On the Problem of Season and Cold Dependence of Calcium Transport by Skeletal Muscle Sarcoplasmic Reticulum

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Sarcoplasmic Reticulum, Calcium Transport, Season and Cold Dependence

Calcium transport of skeletal muscle sarcoplasmic reticulum from golden hamsters was studied in January and in June on animals kept at 22 °C under natural photoperiod and in January after cold-acclimation at ±2 °C in the dark for 55 days. Crude homogenates from psoas and soleus muscles and from mixed skeletal muscles were used.

No differences were observed in the calcium storing capacity of sarcoplasmic reticulum among the three groups of animals. Kinetic studies on the dependence of the calcium uptake rate on the concentration of free calcium revealed a significant increase of the uptake rates and a decrease of the calcium affinity in the control animals sacrificed in winter as compared to those killed in June. Cold-acclimation in winter leads to a further small reduction of the calcium affinity.

This shift of calcium uptake rate and affinity in the sense of that of a fast-twitch muscle may be related to the functional demands of the cold season and cold-acclimation respectively.

Introduction

The possibility of seasonal variations in the calcium transporting system of sarcoplasmic reticulum has always to be taken into account when results obtained with the same species in various laboratories or data from the same institution concerning different species are compared [1].

Various experimental animals respond to seasonal change in climate – usually temperature – differently. A species may not display any important behaviour change, or may undergo acclimation, or adaptation, or even hibernation (*cf.* [2]). On the other hand it is well established that significant shifts of calcium concentration in serum and within the fibres of skeletal and heart muscle occur in the cold-acclimated and hibernating hamsters [3]. Hence, it is reasonable to think that in cold-acclimated and hibernating animals the calcium transport ATPase of sarcoplasmic reticulum is correspondingly modulated in order to regulate the intracellular calcium concentration in a range compatible with muscle activity and cell integrity [4].

In order to test this hypothesis and to demonstrate predictable seasonal differences we investigated the calcium uptake function of sarcoplasmic reticulum in the Syrian hamster in sommer, in winter and after exposure to the cold. It is known that this easy to raise animal is affected by seasonal changes and may enter into hibernation also in laboratory [3, 5, 6]. It will be shown that although our animals do not hibernate, their sarcoplasmic reticulum calcium transporting system exhibits changes similar to those observed in the hibernating European hamster [7].

Materials and Methods

Animals

The experiments were done with adult male golden hamsters (Syrian hamster, *Mesocricetus auratus*) breed in our animals laboratory, which originated from a strain of the Sprague-Dawley Laboratory (Madison, Wisconsin, U.S.A.). Twenty animals aged 100–120 days weighing 155 ± 22.3 g were housed separately on the beginning of December. Ten of them were randomly selected and put in a cold chamber without light at a temperature of ± 2 °C and a relative humidity of 90 ± 5%, and free access to tap water and food ("Altromin 1324", Dry Food, Altromin International, Lage, F.R.G.). The other ten hamsters were kept as controls in the laboratory at 22 °C, relative humidity 55 ± 5% and natural photoperiod. Control and cold-acclimated animals were killed by decapitation between 52 and 58 days after beginning of the experiment.

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For comparison ten golden hamsters aged 100–120 days and weighing 135 ± 16.2 g were housed separately in the beginning of May and also kept for about 55 days in the laboratory as the previous group of animals before being killed by the end of June.

Histochemistry and morphometry

Psoas and soleus muscles from three animals kept at 22 °C under natural photoperiod and killed in June and respectively in January, and from three cold-acclimated hamsters were removed in a slight stretched position and frozen in melting methylbutane at −160 °C as composite block.

Serial 5 and 15 μm cryostate microtome sections were usually stained with haematoxylin and eosin and assayed for the standard myofibrillar myosin ATPase reaction at pH 9.4 after formaldehyde fixation and alkaline preincubation at pH 10.4 according to Guth and Samaha [8].

For morphometric evaluation of muscle fibres measurements were done with transverse muscle sections in a Leitz Orthoplan Microscope connected to the Leitz Semiautomatic Image Analyzer ASM 68 K (Leitz, Wetzlar, F.R.G.).

