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Cyclic Structural Changes of Endoplasmic Reticulum and Golgi Complex in Hippocampal Neurons of Ground Squirrels during Hibernation

L. S. Bocharova[a], R. Ya. Gordon[b]*, V. V. Rogachevsky[b], D. A. Ignat'ev[b], and S. S. Khutzian[a, b]

[a] *Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow region, Russia*
[b] *Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow region, Russia*
**e-mail: ritagordon@mail.ru*

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Abstract—Repetitive remodeling and renewal of the cytoplasmic structures realizing protein synthesis accompanies the cycling of the ground squirrels between torpor and arousal states during hibernation season. Previously, we have shown the partial loss of ribosomes and inactivation of the nucleolus in pyramidal neurons in the hippocampus CA3 area at each bout of torpor with their rapid and full recovery after warming up. In the present paper, we describe reversible structural changes of the endoplasmic reticulum (ER) and Golgi complex (GC) in these neurons. The transformation of the ER form from mainly granular stacks of flattened cisternae to smooth tubules occurs at every entrance in torpor, while the reverse change happens at arousal. The torpor state is also associated with GC fragmentation and loss of their flattened cisternae, i.e., dictiosomes. In neurons, the appearance of the autophagosomal vacuoles containing fragments of membrane structures and ribosomes in torpor state is a sign of the partial destruction of ER and GC. Granular ER restoration, perhaps through assembly from the multilamellar membrane structures, bags or whorls begins in the middle of the torpor bout, while GC dictiosomes reappear only during warming. The ER and GC completely restore their structure 2–3 h after the beginning of arousal. Thus, hibernation represents an example of the structural adaptation of the nerve cell to deep changes in functional and metabolic activity through both the active destruction and renewal of ribosomes, ER, and GC. Perhaps, namely the incomplete ER autophagosomal degradation in torpor provides its rapid renewal at arousal through the reassembly from the preserved fragments.

Keywords: assembly of endoplasmic reticulum, Golgi complex, ribosomes, autophagy, neurons, hibernation,
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Abbreviations used: GC, Golgi complex; AP, autophagosomes; LF, lipofuscin; Tb, brain temperature; ER, endoplasmic reticulum

INTRODUCTION

Cytoplasmic membrane structures constantly change their shape and undergo renewal in response to changing external influences. The structural changes of the ER and GC, which accompany cell division, growth, and differentiation, are currently well understood (Pannese, 1968; Powell, Latterich, 2000; Voeltz et al., 2002; Demakova, Kiseleva, 2003; Fedorovich et al., 2005; Snigirevskaya et al., 2006; Puhka et al., 2007) and so are effects of various disorders and disturbances of cell metabolism (Pathak et al., 1986; del Valle et al., 1999; Bejarano et al., 2006; Borgese et al., 2006; Landhans et al., 2007). At the same time, how the structures of the ER and of GC depend on the level of the cell functional activity are much less clear. Thus, the electrical stimulation of neurons was shown to lead to the marked proliferation of rough ER (Bocharova et al., 1972); however, it remains unclear whether the ER and GC structures are also affected by the decrease in the neuronal activity level.

The hibernation of ground squirrels is a convenient model for studying function-dependent changes in the ultrastructure of brain nerve cells. The hibernation season of these animals consists of periods (bouts) of a cold torpor state alternated with brief episodes of spontaneous warming up to 36°C; during the hibernation season, they undergo up to 20–25 torpor–arousal cycles (van Breukelen, Martin, 2002; Carey et al., 2003; Drew et al., 2007). In torpor animals, the neuron spike electrical activity of the majority of brain regions ceases; however, it is restored between bouts (Shtark, 1970; Heller, 1979; Krilowicz et al, 1988; Drew et al., 2007). Consequently, neurons in the brains of ground squirrels repeatedly transfer from the state of the profound inhibition to the generalized excitation. In hippocampal pyramidal neurons, these transitions are accompanied by marked changes in cell

volume, the number of ribosomes and their synthetic activity, the intensity of RNA synthesis, as well as by changes in the size and structure of dendrites and synaptic contacts (Bocharova et al., 1992a, 1992b; Popov, Bocharova, 1992; Popov et al., 1992; Gordon et al., 1997, 2006).

The goal of the present work was to study the changes in ER and GC structures in ground squirrel hippocampal neurons in the torpor–arousal cycle. In the pyramidal neurons in CA3 field of hippocampus, entrance into torpor has been shown to be accompanied by a pronounced structural transformation and the reduction of the ER and GC in parallel with the accumulation of autophagosomes (APs) that contain fragments of cytoplasmic membrane structures and ribosomes. The signs of the formation of a new ER from multilamellar membranous structures can be seen as soon as in the middle of the torpor bouts, while the GC cisternae only reappear after the animal is warmed up. The structure of both the ER and GC in awakened squirrels is completely restored after 2 h.

MATERIALS AND METHODS

Animals. The ground squirrels *Citellus undulatus* caught during summer in Siberia were kept in vivarium. Animals of both sexes weighing 500–800 g were taken for experiments. During winter, the ground squirrels were placed into individual wooden boxes measuring 15 × 15 × 20 cm and kept in darkness in a sound-proof cold chamber at a temperature optimal for hibernation (1–3°C). Thermosensors were built into the bedding of boxes to monitor the state of the animals. When ground squirrels were in the torpor state, the temperature in the nest corresponded to the temperature in the chamber, while during awakening, it increased to 12–14°C. Just before decapitation, the animal's state was controlled by measuring the brain temperature and heart rate. The brain temperature was measured by a sensor of original construction (with diameter of 1 mm, length of 10 mm, and accuracy of measurement 0.2°C (manufactured at the Institute for Biological Instrumentation, Russian Academy of Sciences, Pushchino), which was placed into the external acoustic meatus near the tympanum.

