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Characteristics of Sarcoplasmic Reticulum Membrane Preparations Isolated from Skeletal Muscles of Active and Hibernating Ground Squirrel *Spermophilus undulatus*

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Abstract—The total Ca-ATPase activity in the sarcoplasmic reticulum (SR) membrane fraction isolated from skeletal muscles of winter hibernating ground squirrel *Spermophilus undulatus* is ~2.2-fold lower than in preparations obtained from summer active animals. This is connected in part with ~10% decrease of the content of Ca-ATPase protein in SR membranes. However, the enzyme specific activity calculated with correction for its content in SR preparations is still ~2-fold lower in hibernating animals. Analysis of the protein composition of SR membranes has shown that in addition to the decrease in Ca-ATPase content in hibernating animals, the amount of SR Ca-release channel (ryanodine receptor) is decreased ~2-fold, content of Ca-binding proteins calsequestrin, sarcalumenin, and histidine-rich Ca-binding protein is decreased ~3-4-fold, and the amount of proteins with molecular masses 55, 30, and 22 kD is significantly increased. Using the cross-linking agent cupric–phenanthroline, it was shown that in SR membranes of hibernating ground squirrels Ca-ATPase is present in a more aggregated state. The affinity of SR membranes to the hydrophilic fluorescent probe ANS is higher and the degree of excimerization of the hydrophobic probe pyrene is lower (especially for annular lipids) in preparations from hibernating than from summer active animals. The latter indicates an increase in the microviscosity of the lipid environment of Ca-ATPase during hibernation. We suggest that protein aggregation as well as the changes in protein composition and/or in properties of lipid bilayer SR membranes can result in the decrease of enzyme activity during hibernation.

Key words: sarcoplasmic reticulum, Ca-ATPase, Ca-binding proteins, calsequestrin, sarcalumenin, histidine-rich Ca-binding protein, ground squirrel *Spermophilus undulatus*

For many small mammals, winter survival is assured by adoption of a special physiological state, i.e., hibernation [1, 2]. In response to cold temperatures, shortening of daylight, and food restriction, the animals enter into a deep state of torpor that is accompanied by a strong depression of metabolic rate (often only 1-5% of normal values) and reduction in body temperature to near ambient [3, 4]. It should be noted that the depression of metabolism during hibernation cannot be explained by the simple effect of low temperatures on the rates of biochemical reactions. Rather, the fall in body temperature during entry into hibernation is the result of a well-coordinated reduction in the rates of all intracellular processes [5, 6]. Moreover, hibernation is not a discontinuous process but consists of a number of periods of deep torpor lasting 2-4 weeks (so-called hibernation bouts) with short (20-30 h) intervals (bouts) of awakening between them [1, 2]. During awakening bouts, the body temperature is increased very rapidly (for 4-6 h) from 0-5 to 37°C and is maintained on this level for some time after which the animals enter into torpor again. The physiological significance of such awakening bouts is still unknown as well as the molecular mechanisms that allow the animals to change the body temperature during a few hours by tens of degrees Celsius. These features of hibernators attract the attention of many researchers because, for most mammals, a drop of body temperature of even a few degrees is lethal.

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During hibernation, the energy metabolism of animals is strongly reduced due to the suppression of both glycolysis [7, 8] and oxidative phosphorylation [9, 10]. Phosphorylation by protein kinases of phosphofructokinase and pyruvate kinase that changes their activity and kinetic characteristics results in a strong suppression of glycolysis rate [11]. In combination with the changes of concentration of different metabolites (allosteric activators and inhibitors of glycolytic enzymes), protein phosphorylation provides effective suppression of carbohydrate catabolism and simultaneously sharply increases the use of lipids as a main fuel during hibernation [12]. Depression of carbohydrate oxidation in the citric acid cycle is provided by phosphorylation of pyruvate dehydrogenase by protein kinases [13] and the total decrease of oxidative processes during hibernation is also connected with the decrease of activity and changes in regulatory properties of a number of citric acid cycle enzymes and respiratory chain components in the inner mitochondrial membrane [9, 10].