Preparation of muscle homogenates

The technique introduced by Briggs *et al.* [9] was applied. Samples from the central part of psoas and soleus muscles as well as mixed skeletal muscles were quickly dissected and immediately immersed in an ice-cold solution containing 300 mM sucrose, 100 mM KCl and 40 mM imidazole buffer pH 6.9. After removal of connective tissue, nerves and blood vessels, muscle tissue was weighed, cut in very thin slices, and homogenized in 10 vol of the above solution for two periods of 10 s with a Polytron Homogenizer at step 7 of velocity. The homogenate was filtered through cheese-cloth, complemented with 1 mM phenylmethylsulfonylfluoride to inhibit protease activity, and used without delay after determination of protein concentration by the biuret method.

Assays of calcium uptake rate and calcium storing capacity of sarcoplasmic reticulum

For the estimation of calcium uptake rate and calcium storing capacity crude homogenate – final concentration 1 mg of protein/ml – was incubated at 20 °C for 20 min in a standard solution containing 5 mM NaN_3 – to inhibit mitochondrial activity –, 100 mM KCl, 40 mM imidazole buffer pH 6.9, 5 mM $MgCl_2$, 5 mM potassium oxalate, 0.6 mM EGTA, 5 mM ATP, and 0.5 mM $^{45}CaCl_2$.

The dependence on free Ca^{2+} concentration of the rate of calcium uptake of sarcoplasmic reticulum was studied at different free calcium concentrations, adjusted with EGTA 0.1, 0.22, 0.3, 0.4, 0.8 and 1.2 mM in an incubation medium as above which contained 0.2 mM $^{45}CaCl_2$; the corresponding free Ca^{2+} concentrations are reported in the legend of Fig. 1, 2 and in Table I. As maximal rates to be used for comparison, values obtained in the presence of 0.1 mM free Ca^{2+} after 1 min incubation were chosen.

Calcium uptake was interrupted 1, 2, 3, 5 and 20 min after the addition of the protein by passing the suspension through a glass nitrocellulose 0.45 μm filter combination (Schleicher & Schuell, Dassel, F.R.G.) protected from clotting by large components of the homogenate by a Whatman GF/C glass microfibre filter. The radioactivity remaining in the filtrate was determined by liquid scintillation counting.

Results

Effect of cold-acclimation on animals

None of the animals kept at 22 °C under natural photoperiod in winter hibernated and only some degree of torpor was occasionally observed in those cold-acclimated. This is not surprising since it is well known that Syrian hamster, when exposed to the cold in laboratory may or may not hibernate [3, 5] or hibernates in an irregular manner [6].

The original weight increased – from 155 ± 22.3 g up to 171 ± 13.4 g – in the control animals in winter, but it decreased up to 135 ± 22.9 g in those exposed to the cold for about 55 days. A weight increase from 135 ± 16.2 g up to 158 ± 18.1 g was observed in the hamsters housed separately in May and killed after 55 days by the end of June.

Morphometric cytochemistry of muscle fibres and calcium transport of sarcoplasmic reticulum of psoas and soleus muscles

As it is known for other experimental animals like f.i. rat, guinea pig and mouse, morphometric

cytochemistry revealed in the control animals killed in January that the fast-twitch-psoas muscle of golden hamster also contain a large amount of type I fibres (25.7 ± 10.4%) and that the slow-twitch-soleus muscle too displays a fibre type pattern of a mixed muscle since it contains a high proportion of type II fibres (29.9 ± 2.8%). Nevertheless, calcium uptake rate after 1 min incubation and calcium storing capacity of sarcoplasmic reticulum from psoas muscle were twice higher than those of soleus muscle and reached values of 0.09 μmol and 0.19 μmol/mg protein respectively.

On comparison with the control animals kept at 22 °C in winter no change in fibre type pattern of psoas and soleus muscles was observed in those killed in June and in the cold-acclimated ones; the latter only displayed some reduction of fibre area, which was, however, not significant. Values of calcium uptake rate and calcium storing capacity of sarcoplasmic reticulum from the above muscles were also similar in the three groups of animals studied. However, calcium transport of soleus muscle sarcoplasmic reticulum could not be properly investigated because of the small amount of competent membranes obtainable from such a small muscle. Since values of calcium transport of sarcoplasmic reticulum from mixed skeletal were very close to those of psoas muscle, we therefore used homogenate from mixed skeletal muscles for the subsequent experiments.