The duration of torpor bouts varies from several days at the beginning and at the end of the hibernation season to two weeks in the middle. The spontaneous warming up of ground squirrels up to 36°C occurs for 2–3 h, whereas periods of normothermia last up to 1.5 days. Between the torpor bouts, animals arouse; however, they do not eat and remain at low activity. The exit of ground squirrels from torpor can be provoked at any bout point by various stimuli. In the present work, to induce arousal, the animals were transferred into a warm room where they awoke up after 2–3 h.

The ground squirrels were taken in January to February at different stages of the torpor–warming up cycle: at the beginning of cooling ($T_b \approx 23$°C), in the middle of the torpor bout ($T_b \approx 4$°C); immediately after provoked warming up ($T_b \approx 23$°C); 2–3 h after the beginning of provoked warming up ($T_b \approx 36$°C); and in the normothermic state between bouts ($T_b \approx 36$°C). As a control, the summer ground squirrels were used. For each state, brains of three animals were used.

Electron microscopy. Ultrastructure of pyramidal neurons of the hippocampus CA3 field was studied by the commonly accepted procedure: after the decapitation of animals, brains were removed and the hippocampus was fixed in 2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer (pH 7.2–7.4) with subsequent postfixation in 1% solution of osmium tetroxide in the same buffer, dehydration, and embedding in epon-araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. Length of ER profiles was measured in scanned microphotographs of various areas of the neuronal cytoplasm in Photoshop software (Adobe Systems Inc.). Data are presented as the means and their standard deviations. Statistical significance of differences of the means was estimated by means of Student's t-criterion.

RESULTS

The general ultrastructure of the cytoplasm of pyramidal neurons in the hippocampus CA3 field in hibernating ground squirrels between torpor bouts and in active summer animals does not differ practically; i.e., rough ER forming Nissl bodies, polyribosomes, and GC (Fig. 1a) predominate. All of these structures are involved in the active synthesis of proteins and their export from neuronal soma. During the cooling of animals, the picture changes fundamentally as soon as at T_b 23°C. The majority of Nissl bodies disappear and the amount of polyribosomes also decreases markedly. Only a small number of rough ER cisternae are saved and they are short; the smooth ER becomes more pronounced (Fig. 1b). In the state of torpor, the overall filling of the cytoplasm with structures, especially with ER and GC, decreases drastically; polyribosomes are rare (Fig. 1c), but numerous small vesicles appear (Fig. 2d), as well as AP and large LF granules (Fig. 1c). Not later than two hours after the beginning of warming, the structure of the neuronal cytoplasm is restored: the rough ER, Nissl bodies, and developed GC reappear again and ribosomes are jointed as polyribosomes (Fig. 1d). At the beginning of the next bout of torpor, the cycle of structural remodeling in the neuronal cytoplasm is repeated.

Transformation of ER of nerve cells in cold ground squirrels appears mainly as a decrease in the length of profiles of flat cisternae covered with ribosomes (Fig. 1, 3b). Whereas, in neurons of warmed ground squirrels, the mean length of the ER profile in section is 1.3 ± 0.8 μm, while in the middle of the bout of torpor, it is reduced to 0.3 ± 0.1 μm. The factor of the initiation of protein synthesis eIF2 in ribosomes is known to be inactivated in the brain of torpid ground squir-