Functional activity of cardiac, skeletal, and smooth muscles during hibernation is also strongly depressed. However, the hearts of hibernators continue to contract at low body temperatures, whereas those of non-hibernating mammals fail to work at only a few degrees Celsius below normal body temperature [14]. It is still unclear which mechanisms allow cardiac muscle of hibernators to operate over such a broad temperature range (from 0 to 37°C) and to change the heart contractile parameters within only a few hours. It is suggested that the main participants of excitation–contraction coupling, i.e., the plasma membrane Ca-channels and proteins of the sarcoplasmic reticulum (SR) membranes, play a key role in the maintenance of heart contractile activity at low temperatures [14-16]. The influx of Ca^{2+} into cardiac cells through the plasma membrane Ca-channels is strongly decreased during hibernation [14, 17]; this is probably connected with the decrease of the phosphorylation of subunits of these channels by endogenous protein kinases [18]. Simultaneously, the role of SR in transient increases of cytoplasmic free Ca^{2+} concentration necessary for heart contraction become more important [15, 16]. Enhanced Ca-accumulating ability of SR preparations from the hearts of hibernating animals was reported [19, 20]; this is probably connected with changes in the properties of Ca-ATPase and/or SR Ca-binding proteins, in particular, calsequestrin [21]. An unusually high content of SR Ca-release channels (ryanodine receptors) with increased sensitivity to activating Ca^{2+} concentrations was also found in SR preparations from the heart of hibernators [21].

Most skeletal muscles are inactive during hibernation; only contractions of the intercostal muscles and diaphragm support the infrequent breathing movements and some tonic muscles are involved in maintenance of the typical posture of hibernating animals [1, 2]. Until now, information about possible adaptations in the properties of skeletal muscle SR for hibernation is essentially absent. However, it is known that SR can provide significant input into non-shivering (at body temperature below 12-14°C) and shivering (at higher body temperatures) thermogenesis during animal awakening between torpor bouts [1, 22]. In addition, during these awakenings the functional activity of skeletal muscles is fully restored. All of these indicate that both the contractile apparatus and SR Ca-release channels and Ca-ATPase, the main participants of excitation–contraction coupling in skeletal muscles, readily regain their functional activity.

Therefore, the main goal of the present study was to analyze the general functional properties of SR preparations obtained from the skeletal muscles of summer active and winter hibernating ground squirrels *Spermophilus undulatus*.

MATERIALS AND METHODS

Fluorescent probes 8-anilino-1-naphthalenesulfonic acid (ANS) and pyrene, ATP, ADP, Mops, NADH, sodium deoxycholate, histidine, imidazole, sucrose, glycine, EGTA, and EDTA were purchased from Sigma (USA). Tris, pyruvate kinase, and phosphoenolpyruvate were from Reanal (Hungary); lactate dehydrogenase was from Ferak (Germany); SDS and the carbocyanine dye Stains-All were from Serva (Germany). All other reagents were "reagent grade" or better.

Adult ground squirrels *Spermophilus undulatus* were collected by live trapping in Yakutiya and were maintained in the Animal Facility of the Institute of Cell Biophysics (Pushchino, Moscow Region) in individual cages at 20-25°C in natural daylight. The animals were supplied with satisfactory food, water, and nest material. In November, the animals were put into a dark room at temperature 2-4°C. For experiments, summer active (body temperature 37°C) and winter hibernating (body temperature 2-5°C) animals were killed by decapitation in June-July and in January-February in the middle of hibernation bout, respectively. Hind leg skeletal muscles were immediately cut off and plunged into liquid nitrogen for transportation. For long storage (for 2-4 weeks), tissues were transferred into a deep freezer (below –70°C). In this study, the data obtained during summer–winter seasons of 1998, 1999, and 2000 are present.

SR fragments from hind leg skeletal muscles of ground squirrels were obtained by differential centrifugation [23] with minor modifications described earlier [24]. The final SR preparations were frozen in liquid nitrogen and stored at –70°C.

Protein concentration was measured according to Lowry et al. [25] using BSA as a standard. The content of phospholipids in SR preparations was measured after

mineralization followed by P_i measurement according to Bartlett [26].

