depression of slow-twitch muscles is clearly visible and the twitch potentiation of fast-twitch muscles is maximal at this temperature (cf. Fig. 1). The muscles were then superfused by a Tyrode solution containing 0.1, 0.2, 0.3, 0.5, 1.0, 2.0, 5.0 or 10.0 mM caffeine and dynamic properties were measured after 1, 2, 3, 4, 6, 8, 10, 12 and 15 min at either temperature. Thereafter, the muscles were allowed to recover from the drug effect in a normal Tyrode solution and the twitches were recorded at either 35 °C or 20 °C at the same intervals as described above to assess the kinetics of the recovery process at both temperatures. Then the muscle bath was either cooled to 20 °C or warmed to 35 °C and the same uniformly timed procedure was repeated. At the end of every set of twitch tension recordings, tetani were evoked by a 500 ms train of supramaximal stimuli at a fusion frequency to determine the maximum tetanic tension and the twitch-tetanus ratio.

To study contractures, muscles were first tested at the lowest caffeine concentration during which records of twitch and base line tensions were obtained (2 mM for the SOL and 5 mM for the EDL muscles; cf. Fig. 5). The concentration of caffeine was subsequently increased in a stepwise manner 2, 5, 10, 20, 40 and 60 mM), always after a maximum contracture developed at the previous lower concentration.

Statistical analysis

Data are presented as means \pm S.D. The paired Student's t-test was used for comparison of the control and experimental data (Weber 1967).

Results

Contractile properties of SOL and EDL at various temperatures

The change of single twitches of SOL and EDL muscles over a range of temperatures of 15-37 °C is illustrated in Figure 1. Cooling of the SOL muscle showed a monotonous gradual fall of the twitch tension (cold depression) over the whole temperature range (Fig. 1A). Cooling of the EDL muscle was followed by a continuous enhancement of the twitch tension (cold potentiation) reaching a maximum at 20 °C (increase by about 60 ± 20 %); however, further cooling below 20 °C decreased the extent of this maximal cold potentiation of the twitch tension (Fig. 1A). A decrease of the temperature of the bathing solution caused a 3 to 5 fold prolongation of the contraction (Fig. 1B) and half-relaxation times (Fig. 1C) in both muscles.

The contractile parameters of SOL and EDL muscles at 35 °C and 20 °C are compared in Table 1 (N-SOL, N-EDL). Upon cooling to 20 °C, the maximum tetanic tension of both muscles decreased by about 10 % on the average. The decline of the tetanic tension and the diverse sensitivity of the twitch tension upon cooling in SOL and EDL muscles (either cold depression or potentiation) increased the twitch-tetanus ratio in fast-twitch EDL muscles, while it remained fairly constant in slow-twitch SOL muscles.

The absolute temperature dependence of SOL and EDL twitch tension was low. f_{10} ranges between 1.02-1.17 in the SOL and 0.63-1.3 in the EDL muscle (Table 2). f_{10} of the tetanic tension of both muscles was 1.07±0.02 over the whole temperature range investigated. The temperature dependence of the contraction and halfrelaxation times was greater than that of the force parameters and both muscles showed Q₁₀ values above 2 (Table 2). They displayed a higher temperature quotient at lower temperatures; the highest temperature dependence of the contraction and especially for halfrelaxation time was noted for the SOL muscle between 15 and 20 °C (Table 2).

By convention, the temperature dependence of a process is expressed as the mean change over a temperature range of 10 °C. According to the rule of van Hoff, the Q_{10} is defined for time-dependent processes, e.g. contraction or relaxation times. Because the amplitude tension (twitch tension or T_0) exerted by a muscle are not time-dependent values, their temperaturedependence (coefficients) are denoted as f_{10}



Fig. 1. Influence of temperature on single twitch properties of isolated rat slow-twitch soleus (empty symbols) and fast-twitch extensor digitorum longus muscles (filled symbols). Data are plotted as percentage of a control twitch at 35 °C (means \pm S.D., n = 29). Twitch tension (**A**), contraction time (**B**), half-relaxation time (**C**).

(mathematically determined in the same manner as Q_{10}). Values of Q_{10} or f_{10} around 1 indicate physical processes. Chemical processes have values of 2 or higher (according to the number and temperature dependencies of the involved processes). The temperature coefficient (f_{10}) for twitch tension of EDL and SOL was calculated as tetanic and twitch forces (not time-dependent processes), i.e. they are not suitable to be expressed as Q_{10} .