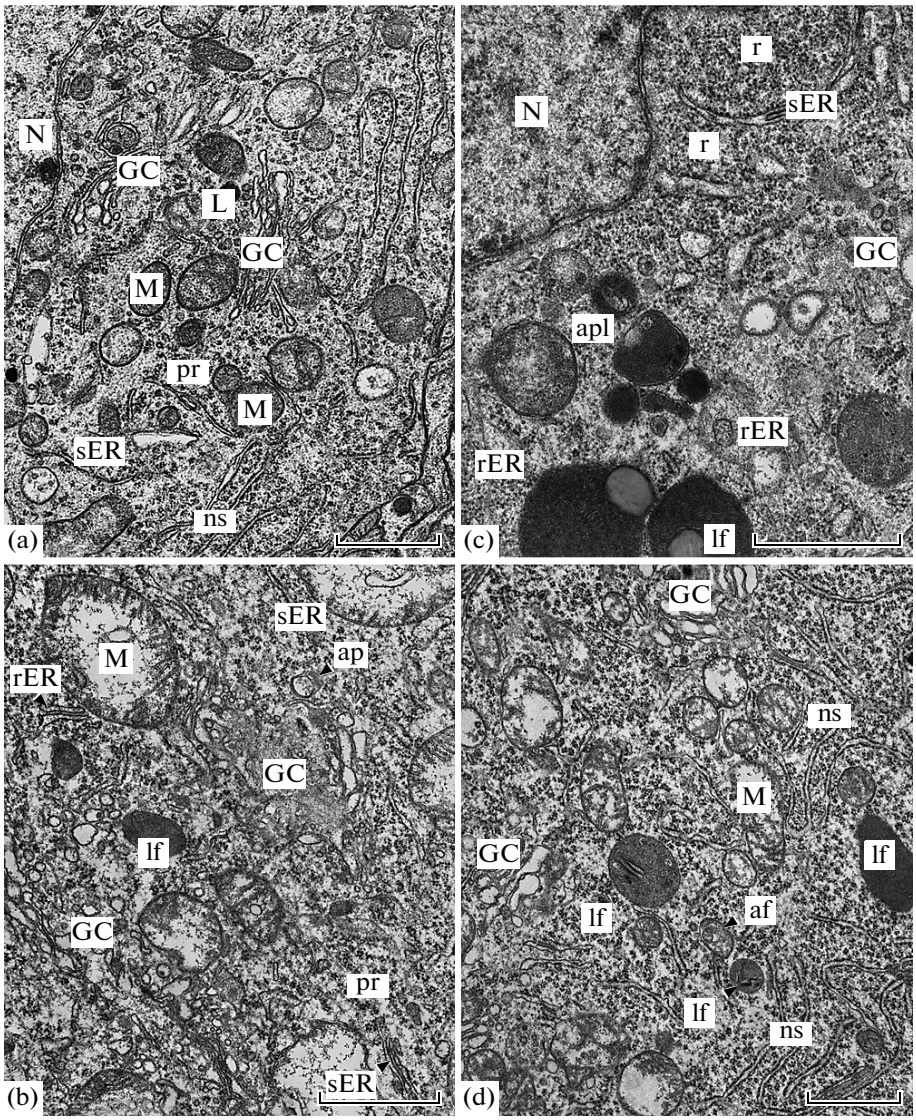

Fig. 1. General view of cytoplasm in the pyramidal neurons of ground squirrel hippocampus CA3 field at different stages of the torpor–arousal cycle. (a) normothermia between bouts of torpor (T_b 37°C); (b) the beginning of cooling (T_b 23°C); (c) the state of torpor (T_b 4°C); (d) 2 h after the beginning of the provoked warming up (T_b 36°C). Designations: A, autophagosome; AL, autophagolysosome; G, Golgi complex; L, lysosome; LF, lipofuscin granule; M, mitochondrion, nis, Nissl substance; pr, polyribosomes; r, ribosomes; sER, smooth endoplasmic reticulum; gER, rough endoplasmic reticulum; N, nucleus. Scale bar: 1 μm.

rels, as well as in animals at just the beginning of cooling (Frerichs et al., 1998; Carey et al., 2003). As a result, ribosomes lost their ability to interact with the ER, while the absence of this interaction might be the main reason for changes in the shape of the ER from mainly cisternal and flat to predominantly tubular.

Reduction of GC is the peculiarity of neurons in the hippocampus of cold ground squirrels. Between bouts, GC has the classic structure: *cis*-, *media*-, and *trans*-flat cisternae are well expressed, as well as the vesiculotubular compartment, and transport vesicles (Figs. 1a, 2a). During torpor, GC undergoes disintegration into vesicles, while stacks of flat cisternae, also called dictiosomes, are nearly absent. The GC remnants are presented by solitary large light vesicles (0.1–0.25 μm) surrounded by numerous small vesicles of various size (0.03–0.06 μm) (Fig. 1c, 2c, 2d, 3d). The reduction of GC dictiosomes in CA3 pyramids is well expressed as soon as T_b decreases up to 23°C (Fig. 2b). The flat cisternae of GC, mainly in *cis*- and medial layers, swell and split into individual large fragments that resemble terminal enlargements of GC flattened cisternae in the cells of active animals (Figs. 2b, 3d) by their sizes, as well as by the absence of electron dense content (Figs. 1a, 2a, 3c). Neighboring large vesicles happen to be connected with short bridges of remnants of flat cisternae by forming dumbbell-like structures (Fig. 2b, insertion). GC is known to be disintegrated into vesicles during the cessation of synthesis and transport of synthesized proteins from