Assays of calcium uptake rate and calcium storing capacity of sarcoplasmic reticulum

Fig. 1 shows the course of calcium uptake of sarcoplasmic reticulum from mixed muscles of control hamsters killed in January. The study was done with six different concentrations of free calcium. It is easy to recognize that calcium uptake rate and calcium storing capacity directly depend on the concentration of free calcium in the medium. The activity increases from minimal values at 0.06 μM (▼) to saturating values obtained at 0.1 μM free calcium (●). The calcium storing capacity, defined as the amount of calcium uptake measured after an incubation period of 20 min, reaches saturation values of about 0.18–0.19 μM·mg protein of muscle homogenate between 0.6 μM and 100 μM free calcium.

Comparison of the various values of calcium storing capacity obtained with sarcoplasmic reticulum from animals killed in June and respectively from the cold-acclimated hamsters with the corresponding amounts of calcium stored by sarcoplasmic reticulum vesicles from control animals killed in January did not reveal any significant difference.

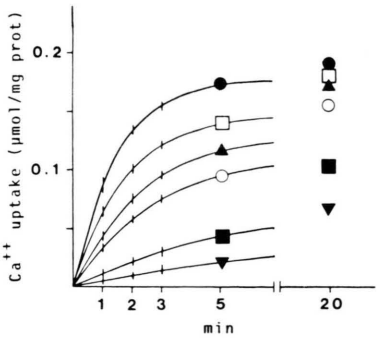

Fig. 1. Calcium uptake of sarcoplasmic reticulum in the presence of various amounts of free calcium in control hamsters killed in January. Values of uptake rate and calcium storing capacity clearly vary with the concentration of free calcium. Calcium uptake was measured with crude homogenate as described in Materials and Methods. Concentrations of free calcium (μM): ● = 100, □ = 3, ▲ = 0.6, ○ = 0.3, ■ = 0.1, ▼ = 0.06. The results are given in $\bar{x}$ ± SEM as indicated by the bars. n = 7.

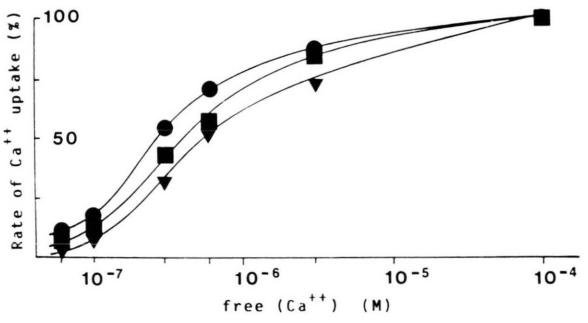

Fig. 2. Comparison of the dependence on free calcium concentration of calcium uptake rate of sarcoplasmic reticulum in June at 22 °C (●), in January at 22 °C (■) and after cold-acclimation at ± 2 °C for about 55 days in winter (▼). The values obtained with the various concentrations of free calcium are given in $\bar{x}$ as percentage of those obtained with 0.1 mM free calcium. n = 7. Calcium affinity in January (■) is 20.7% and 17.9% lower than in June (●) at 0.3 μM and 0.6 μM free calcium respectively, and further decreases in cold-acclimated animals (▼) (s. Table I). Profile of uptake rates curve in January (■) and of cold-acclimated hamster (▼) differs from that of animals killed in June (●) and resembles that of a fast-twitch-muscle.

In contrast, the dependence of the calcium uptake rate on free calcium concentration showed (Table I) that the relative calcium uptake rates obtained in June (June 22 °C) after 1 min incubation at non saturating concentrations of free calcium applied are significantly higher then those of control animals killed in January (January 22 °C). As it is also shown in the Table I, that difference is even more pronounced when comparison is done between animals killed in June (June 22 °C) and cold-acclimated ones (Winter 0 °C). This indicates that the calcium affinity of skeletal muscle sarcoplasmic reticulum decreases in winter-hamsters and even more during cold-acclimation.

The above differences are well outlined in the Fig. 2 which reveals that the profile of the uptake rates curve obtained with the values of animals killed in June (●) is very similar to that of the well known curve of a slow-twitch-muscle with high calcium affinity like the soleus muscle, while the profile of the corresponding curve of cold-acclimated animals (▼) rather resembles that a fast-twitch-muscle with low calcium affinity like the psoas muscle. This difference between soleus and psoas muscle was already established in the rabbit (*cf.* [10]).