Ca-ATPase activity was measured using a coupled enzyme system (pyruvate kinase + lactate dehydrogenase) as described in full details earlier [24].

SDS-PAGE was carried out according to Laemmli [27] using 3% stacking and 3-20% gradient running gels [28]. Myosin heavy chains (205 kD), β-galactosidase (116 kD), phosphorylase *b* (97.4 kD), BSA (66 kD), ovalbumin (45 kD), and carboanhydrase (29 kD) were used as protein markers for measurement of molecular masses of SR proteins. After electrophoresis, the gels were fixed and washed 3 times in 25% ethanol and stained with a cationic carbocyanine dye (Stains-All) in a solution containing 25% ethanol, 7.5% formamide, 0.0025% Stains-All, and 30 mM Tris, pH 8.8, for 36-48 h in the dark. The gels were briefly destained in 25% ethanol in the dark and scanned on an UltroScan XL laser densitometer at 595 nm (LKB, Sweden). The gels were finally destained in 25% ethanol in the light. Subsequently, the gels were stained with Coomassie Brilliant Blue R-250 and destained by a standard procedure. Then they were scanned on an UltroScan XL laser densitometer at the same wavelength. The protein peak areas were calculated using the GelScanXL program (LKB, Sweden). Treatment of SR preparations with cupric–phenanthroline was carried out as described previously [29].

In the present study, ANS and pyrene were used as fluorescent probes. ANS (5-100 μM) was added to SR membranes (0.2 mg/ml) in 10 mM Mops, pH 7.0. Sample fluorescence was excited at 360 nm and registered at 480 nm with the spectral width of the exciting and analyzing monochromator slit set for 5-nm resolution using a Hitachi F-3000 spectrofluorimeter. Apparent K_d and F_{max} values for ANS were calculated using double reciprocal ($1/F$ versus $1/[ANS]$) plots [30]. Pyrene fluorescence was excited at 335 nm (total fluorescence) and at 285 nm (induced fluorescence) with the spectral width of the exciting monochromator slit set for 5-nm resolution and recorded at 350-470 nm with the spectral width of the analyzing monochromator slit set for 1.5-nm resolution. The medium contained 10 mM Mops, pH 7.0, 100 mM KCl, 0.5 mg/ml SR protein, and 12 μM pyrene. The degree of pyrene excimerization was calculated as the ratio of fluorescence intensity at 465 nm (excimeric form) and 373 nm (monomeric form). All calculations were carried out using standard methods [30].

Each parameter for every individual SR preparation was measured at least 3 times, and the mean values for the parameters were calculated for further statistical calculations. In the tables, the mean values for a number of individual SR preparations isolated from different animals are presented ± standard deviation (mean ± SD). The number of preparations used is indicated in parentheses. Statistical calculations were made using Student's *t*-criterion [31].

RESULTS AND DISCUSSION

The Ca-ATPase activity in SR membrane fraction isolated from skeletal muscles of winter hibernating ground squirrels was ~2.2-fold lower than that in SR preparations from summer active animals (Table 1). The yield of SR protein from skeletal muscles of active and hibernating animals was practically the same, but the phospholipid/protein ratio in SR membranes of winter hibernating ground squirrels was significantly lower in comparison with summer active animals (Table 1). This is probably connected with the decrease of phospholipid content in SR membranes during hibernation, whereas the total protein content remained unchanged.

The decrease of the content of Ca-ATPase protein in SR membranes during hibernation may be one of the reasons of the low enzyme activity. SDS-PAGE was used to examine this suggestion. It was found that the content of Ca-ATPase protein (molecular mass 105 kD) is in fact slightly lower in SR preparations of hibernating ground squirrels than that in SR of summer active animals (Fig. 1 and Table 2). However, the decrease of Ca-ATPase protein content in SR membranes is not the main reason for the low enzyme activity because the Ca-ATPase specific activity calculated with correction for its real protein content was still ~2-fold lower in SR preparations of winter hibernating ground squirrels (Table 1). Therefore, the

Table 1. Yield of protein, content of phospholipid phosphorus, and Ca-ATPase activity in SR preparations of summer active and winter hibernating ground squirrels