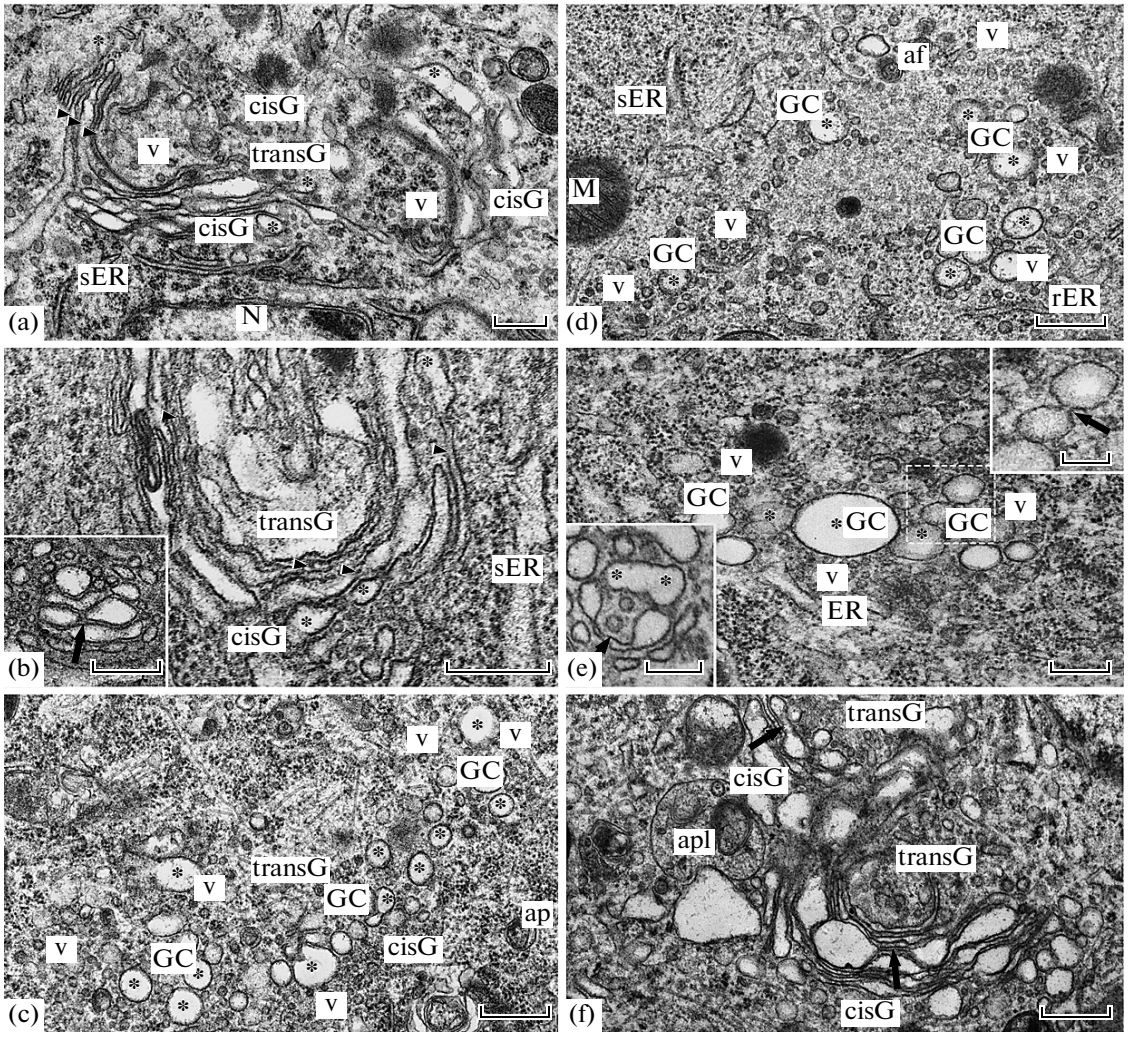

Fig. 2. Reduction of GC in pyramidal neurons of ground squirrel hippocampus CA3 field during entrance in torpor and restoration of GC structure at arousal. (a) GC structure between bouts of torpor (T_b 37°C); (b) at the beginning of cooling (T_b 23°C); (c) at the beginning of the torpor bout (T_b 4°C); and (d) in the middle of the torpor bout (T_b 4°C); (e and f) during the process of warming up (T_b 13 and 23°C, respectively). Designations: *cis*, *trans*, the *cis*- and *trans*-sides of Golgi stacks; v, vesicle; arrow heads (a, b) flattened cisternae; asterisk (a, b)dilated cisternae endings; asterisks (c, d, e) large transparent vesicles; arrow (b insertion, e insertion, f) dumbbell-like structures composed by large vesicles and residual fragments of flattened cistern in reduction of GC at entrance in torpor and arrows (d, e inserts) reassemble at arousal from torpor; other designations as those in Fig. 1. Scale bar: in all images excluding insertions; 0.5 μm, 0.5 and 0.25 μm (b, e, insertion).

ER (Altan-Bonnet, Lippincott, 2005; Persico et al., 2009). The same consequences are produced by cell ATP deficit (del Valle et al., 1999) and by the inhibition of activity of protein kinase A as well (Bejarano et al., 2006). In ground squirrel neurons, the change in the form of the GC certainly results from its dysfunction after the entrance of animals into torpor.

Partial destruction of ER and GC also may occur concurrently with their structural transformations. There are a number of observations that indirectly support this point of view. In torpor state the volume of cytoplasm in the CA3 pyramids is by 30–50% lower than that between bouts and at the same time these cells lose up to 40% of cytoplasmic rRNA that corresponds with destruction of a considerable part of ribosomes (Bocharova et al., 1992b). In nerve cells of torpor ground squirrels, the protein content is also significantly reduced (Demin et al., 1988; Golovina, 1988). At the same time, cytoplasm of neurons is markedly less filled with all membranous structures (Figs. 1c, 2c, 2d; 3b, 3d). These facts indicate the activation of catabolic processes in the brain of cold ground squirrels and allow one to suggest that degradation involves not only ribosomes, but also some membrane cytoplasmic structures. During cooling and at the beginning of the torpor state, many APs that contain both ribosomes and fragments of membranous structures are present in the cytoplasm (Figs. 4a–4c); by the middle of the bout, they are transformed into autophagolysosomes (Figs. 4e, 4f). The possible sequence of transforma-