Discussion

The results provide evidence that the cold season elicits a significant decrease of calcium sensitivity of the calcium transport ATPase of sarcoplasmic reticulum from golden hamster skeletal muscle, and that this alteration is enhanced when the animals are exposed to cold although they do not hibernate. Yet, neither the calcium uptake capacity nor the maximal rate of calcium uptake are augmented. This is in contrast to the behaviour of the European hamster *(Cricetus cricetus)*, which hibernates and exhibits change of the three studied calcium transport parameters calcium capacity, maximal calcium uptake rate and calcium sensitivity [7].

While calcium uptake capacity and maximal rate of calcium uptake must be considered to be extrinsic properties of the calcium transport system, calcium sensitivity constitutes an intrinsic quality. An increase of calcium capacity and of maximal uptake rate may result from a proliferation of the sarcoplasmic reticulum leading to an increased yield of the sarcoplasmic reticulum vesicles in the homogenate. In contrast, calcium sensitivity as an intrinsic property of the calcium transport-

Table I. Calcium uptake rates of sarcoplasmic reticulum in the presence of various concentrations of free calcium (s. Fig. 1 and 2). Values – μmol·mg protein of muscle homogenate^{-1}·min^{-1} – are given in $\bar{x} \pm$ SEM. $n = 7$. Uptake rates of animals killed in summer (June, 22 °C) and respectively of the cold-acclimated animals (winter, ± 2 °C) were compared with the corresponding values of those sacrificed in winter (January, 22 °C) using the Student's t-test. $P < 0.05$ was considered significant. n.s. = not significant.

Free Ca^{2+} [μM]	Groups of animals January 22 °C [μmol·mg^{-1}·min^{-1}]	Winter ± 2 °C	June 22 °C
100	0.086 ± 0.005	0.090 ± 0.006 n.s.	0.105 ± 0.013 n.s.
3	0.070 ± 0.003	0.068 ± 0.002 n.s.	0.091 ± 0.007 P < 0.01
0.6	0.051 ± 0.004	0.050 ± 0.002 n.s.	0.073 ± 0.005 P < 0.01
0.3	0.037 ± 0.003	0.033 ± 0.002 n.s.	0.057 ± 0.005 P < 0.005
0.1	0.012 ± 0.002	0.007 ± 0.001 P < 0.01	0.018 ± 0.004 n.s.
0.06	0.006 ± 0.002	0.002 ± 0.001 P < 0.05	0.012 ± 0.002 P < 0.01

ing protein reflects qualitative change in the calcium pumping protein. Thus, in the non hibernating animals only the intrinsic parameter of the calcium transport persists as a season-dependent change in the properties of the calcium transport enzyme.

Wickler *et al.* [5] compared hibernating with cold-acclimated golden hamsters and found a significant increase of citrate synthase (an indicator of oxidative capacity) and of β-hydroxyacyl-CoA-dehydrogenase (an enzyme marker of β-oxidation activity) in the skeletal muscle and in the myocardium of the hibernating animals. On the opposite, these enzymes were not significantly increased in the muscle of cold-acclimated animals which did not hibernate. Comparison of our observations on the calcium transport ATPase of sarcoplasmic reticulum with these results on the oxidative capacity points out the high sensitivity of the calcium transporting enzyme the properties of which are just season-dependent.

Calcium concentration increases in muscle tissue in cold-acclimated and even more in hibernating hamsters [3, 11]. During hibernation muscle is characterized by pronounced inactivity with very low levels of metabolism and body temperature. Muscle contraction is then very important for thermogenesis upon arousal. It appears reasonable to think that the change of the calcium transport ATPase described in this contribution is related to the necessity of maintaining the calcium transport function at low temperature, which in turn warrants ion homeostasis [12–15]. In connection with these considerations it would be interesting to know if the contractile proteins undergo similar changes of their calcium sensitivity.

Notes

1. Studies on cold-acclimation were done with the approval of the Regierungspräsidium Karlsruhe, Karlsruhe, B.R.D. (License N. 37-9550 from March 23rd 1984 extended on June 6th 1986).
2. Luisa De Martino is a research fellow of the Max-Planck-Gesellschaft, München, B.R.D.

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