Parameter	Summer active ground squirrels ($n = 6$)	Winter hibernating ground squirrels ($n = 8$)
Protein yield, mg/g tissue	0.91 ± 0.04	0.85 ± 0.07
Phosphorus content, μmole P_i/mg SR protein	1.04 ± 0.07	0.75 ± 0.05*
Ca-ATPase activity, μmole/min per mg SR protein	5.2 ± 0.4	2.4 ± 0.1*
Ca-ATPase activity, μmole/min per mg Ca-ATPase protein	14.2 ± 0.4	7.8 ± 0.9*

Note: The number of SR preparations used for analysis is shown in parenthesis. Asterisks indicate values for SR preparations of winter hibernating animals that are significantly different from the corresponding value for SR preparations of summer active ground squirrels.

* $p < 0.01$.

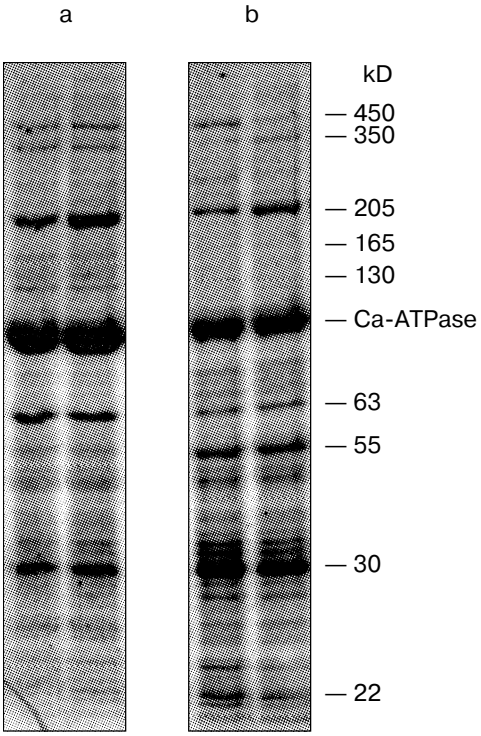

Fig. 1. Electrophoregrams of SR preparations isolated from skeletal muscles of two summer active (a) and two winter hibernating (b) ground squirrels. Gels were stained by Coomassie R-250. In each case 30 μg of SR protein was loaded.

turnover number of Ca-ATPase in skeletal muscle SR of hibernating ground squirrels is significantly lower than in SR of summer active animals (7,400 versus 13,500, respectively).

SDS-PAGE also demonstrated that in addition to the difference in Ca-ATPase protein content the SR preparations are different in the contents of a number of other protein components: in SR membranes of hibernating ground squirrels the contents of proteins with molecular masses 450, 350, 205, 165, 130, and 63 kD is significantly decreased and the content of proteins with molecular masses 80, 72, 55, 44, 30, and 22 kD is increased (Figs. 1 and 2a). The content of some of these proteins in SR preparations of active and hibernating ground squirrels is different with a high level of significance (Table 2).

Calsequestrin, sarcalumenin, and histidine-rich Ca-binding protein were identified in SR preparations using the anionic carbocyanine dye Stains-All. Stains-All preferentially stains in a dark blue color only Ca-binding proteins, glycoproteins, and phosphoproteins. In SR preparations obtained from skeletal muscles of rabbits, rats, and ground squirrels, most proteins are stained by Stains-All in red color, but only the three above-mentioned proteins are stained in dark blue color [24]. As seen from Figs. 2b and 2c, in SR preparations of both summer active and winter hibernating ground squirrels only three proteins are stained by Stains-All in dark blue color. In accordance to their electrophoretic mobility and blue staining, these proteins were identified as Ca-binding glycoproteins: calsequestrin (63 kD), sarcalumenin (130 kD), and histidine-rich Ca-binding protein (165 kD) [24, 32]. The content of all of these proteins was significantly lower in SR preparations of hibernating ground squirrels (Fig. 2, b and c; Table 2). The decrease of the content of proteins with molecular masses 63, 130, and 165 kD was also found after gel staining by Coomassie R-250 (Figs. 1 and

Table 2. Contents of the main proteins in SR membranes of summer active and winter hibernating ground squirrels