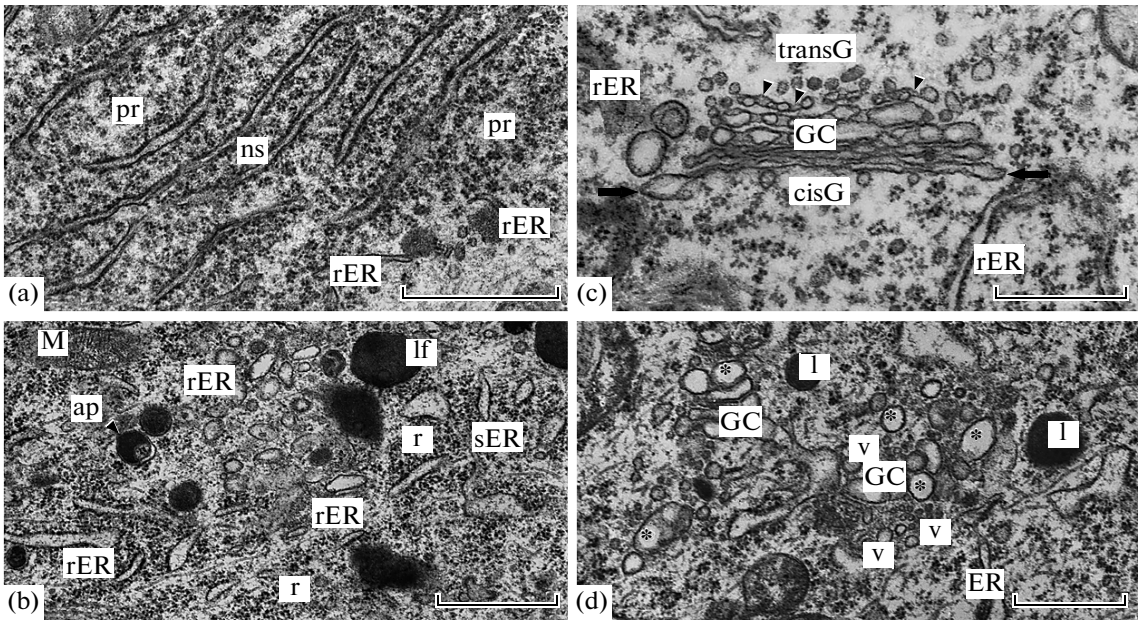

Fig. 3. Transformation of structure of ER (a, b) and GC (c, d) in pyramidal neurons of the ground squirrel hippocampus CA3 field during transition from normothermia (a, c, T_b 37°C) to the cold torpor state (b, d, T_b 4°C).
During cooling, ribosomes are shed from ER and length of its profiles decreases (a, b). GC is disintegrated into vesicles, dictiosomes disappear. Designations: cis, trans, the cis- and trans-sides of Golgi stacks; v, vesicles; arrows (c) terminal enlargements of GC cisternae; arrow heads (c) vesiculotubular GC compartment; other designations as those in Fig. 1 and 2. Scale bar: 0.5 (a–c) and 1 (d) μm.

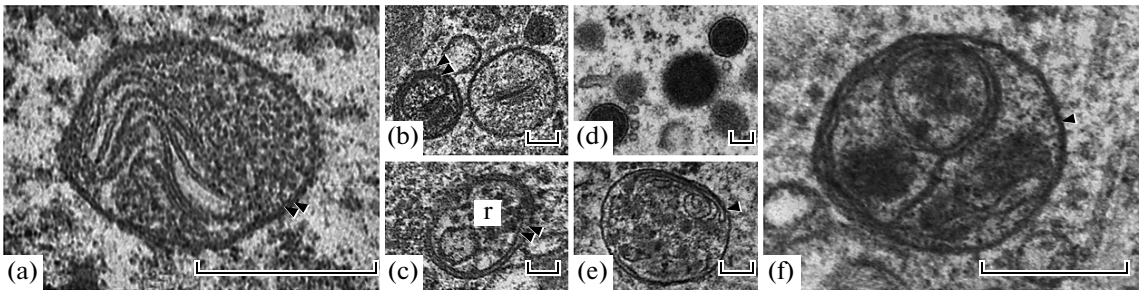

Fig. 4. Autophagosomal degradation of membranous structures and ribosomes in pyramidal neurons of ground squirrel hippocampus CA3 field during cooling and in the beginning of torpor. Autophagosomes (a–c); primary lysosomes (d); autophagolysosomes (e, f); r, ribosomes. Arrowheads, double and single external membranes of these structures. Scale bar: 0.25 (a, f) and 0.1 (b–e) μm.

tions of the ER and GC in CA3 pyramids during the transition of ground squirrels from normothermia between bouts to torpor may be represented as follows: shedding of ribosomes from ER ⟶ transformation of flat rough ER cisternae into tubular structures ⟶ cessation of transport of newly synthesized proteins from ER to GC ⟶ the disintegration of GC into vesicles ⟶ the uptake of fragments of smooth ER and GC by AP ⟶ the partial degradation of these fragments and ribosomes in autophagolysosomes.

The restoration of the ER begins even before ground squirrels are completely warmed up. Starting in the second part of the torpor bout, the CA3 pyramids are characterized by the presence of several types of specific multilayer membranous structures in their cytoplasm that are all in direct contact with flat rough ER cisternae (Fig. 5). Here, large (2–3 μm) multilayer structures merit special attention; they are formed by concentrically arranged, tightly connected membranes 6–7 nm in thickness grouped as layers of 10–15 single-membrane sheets (Figs. 5a, 5b). These structures are known as myelin-like bodies. These structures have signs of similarity, but are not identical, to myelin (Komissarchik, 1975; Nunomura, Miagishi, 1993; Semakova, Kiseleva, 2003). However, in these structures, in addition to sites with dense myelin-like packing, areas are also present in which the neighboring membranes diverge and a 12–20-nm-wide gap is