Action of caffeine at 35 °C

A representative example (Fig. 2A) of the effect



Fig. 2. Representative records of single twitches of isolated rat slow-twitch soleus and fast-twitch extensor digitorum longus (EDL) muscles in standard Tyrode solution and the effect of 2 mM caffeine at 35 °C (**A**) and at 20 °C (**B**). Note the different time scale for the EDL and soleus muscles at both temperatures.

of 2.0 mM caffeine on the time course of single twitches of EDL and SOL muscles at 35 °C shows an increase of the twitch tension as well as a prolongation of contraction and half-relaxation times. These effects of caffeine are confirmed by quantitative results obtained at different caffeine concentrations (Table 1, C-SOL, C-EDL; Fig. 3A). After application of 5 mM caffeine, the twitch tension increased by about 40 % in both muscles, while the maximum tetanus force increased in both muscles only by about 10 %; the twitch/tetanus ratio thus increased by almost 40 % in the SOL and by about 25 % in the EDL muscles (Table 1). The obtained values of twitch tensions showed that the SOL muscle was more sensitive to caffeine than the EDL muscle. In the SOL muscle, a significant twitch potentiation (p<0.05) was already observed at a caffeine concentration of 0.3 mM, whereas in the EDL muscle it was initiated only at higher, 1.0 mM caffeine concentrations (Fig. 3A). Caffeine application led to potentiation and prolongation of the contraction and half-relaxation times of both muscles,

more pronounced in the SOL muscles (Fig. 3C). Lower concentrations of caffeine showed a potentiation of the twitch tension without affecting the resting tension of the preparations. At medium and higher concentrations, a dose-dependent increase appeared in the baseline tension (Fig. 5). However, a continuation of the twitch potentiation throughout the contracture was observed around these threshold values. At higher caffeine doses (>5 mM in SOL and >20 mM in EDL muscles, respectively), the amplitude of the evoked twitch diminished.

Action of caffeine at 20 °C

A representative example of the effect of 2.0 mM caffeine (Fig. 2B), as well as quantitative evaluation at different caffeine concentrations (Fig. 3B) on the time course of single twitches at 20 °C shows, similarly as at 35 °C, that the SOL muscle exhibits higher caffeine sensitivity and more pronounced increase in twitch tension in comparison to the EDL muscle. Application of



Fig. 3. Dose-response curves of the action of caffeine on single twitch contractions of isolated rat slow-twitch soleus (open symbols) and fast-twitch extensor digitorum longus (filled symbols) muscles at temperatures 35 °C (**A**, **C**) and 20 °C (**B**, **D**). **A**, **B**: Action on twitch tension (circles; potentiation after caffeine is expressed as percentage of control twitches recorded in normal Tyrode solution). **C**, **D**: Action on contraction (triangles) and half-relaxation (squares) times (means \pm S.D., n = 5-6).

5 mM caffeine significantly increased the twitch tension (more in the SOL than in the EDL muscle), even to a greater extent than at 35 °C especially in the SOL muscle; the maximal tetanic force was also increased compared to normal Tyrode solution, but the maximum was still lower than at 35 °C (Table 1 C-SOL, C-EDL). The twitch/ tetanus ratio thus increased in the SOL muscle at 20 °C similarly to that at 35 °C, whereas in the EDL muscle, the twitch/tetanus ratio remained at 20 °C as in the control solution without caffeine. Caffeine application also led to a prolongation of the contraction time and especially of the half-relaxation time (Fig. 3D, see also Fig. 2B and Table 1), similarly as at 35 °C. Although the EDL and SOL muscles behaved at 20 °C similarly as at 35 °C, a higher threshold dose 0.5 mM caffeine for the SOL and 2 mM for the EDL was necessary to evoke a significant (p<0.05) potentiation, the extent of potentiation was practically comparable with the situation at 35 °C in the EDL. In the SOL, however, the extent of changes, especially after concentrations >1 mM, was much more prominent at 20 °C than at 35 °C (cf. Figs 3A and 3B).

Kinetics of caffeine potentiation and its reversibility

Both the SOL and EDL muscles demonstrated a progressively increasing twitch tension at 35 °C when superfused with 2 mM caffeine. The maximum increase, was followed by a plateau, and was achieved after 6-8 min (Fig. 4A). When tested at 20 °C, both muscles demonstrated a similar course of twitch potentiation, the maximum twitch potentiation being attained after 12-15 min (Fig. 4B). The augmentation after application and reversal of the twitch tension to the control level upon withdrawal of caffeine thus proceeded more rapidly at 35 °C than at 20 °C (Fig. 4A and 4B).