SR proteins	Summer active ground squirrels ($n = 6$)	Winter hibernating ground squirrels ($n = 8$)
Stains-All staining		
Histidine-rich Ca-binding protein (165 kD)	100 ± 9.1	39.8 ± 6.7**
Sarcalumenin (130 kD)	100 ± 6.4	39.9 ± 4.8**
Calsequestrin (63 kD)	100 ± 5.0	28.7 ± 2.8**
Coomassie R-250 staining		
450 kD-protein (ryanodine receptor?)	2.0 ± 0.4	1.0 ± 0.4**
Histidine-rich Ca-binding protein (165 kD)	1.6 ± 0.1	0.6 ± 0.1**
Sarcalumenin (130 kD)	2.0 ± 0.1	0.6 ± 0.1**
Ca-ATPase (105 kD)	42.0 ± 3.1	29.4 ± 2.8*
Calsequestrin (63 kD)	10.3 ± 1.1	3.3 ± 0.5**
55 kD-protein (calreticulin?)	5.7 ± 0.9	8.7 ± 0.9*
30 kD-protein	11.0 ± 1.9	17.0 ± 0.9*
22 kD-protein	4.7 ± 1.6	10.6 ± 2.7*

Note: Data obtained from scanning gels stained by Stains-All are expressed relative to the mean intensity for the peak area of each protein in SR preparations of summer active ground squirrels. Data obtained after staining the gels with Coomassie R-250 are expressed as percentages of the total peak areas. The number of SR preparations used for analysis is shown in parenthesis. Asterisks indicate values for SR preparations of winter hibernating animals that are significantly different from the corresponding value for SR preparations of summer active ground squirrels.

* $p < 0.05$.