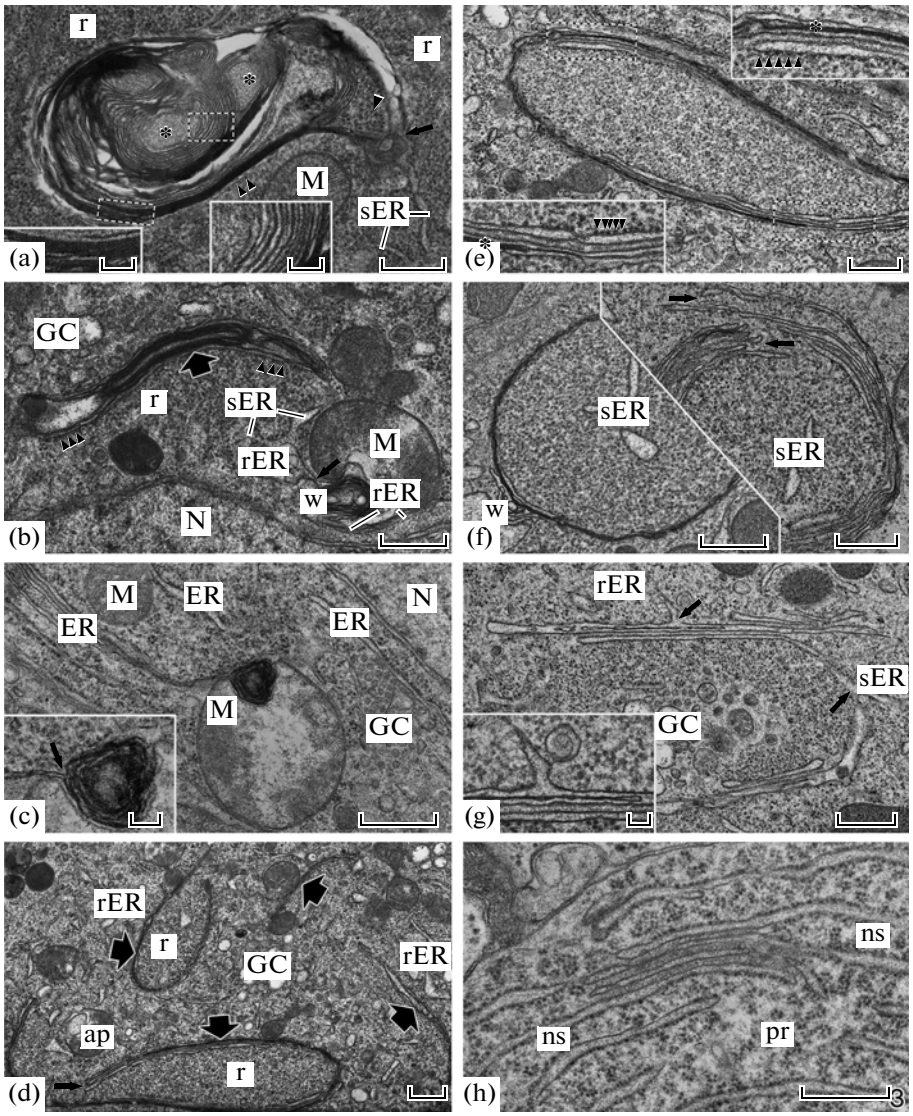

Fig. 5. Restoration of ER in pyramidal neurons of ground squirrels hippocampus CA3 field before their complete warming up. Multilamellar membranous structures and novel ER at the second half of the torpor bout (a–g) and 2 h after warming up (h). Thick transparent arrow (a, b) dense myelin-like structures; w (b, c-insertion, f) whorls; thick black arrows (d) multilamellar membranous bags with sites of orderly ER packing; thin arrows (a–c) - contact sites of the outer cisternae of myelin-like structures and bags to cisternae of rough ER; arrowheads (a, b, e) ribosomes attached to the outer surface of the myelin-like structure and the inner bag surface; arrows (d, f the right) enlargements of ER at ends of bags (d, f on the right); arrows; (g) side branchings of cisternae of the new formed ER; asterisks (a) unstructured matrix between membranes in the myelin-like stack; black triangles (e) dense matrix filling space between membranes; other designation as those in Fig. 1. Scale bar: 0.5 μm, in insertions, 0.1 μm.

formed between them that is filled with a finely structured matrix (middle and right insertions in Fig. 5a). In these areas, the structure already resembles a regularly packed smooth ER.

There is another kind of multilamellar structure that may also be related to restoration of ER in neurons of cold ground squirrel we designated them as multilayer bags (Fig. 5d–5f). They may be observed more frequently than the above-described ones, but also only in the middle and in the second half of the bout. The sizes of the membrane bags are 1.5–5 μm. Unlike the dense myelin-like structures, the bags are not closed (Figs. 5a, 5b). Inside of them, only monoribosomes and sometimes short fragments of rough ER are present (Figs. 5d–5f). The majority of bags represent orderly packed stacks of expanded flat cisternae of predominantly smooth ER. These stacks also contain sites of myelin-type structures (Fig. 5e, arrowheads). It is important that, from the very beginning of the new reticulum formation, these bags contain sites of rough ER. Ribosomes are bound to the cisterna surfaces contacting with the cytoplasmic matrix from both the inner and outer bag sides (Fig. 5e). In dense myelin-like structures, ribosomes are only present on the outer

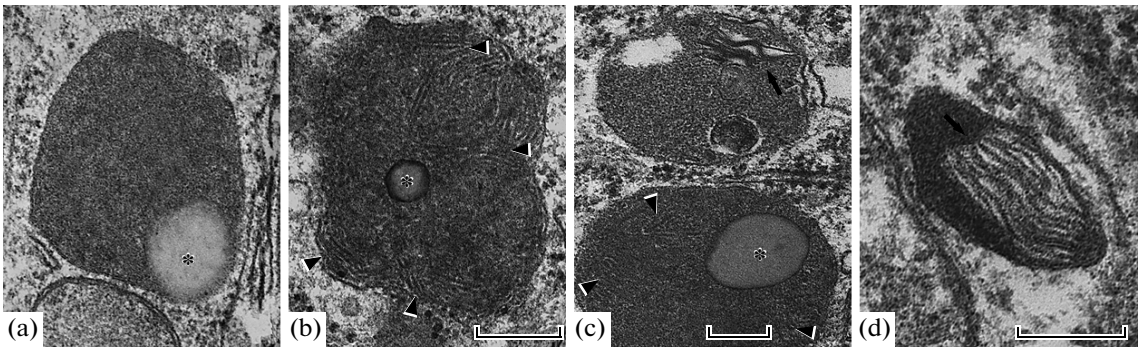

Fig. 6. Structure of lipofuscin granules in pyramidal neurons of hibernating ground squirrel hippocampus CA3 field in the hippocampus CA3 field pyramidal neurons.

Long arrows, stacks of myelin-like membranes; arrowheads, single membranes; asterisks, lipid droplets. Scale bar: 0.25 μm.

surface of forming cisternae (Figs. 5a, 5b, arrowheads). New ER has a more mature structure at the tips of bags, where cisternae already partly covered with ribosomes are diverging (Figs. 5d, 5f, arrows on the right). Before the beginning of animal warming, the new rough reticulum already has branching cisternae; however, it retains its general packing in the form of stacks (Fig. 5g). Two hours after being transferred to a warm room, the ground squirrels are awakened and the rough ER in the cytoplasm of CA3 pyramids is developed very well and forms Nissl bodies (Figs. 1d, 5h).

Finally, the third type of structure, whorls, are assumed to be related to the ER restoration in squirrel neurons (Le Beux et al., 1969). These structures are small (0.2–0.3 μm) with concentrically arranged and tightly packed membranes (Figs. 5b, 5c, 5f) and it is typical for them to have swollen mitochondria in their vicinity (Figs. 5b, 5c). Sometimes, the outer layer sheets of the whorls are also connected with the ER cisternae (Fig. 5b, 5c in insertion, arrows).