Caffeine contractures in SOL and EDL muscles at different temperatures

In the SOL muscle, 2 mM and 5 mM caffeine regularly evoked a contracture at 35 °C and 20 °C, respectively. The threshold concentration in the EDL muscle was considerably higher, about 20 mM of caffeine at both temperatures (Fig. 5). The onset of the contracture began almost immediately after exposure of the muscles



Fig. 4. Time courses of the maximal twitch tension of soleus (open circles) and extensor digitorum longus (filled circles) muscles after application (\uparrow) and washing (\downarrow) of 2 mM caffeine at 35 °C (**A**; n = 6) or at 20 °C (**B**; n = 5). The potentiation is expressed as percentage of control twitches recorded in normal Tyrode solution (means ± S.D.).

to the appropriate drug concentration, but the time to maximum contracture varied depending on the caffeine concentration.

In the SOL muscles, the maximum contracture tension at 35 °C and 20 °C was already obtained at 20 and 40 mM caffeine, respectively, whereas EDL muscles required 40 mM and 60 mM caffeine for the same effect (Fig. 5). After reaching their maximum, the contractures declined in both muscles despite a further rise in the caffeine concentration. In general, both muscles demonstrated four distinct phases of reaction to increased caffeine concentrations (cf. Fig. 5): 1) Twitch tension potentiation with no detectable change in the resting tension (SOL: 2-5 mM, EDL: 5-10 mM), 2) caffeine contracture appearing parallel to a further potentiation of twitch tension (SOL 5-10 mM, EDL: 10-20 mM), 3) a decline of the twitch tension with a further rise in

contracture tension (SOL: 20-40 mM, EDL: 20-60 mM), and 4) a decrease of the contracture despite a further increase in the caffeine dose (SOL: > 40 mM, EDL: > 60 mM).

Discussion

Our results suggest that the potentiation effect of caffeine is also a temperature-dependent process and that at both temperatures it is more pronounced in the slowtwitch SOL than in the fast-twitch EDL muscle. It is also evident from the results that twitch tension changes in the SOL muscles are more susceptible to caffeine and that the contractures in the SOL muscles appear at lower concentrations of caffeine than in the EDL muscles (cf. also Anwyl et al. 1984, Singh and Dryden 1989, Pagala et al. 1994, Choisy et al. 2000, Lamb et al. 2001). On the other hand, caffeine produced only a relatively small increase (10-14 %) in maximum tetanic tension, irrespective of the muscle type and temperature. These data are in good agreement with the suggestion of Fryer and Neering (1989) who stated that it is likely that the myofilaments are saturated with Ca²⁺ during the tetanus and that a further increase in intracellular Ca^{2+} level would produce no further increase in force.

The observed effects of caffeine were completely reversible. Sorenson et al. (1986)demonstrated that the Ca²⁺-release from the sarcoplasmic reticulum and the inhibition of Ca²⁺-uptake induced by caffeine are rapidly reversible in skinned psoas and EDL muscle fibers. Nevertheless, there is a distinct dependence of the reversibility on temperature, as we have shown that cooling slowed down the speed of muscle recovery after caffeine treatment apparently due to a slower reuptake of Ca^{2+} into the SR by the Ca^{2+} pump.

A further difference between the SOL and EDL muscles emerged when we compared extent of the twitch tension potentiation produced by caffeine at 35 °C and 20 °C (cf. Table 1). The interaction of these two muscle tension modulating factors showed that despite the cold depression by about 10 %, the extent of potentiation of the twitch tension by caffeine in the SOL muscle was higher at 20 °C than at 35 °C (cf. Table 1 N-SOL and C-SOL). On the other hand, in the EDL muscle, caffeine further increased cold potentiation of both temperatures (Table 1 N-EDL, C-EDL). Furthermore, the difference in the extent of twitch potentiation of the SOL muscle between 35 °C and 20 °C increased with increasing



Fig. 5. Typical changes in baseline tension and the twitch amplitude of a rat extensor digitorum longus muscle exposed to different caffeine concentrations (5-60 mM) at 35 °C. Exchanges of normal Tyrode solution to solutions with stepwise increasing caffeine concentration are indicated by arrows.

concentration of caffeine and was thus most evident at 5 and 10 mM caffeine concentrations (cf. Figs 3A and 3B). This may indicate that the increased intracellular Ca^{2+} -concentration by caffeine would outweigh the cold depression in the slow SOL muscle, probably by providing additional Ca^{2+} for myosin phosphorylation and transition of cross-bridges into the force-generating state (Metzger *et al.* 1989, Sweeney and Stull 1990).