** $p < 0.01$.

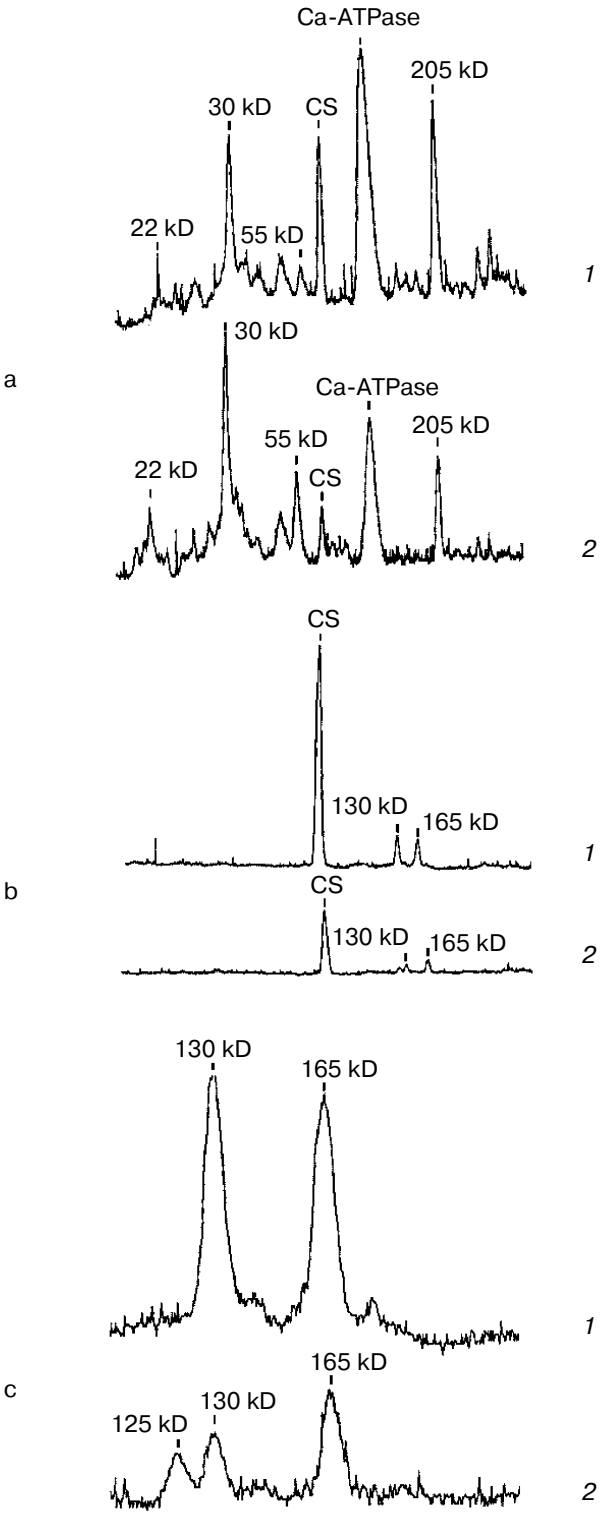

Fig. 2. Densitograms of SR preparations from skeletal muscles of summer active (*1*) and winter hibernating (*2*) ground squirrels separated by SDS-PAGE and stained by Coomassie R-250 (a) and Stains-All (b), and an enhanced view of the densitogram region containing proteins of 100-200 kD after Stains-All staining (c). In each case, 30 μg of SR protein was loaded (CS, calsequestrin).

2a; Table 2). In addition to the decrease of the content of sarcalumenin and histidine-rich Ca-binding protein in SR preparations of hibernating ground squirrels, a new protein band with molecular mass ~125 kD that is stained in blue color by Stains-All is appeared in these preparations (Fig. 2c). This is a probably a proteolytic fragment of sarcalumenin or its modification in the carbohydrate part.

It should be noted that calsequestrin, sarcalumenin, and histidine-rich Ca-binding protein, the content of which is significantly decreased in SR membranes of hibernating ground squirrels, are directly involved in the regulation of SR Ca-release channels [32, 33]. Calsequestrin interacts with ryanodine receptor providing high calcium concentration near the intralumenal domain of the Ca-release channel, and the removal of calsequestrin affects Ca^{2+} release from SR [33]. Re-association of calsequestrin and ryanodine receptor restores the operation of the Ca-release channel. Sarcalumenin and histidine-rich Ca-binding protein probably also interact with the ryanodine receptor molecule; moreover, phosphorylation of these proteins by endogenous protein kinases inhibits the activity of rabbit SR Ca-release channels [32].

An unequivocal identification of other proteins the content of which in SR preparations of summer active and winter hibernating animals is significantly different is rather complicated. However, the protein with molecular mass 450 kD is probably the ryanodine receptor, and the protein with molecular mass 350 kD is probably its proteolytic fragment [34]. The protein with molecular mass 205 kD has the same electrophoretic mobility as myosin heavy chains; we have described the unusually high content of this protein in SR preparations of the ground squirrels earlier [24]. The 55 kD protein is most probably the Ca-binding protein calreticulin [35]. It should be noted that the ratio of SR Ca-binding proteins calsequestrin and calreticulin is significantly changed during skeletal muscle development. In the early stages of embryonic development calreticulin is a dominant Ca-binding protein of SR membranes, but in mature skeletal muscle fibers it is replaced by calsequestrin [36]. This may suggest that the opposite process occurs during hibernation: the decrease of calsequestrin content in skeletal muscle SR membranes is accompanied by an increase in the content of calreticulin.

Additional investigations should be carried out to identify other protein components of SR membranes the content of which in SR preparations of summer active and winter hibernating ground squirrels is significantly different (Table 2). However, the existing data show clearly that the protein composition of SR membranes in skeletal muscles of the ground squirrels is significantly changed during hibernation. These changes in SR protein composition are probably one of the reasons for the decrease of Ca-ATPase activity because calsequestrin and

calreticulin have been considered in recent years as probable regulators of its enzymatic activity [35].