A possible sequence of events at the restoration of ER in ground squirrel CA3 pyramids may be presented as follows: the appearance of large myelin-like multilamellar structures with densely packed membranes ⟶ the penetration of the cytoplasmic matrix inside these structures and their untwisting in the form of bags ⟶ the conversion of myelin-like structures into stacks of flat cisternae of smooth ER with orderly packing ⟶ the binding of ribosomes on the cisterna surfaces that faced the cytoplasm ⟶ the formation of side branchings of ER cisternae. Unfortunately, it does not seem possible to trace the real sequence of ER transformations in the nerve cells of ground squirrel brain during hibernation *in situ*. Therefore, all proposed sequences of organelle transformations are forcedly speculative.

The key question for understanding mechanism of the ER formation in the neurons of ground squirrels is the origin of the multilamellar membranous structures. Remarkable is the fact that in course of the hibernation bout the structure of lipofuscin (LF) granules changes (Fig. 1c, Fig. 6). It is possible that the processes of ER renewal and the reorganization of LF granules are interconnected. The LF granules are derived from autophagolysosomes, but they are much larger than the latter (Fig. 4b). In neurons of torpor ground squirrels, LF-granules are very abundant; they frequently merge and form large groups (Fig. 1c). In summer and between bouts of torpor, in these neurons, LF-granules are very rare. The structure of LF-granules in neurons is quite diverse (Boellaard, Schlote, 1986); this is manifested strikingly in the hippocampal pyramids of ground squirrels (Fig. 6). From the middle of torpor and later, there are pronounced signs of membrane structuring in LF and the majority of LF-granules contain membranes bound to lipid droplets (Figs. 6b, 6c). At the end of the bout of torpor and during warming up, the LF granules appear to contain multilamellar structures of the myelin type (Figs. 1d, 6c, 6d), which resemble the structures connected with the new ER.

Restoration of GC in neurons begins only in warmed-up ground squirrels. We revealed the first signs of this process in the animals whose T_b rose up to 13°C (Fig. 2e). In the CA3 pyramids the GC reconstruction during warming up looks like a mirroring of its reduction process at cooling: large transparent vesicles fuse (Fig. 2e, asterisks), the bridges reminding the fragments of dictiosome flat cisternae connect the adjacent large vesicles (Fig. 2e, arrows). In ground squirrels with T_b 23°C, large vesicles, their aggregates, and dumbbell-like structures are already present, as are normal dictiosomes (Fig. 2f). Immediately after arousal (T_b 36°C), the GC structure is the same (Fig. 1d) and only on the next day large transparent vesicles disappear (Fig 3a). We did not see features of the GC formation *de novo* described in the literature (Glick, 2002; Landhans et al., 2007) in either cooled or warmed squirrels. Therefore, it seems likely that GC renewal occurs just as a restoration of already existing structures by addition of the components lost at cooling after their new production.

DISCUSSION

Study of hibernation of ground squirrels shows with certainty that both enhancement and cessation of electrical activity of nerve cells is coupled with changes in their cytoplasmic structure. Each time when ground squirrels enter the torpor state and when they are warming up, the structure of ER and GC in pyramidal neurons of the hippocampus CA3 field undergo alterations. In cooled animals neurons are in the state of profound inhibition, with significantly reduced ER and GC. While warming up, the nerve cells are activated and these organelles are restored.

It is widely accepted that the adaptation of hibernating animals to surviving periods of profound hypothermia is based on a reduction in the rates of all processes, as well as on the partial conservation of cell organelles (van Breukelen, Martin, 2002; Carey et al., 2003; von der Ohe et al., 2007). However, this conclusion seems to be in doubt due to the dynamicity of the ER and GC structure in the neurons of the hippocampus CA3 field. Numerous observations show that functional state of the nerve cell in the brain of a hibernating animal is under more complex regulation. Thus, both the production and expenditure of energy in the brain of torpor ground squirrels are balanced and the ATP concentration does not decrease (Lust et al., 1989; Drew al., 2007; Henry et al., 2007). The transport of nucleic acid precursors into brain cells of cooled and warmed animals occurs at identical rates (Bocharova et al., 1992a). RNA degradation in neurons at the initial torpor phase accelerates sharply compared to the mean values for rodents (Stoykova et al., 1983; Bocharova et al., 1992b). In the brains of hibernators, not only the rates of processes change differently during cooling, but these changes in different brain regions do not coincide in either value or direction (Demin et al., 1988; Bocharova et al., 1992b). The functional dependence of these differences is clearly seen in the hippocampus. Thus, all CA3 field pyramids lose up to 40% of ribosomes in the cytoplasm by the middle of the torpor bout. By the end of the bout, in dorsal hippocampus cells, the number of ribosomes rises 1.5 times, whereas in the similar neurons of the ventral hippocampus, it hardly changes at all. There, the resource of ribosomes is only restored during the arousal of the ground squirrels (Bocharova et al., 1992c). This can be explained by the difference in functions of the dorsal and ventral parts of the hippocampus (Fanselow, Dong, 2010). The ventral hippocampus neurons predominantly affect emotional behavior; therefore, their activation is only important under normothermia. It is obvious that the level of neuronal activity affects their metabolism and the state of the cell structure more deeply than temperature.