Caffeine opens Ca²⁺-channels or increases the probability of opening the channels via the RyRs. At low doses this effect is small and leads to twitch potentiation by normal depolarization or by direct stimulation, but not to a contracture. A subcontracture dose of caffeine potentiates the twitches of both muscles in a concentration-dependent manner and the first measurable twitch potentiation was observed at 0.3 and 1.0 mM caffeine for SOL and EDL muscles, respectively, which appears to be consistent with earlier reports (Fryer and Neering 1989, Choisy et al. 2000, Lamb et al. 2001). Using isolated skinned muscle fibers, these authors demonstrated dose-dependent enhancement of the force and slowing of relaxation. Furthermore, they argued that these effects are the result of an increased Ca²⁺-release and decreased Ca²⁺-uptake of the SR as well as Ca²⁺-buffering diminished myoplasmatic or a combination of these factors. It was suggested that caffeine induces a release of Ca2+ from calsequestrin, which is the main Ca²⁺-binding protein of the SR, with a high capacity and a low affinity for Ca^{2+} (Scott *et al.* 1988, Ikemoto et al. 1991). From these experiments it was concluded that the Ca2+-dependent conformational change of calsequestrin causes a change in the shape of SR membrane proteins, including the RyRs, and that

calsequestrin, exclusively when phosphorylated, led to a fivefold increase of open probability of the RyRs and caused a twofold increase of the RyRs open time (Szegedi *et al.* 1999). As calsequestrin exists in fast and slow/cardiac isoforms, similarly as some other Ca^{2+} binding proteins (for review see Berchtold *et al.* 2000), it is suggestive that different calsequestrin isoforms can partly contribute to the observed differences of caffeine effect in slow- and fast-twitch muscles.

At higher doses, the liberation of Ca^{2+} from the SR is so high that it couples directly to the contractile apparatus and evokes tension without any electrical stimulation (contractures). The resting tension of a muscle, however, is enhanced with an increasing dose of caffeine. This progression of the twitch potentiation accompanied with contractures elicited by intermediate caffeine doses (cf. also Connett *et al.* 1983) suggests that potentiation and contractures are probably caused by related mechanism(s) of the drug effect on sarcoplasmic Ca^{2+} -transport. To verify this, skinned fiber preparations in which Ca^{2+} -transients would be simultaneously recorded at various temperatures should be used.

The present results have confirmed that the temperature sensitivity of the rat EDL and SOL muscles markedly differs (Close and Hoh 1968a, Buller *et al.* 1968, 1984, Ranatunga 1984, Ranatunga and Wylie 1989, Asmussen and Gaunitz 1989, Barnes 1993). The enhancement of single twitch tension is a typical response of the rat EDL muscle upon cooling to 20 °C. On the contrary, cooling decreases the twitch tension of the rat SOL muscle by about 10 %. A cold depression of the SOL muscle of various mammals was reported, whereby the depression was directly proportional to the fiber

| | N-SOL (n = 30) | | C-SOL (n =6) | | N-EDL $(n = 29)$ | | C-EDL (n= 6) | |
|-----------------|----------------|-----------------|-----------------|-----------------|------------------|-----------------|-----------------|-----------------|
| Parameters | 35 °C | 20 °C | 35 °C | 20 °C | 35 °C | 20 °C | 35 °C | 20 °C |
| CT [ms] | 29.0±2.7 | 100.2±5.6 | 57.9±4.9 | 175.6± 4.7 | 12.5±1.5 | 52.9±0.1 | 18.7±2.3 | 59.1±3.8 |
| HRT [ms] | 32.3±2.8 | 138.0±20.1 | 81.9±5.1 | 245.4±12.8 | 13.2±1.6 | 59.3±11.2 | 21.1±2.9 | 102.3±4.6 |
| P_t/P_{t35}^l | 1 | 0.89 ± 0.03 | 1.46 ± 0.04 | 1.51±0.05 | 1 | 1.61±0.18 | 1.39±3.8 | 1.99±6.9 |
| T_0/T_{035}^2 | 1 | 0.90 ± 0.04 | 1.11 ± 0.04 | 1.08 ± 0.05 | 1 | 0.87 ± 0.04 | 1.10 ± 0.03 | 1.04 ± 0.4 |
| Twitch/tetanus | 0.13±0.03 | 0.14 ± 0.02 | $0.19{\pm}0.04$ | 0.20 ± 0.04 | 0.15±0.06 | 0.29 ± 0.07 | 0.19 ± 0.04 | 0.29 ± 0.07 |