Because Ca-ATPase is present in SR membranes both in monomeric and oligomeric (consisting of a few enzyme molecules [37]) forms, some researchers suggest that the monomer–oligomer transitions might be one of the ways of regulating the activity of the enzyme; the formation of oligomeric Ca-ATPase complexes leads to its inhibition [38, 39]. To evaluate the Ca-ATPase oligomeric state in SR membranes of summer active and hibernating ground squirrels, the preparations were treated by the cross-linking agent cupric–phenanthroline, which catalyses the formation of covalent S–S-bonds between neighboring SH-groups [28, 29]. As seen from Fig. 3, the treatment of SR membranes by cupric–phenanthroline leads to a the progressive decrease of the peak area of the Ca-ATPase monomers (protein band 105 kD). Simultaneously, new protein bands with high molecular masses (from ~200 to ~500 kD) corresponding to the covalently linked Ca-ATPase oligomeric complexes appear on electrophoregrams (data not shown). However, the rate of cross-linking of enzyme molecules in SR preparations of summer active and hibernating ground squirrels is significantly different. In the SR preparations of summer active animals the peak area of Ca-ATPase monomers is ~20% decreased for 30-min treatment by cupric–phenanthroline, and in SR preparations of hibernating ground squirrels only 10-min incubation already leads to ~60% decrease of the peak area of enzyme monomers (Fig. 3). Therefore, in SR membranes of hibernating animals the Ca-ATPase is present in a more aggregated state in comparison with membranes of summer active ground squirrels that, probably, results in the decrease of enzymatic activity.

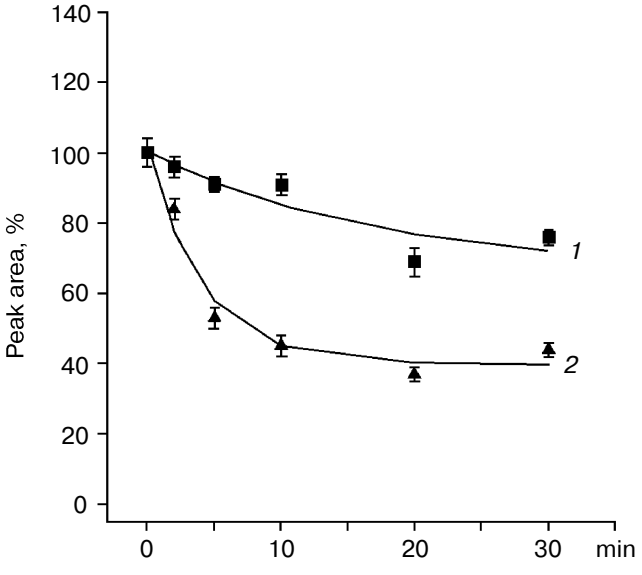

Fig. 3. Dependence of monomeric Ca-ATPase peak area (protein band at 105 kD) on the incubation time of SR membranes from summer active (*1*) and winter hibernating (*2*) ground squirrels with cupric–phenanthroline. Details of the experimental procedure are described in "Materials and Methods".

Table 3. Parameters of ANS and pyrene fluorescence in SR membranes of summer active and winter hibernating ground squirrels

Parameter	Summer active ground squirrels ($n = 6$)	Winter hibernating ground squirrels ($n = 8$)
ANS		
Apparent K_d, μM	29.1 ± 2.7	21.3 ± 1.6*
F_{max}, arbitrary units	230 ± 34	192 ± 20
Pyrene		
I_e/I_m, λ_{ex} = 335 nm (total lipids)	0.54 ± 0.04	0.50 ± 0.01
I_e/I_m, λ_{ex} = 285 nm (annular lipids)	0.54 ± 0.01	0.44 ± 0.01*

Note: The number of SR preparations used for analysis is shown in parenthesis. Asterisks indicate values for SR preparations of winter hibernating animals that are significantly different from the corresponding value for SR preparations of summer active ground squirrels. I_e/I_m is the ratio of fluorescence intensity of excimeric and monomeric forms of pyrene.

* $p < 0.05$.

Increased Ca-ATPase aggregation in SR membranes of hibernating animals is probably connected in part with the decreased lipid/protein ratio in these preparations. On the other hand, it was demonstrated recently that lateral separation of proteins and lipids occurs during hibernation in endoplasmic reticulum of nervous tissue cells: lipid areas free of proteins as well as areas with high content of aggregated proteins appeared in the membranes [40]. A similar process probably occurs in skeletal muscle SR membranes.