Table 1. Temperature dependence of contractile properties of rat slow-twitch soleus (N-SOL) and fast-twitch extensor digitorum longus (N-EDL) muscles and the additional influence of 5 mM caffeine (C-SOL and C-EDL)

Data are means \pm S.D. CT = contraction time, HRT = half-relaxation time, P_t = twitch tension, ¹expressed as a fraction of the twitch tension developed at 35 °C in normal Tyrode, T₀ = tetanic tension, ²expressed as a fraction of the tetanic tension developed at 35 °C in normal Tyrode, significant difference (P<0.01) between 20 °C and 35 °C of homologous muscles were found in all cases.

Table 2. The temperature coefficient (f_{10}) of muscle twitch force and the temperature quotient (Q_{10}) of time parameters at different temperature ranges.

| | 15-20 °C | | 21-30 °C | | 31- 37 °C | |
|--|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Temperature dependency ratio | EDL | SOL | EDL | SOL | EDL | SOL |
| f ₁₀ [P _d] Q ₁₀ [CT] Q ₁₀ [HRT] | 1.30±0.11 3.12±0.17 3.64±0.26 | 1.17±0.12 3.25±0.22 5.80±0.32 | 0.71±0.13 2.73±0.15 3.12±0.24 | 1.10±0.10 3.10±0.17 4.03±0.26 | 0.63±0.13 2.11±0.15 2.81±0.20 | 1.02±0.10 2.09±0.16 2.01±0.20 |

Data are means \pm S.D., n = 29. EDL = extensor digitorum longus muscle, SOL = soleus muscle, P_t = twitch amplitude, CT = contraction time, HRT = half-relaxation time

composition of muscles, i.e. to the percentage of slow and fast twitch fibers (Asmussen and Gaunitz 1989, Wondmikun et al. 2003; for fiber type composition, see e.g. Soukup et al. 1979, 2002, Vadászová et al. 2004, 2006, Zachařová et al. 2005). Cold depression is more pronounced in SOL muscles of cats and guinea pigs composed almost exclusively of slow-twitch fibers (Henneman et al. 1968, Asmussen and Gaunitz 1989). We have confirmed that the tension developed during unfused or fused tetanic contractions is also temperature sensitive. The tension output of fused tetani of both muscles tested was about 10 % higher at 35 °C than at 20 °C. Investigation of tetanic contractions over a wide range of temperatures have shown that muscle tension is nearly constant between 30-37 °C and decreases progressively below 30 °C (Bennett 1984, Kössler and Küchler 1987, Asmussen and Gaunitz 1989). Temperatures between 30-37 °C are physiological in mammalian muscles and the constancy of their force output in this range is of functional significance because motor units mainly use tetanic contractions in physiological movements in vivo.

The temperature dependence of various mechanical parameters of muscle contraction is very different. The observed Q_{10} and f_{10} values range from nearly 0.5 to 5.8 (from a negative up to very strong positive temperature dependence). The positive and negative temperature effects on muscle force are, however, small, f_{10} lying close to 1.0 (0.6-1.3; Table 2, cf. Bennett, 1984). The Q₁₀ values for the contraction and half-relaxation times found in this study, were above 2, indicating a moderate to high temperature sensitivity (cf. Rall and Woledge 1990). There is evident correlation between temperature dependent isometric twitch potentiation and a myosin phosphorylable light chain content (Mannig and Stull 1982, Palmar and Moore 1989, Moore et al. 1990), however, the basis of the different temperature sensitivity between fast-twitch and slowtwitch fibers are not fully understood. Apart from the phosphorylation mechanism which would possibly explain the change in muscle force parameters, there may exist some other biochemical factors such as the myosin-ATPase, the Ca²⁺-ATPase of the SR (SERCA) with different temperature-sensitivities operating in parallel

and promoting muscle contraction and relaxation processes. The reaction rates of these factors are subject to temperature regulation, as biochemical reactions are slowed by reduced temperature with a $Q_{10} > 2.0$ (Prosser 1973). This reduction affects the rate at which actin and myosin filaments can slide past one another and this presumably accounts for changes in contraction and a half of relaxation times (Barnes 1993).

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