It is also well known that the activity of Ca-ATPase depends on the composition and properties of the phospholipid bilayer [41]. To investigate some characteristics of SR membranes from summer active and hibernating ground squirrels, fluorescent probes ANS and pyrene were used (Table 3). Binding of the hydrophilic probe ANS with the membrane surface involves both electrostatic and hydrophobic interactions [30, 42]. It was found that the affinity of SR membranes from hibernating animals to ANS is significantly higher (the K_d for ANS is 1.5-fold lower) and the maximal ANS fluorescence is slightly lower than those in SR preparations of summer active ground squirrels (Table 3). An increase of affinity of SR membranes to ANS during hibernation probably reflects

a decrease of the negative surface charge of the membranes that in turn is connected with the changes in SR protein composition and lipid/protein ratio [29]. The lower maximal fluorescence level probably reflects the decrease in the number of ANS-binding sites on SR membranes or the changes of polarity of the microenvironment of the probe.

The hydrophobic fluorescence probe pyrene penetrates into the center of the lipid bilayer where the phospholipid hydrocarbon chains are located, and its fluorescence parameters can be used to characterize the microviscosity of the surrounding of the probe and the hydrophobic volume of the membrane [30, 42]. The most sensitive fluorescence parameter is the degree of pyrene excimerization, the ratio of fluorescence intensity of its excimeric (dimeric) and monomeric forms, because formation of pyrene dimers at its fixed concentration is controlled by the rate of diffusion and directly depends on membrane microviscosity and hydrophobic volume. In addition, pyrene allows estimation of the properties of annular lipids surrounding the hydrophobic domain of the Ca-ATPase molecule because resonance energy transfer from tryptophan to pyrene is possible. After excitation of fluorescence of tryptophan residues in a protein molecule, some part of the energy is transferred to the probe molecules located within the Förster radius that results in the appearance of so-called "induced" pyrene fluorescence [30, 42]. Because 18 of 19 tryptophan residues of Ca-ATPase are located in the hydrophobic domain of the enzyme molecule and the content of Ca-ATPase protein in SR membranes is relatively high (Table 2), the "induced" pyrene fluorescence characterizes mainly the state of the lipids surrounding the enzyme [43].

As seen from Table 3, the degree of pyrene excimerization in SR preparations of winter hibernating ground squirrels is lower than that in SR preparations of summer active animals. This probably indicates an increase of viscosity of the microenvironment of the probe and/or an increase of membrane hydrophobic volume. These differences are higher for annular lipids surrounding the transmembrane domain of Ca-ATPase molecule. Since the content of lipids in SR membranes of winter hibernating ground squirrels is significantly lower than that that in membranes of summer active animals, the hydrophobic volume in the former SR preparations should be decreased. Therefore, the decrease in pyrene excimerization in SR membranes of winter hibernating animals indicates an increase of membrane lipid microviscosity, especially for the annular lipids surrounding Ca-ATPase. This is in good agreement with our data that Ca-ATPase in SR membranes of hibernating ground squirrels is in a more aggregated state (Fig. 3) because membrane proteins, and Ca-ATPase in particular, provide "structurization" of surrounding lipids [42]. Thus, the decrease of lipid/protein ratio in SR membranes of hibernating animals results in an increase of microviscosity of both total and annular lipids. Therefore, the properties of the phospholipid bilayer of the SR membranes of summer active and winter hibernating ground squirrels are different, and this probably plays a particular role in the change of Ca-ATPase activity.

As mentioned above, the changes of activity of many enzymes during hibernation is connected with their phosphorylation by protein kinases [13]. It was demonstrated recently that the sharp decrease of Na,K-ATPase activity in skeletal muscle plasma membranes of ground squirrels in winter is the result of such enzymatic phosphorylation [44]. Preliminary experiments conducted in our laboratory have shown that SR preparations from skeletal muscles of ground squirrels possess relatively high endogenous protein kinase activity. The total level of phosphorylation and the pattern of phosphorylated proteins are different in SR preparations of summer active and winter hibernating animals (A. N. Malysheva, A. M. Rubtsov, unpublished data). Phosphorylation of Ca-ATPase protein and/or some regulatory proteins in SR membranes may be one of the ways in which the changes in enzyme catalytic activity occurs, but further, more detailed investigations are necessary to answer this question.

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