Changes in the functional state of hippocampal pyramid neurons from deep inhibition to generalized excitation concurrently with the passage of ground squirrels between torpor and arousal phases of hibernation are accompanied by structural changes, not only in ER and GC, but also ribosomes (Gordon et al. 1997) and nucleoli (Gordon et al., 2006). In other words, a generalized reaction of the entire system of protein synthesis, maturation, and transport develops these. Most often, the cessation of syntheses during torpor is considered only as a way to save resources of cells (van Breukelen, Martin, 2002; Carey et al., 2003). However, it is more probable that this reaction is the manifestation of a universal, nonspecific adaptation syndrome and is a way of overcoming cellular stresses (Braun, Mozhenok, 1987). Both the cessation of protein synthesis (Frerichs et al., 1998; Carey et al., 2003; Gordon et al., 2006) and the remodeling of ER and GC (Figs. 1, 2, 5) occur in ground squirrel neurons before torpor develops as early as at a decrease in T_b to 23–18°C. It is obvious that this is not the result of torpor, but rather a stage in the cell's process of preparing for survival at this specific state. The cessation of synthesis is accompanied by the shedding of ribosomes from the ER and by the transformation of part of its flat cisternae into tubular structures (Figs. 1 and 3). A similar ER reaction occurs in response to diverse stimuli in various cells (Powell, Latterich, 2000; Fedorovitch et al., 2005; Snapp, 2005; Shibata et al., 2009), including the cessation of protein synthesis (Shibata et al., 2006; Puhka et al., 2007). Both the inhibition of protein synthesis and the transformation of ER and GC should be attributed to typical cell adaptation reactions.

The unusually rapid accumulation of a large amount of AP in neurons during the entry of ground squirrels into torpor (Fig. 1, 4a–4c) is not a manifestation of any cell pathology (Sulzer et al, 2008; Tooze, Schiavo, 2008), since this accumulation is reversible; i.e., between bouts and in summer animals the number of APs and their derivatives LF granules decreases sharply. It is hardly probable that APs appear as a result of the starvation of neurons (Ushiyama et al., 2008; He, Klionsky, 2009), since this occurs before the torpor state develops. Most likely, the enhancement of autophagy is caused by the stress of the ER. The most important manifestation of this form of cellular stress consists in the formation of toxic misfolded proteins (Ron, 2002; He, Klionsky, 2009). ER stress induces the appearance of AP, where misfolded proteins are destructed. Thus, the cells protect themselves from apoptotic death (Høyer-Hansen, Jäättelä, 2007; Kawakami et al., 2009). The protective action of autophagy on nerve cells is well known (Ogata et al., 2006; Komatsu et al., 2007). As far back as in 2002, it was suggested that the ER stress in brain cells of hibernators accompanied entrance into torpor (Ron, 2002). The destruction of misfolded proteins in AP under extreme conditions of the intermittent course of hibernation provides a decrease in the probability of neuronal death (Høyer-Hansen, Jääattelä, 2007; Kawakami et al., 2009).

The idea that part of the ER, along with mitochondria and peroxysomes, can be eliminated by means of autophagy has been expressed repeatedly (Fedorovitch et al., 2005; Mijaljica et al., 2006; Yorimitsu, Klionsky, 2007, Klionsky, 2009); however, this was only demonstrated in yeast cell culture during the development of reticular stress under excess protein production (Bernales et al., 2006; Hu et al 2009). The accumulation of AP with fragments of membranous structures in them in neurons of torpor ground squirrels (Fig. 4) indicates that this mechanism of the regulation of the ER content also operates in mammalian cells. However, whereas yeast eliminates thereby polyribosomes and the rough ER formed for the synthesis of inducible proteins (Bernales et al., 2006), in neurons, APs capture and, later, degrade fragments of smooth ER and single ribosomes (Figs. 2, 4a–4c). No excess ER is present at any of stages of hibernation.

Ribosomes are ascribed to the most stable cellular structures; however, recently, the publications that demonstrate their autophagy appeared (Beau et al., 2008; Kraft et al., 2008); nonworking ribosomes are eliminated (Beau et al., 2008; Nakatogawa, Ohsumu, 2008). During torpor, many APs with ribosomes are observed in nerve cells (Figs. 4a, 4b); there are free ribosomes that do not form polyribosomes, i.e., their destruction occurs in AP. It is still far from the clear understanding of cell structures turnover mechanism, but the basic principle has been already outlined: cells digest excessive or non-functioning structures (Mijaljica et al., 2006; Beau et al., 2008). In the course of hibernation, a great part of ribosomes and ER are removed from the cytoplasm of neurons and eliminated in AP.

The process of the very fast restoration both of the number and of structure of cell organelles following ground squirrel arousal from torpor undoubtedly may be considered as an unique reaction (Figs. 1–3, 5). During the season of hibernation, the renewal of organelles is repeated many times. Classic biosynthetic processes are not quick enough to achieve this result; moreover, they require a huge expenditure of plastic and energetic resources. Reutilization, i.e., the repeated use of precursors of nucleic acids, proteins, and lipids that accumulate in autophagosomes after the destruction of organelles, on its own cannot provide such fast formation of novel organelles. Thus, even at normal temperature, in rodent brains, an average of 5% of ribosomes are renewed daily (Stoykova et al., 1983), while, in ground squirrels, in hippocampal neurons, their amount almost doubles after just 2 h of warming (Bocharova et al., 1992b). The restoration of membranous cellular structures also occurs very quickly and is completed mainly before the final arousal of ground squirrels.

The assembly of structures from fragments can be an alternative of synthesis (Misteli, 2001). Structures similar to both smooth and rough ERs can be formed from lipids and a few proteins in a cell-free system (Lavoie et al., 1996; Shnyrova et al., 2008). In cells, membranes and their complexes aggregate in the APs and LF granules (Boroviagin et al., 1972; Feldman et al., 1981; Nunomura, Miagishi, 1993; Hariri et al., 2000). The probability of the assembly of multilamellar structures into AP depends critically on the level of activity of hydrolases therein (Nunomura, Miagishi, 1993; Hariri et al., 2000; Ushiyama et al., 2008). Multilamellar structures in autophagolysosomes are not present at either the high activity of lysosomal hydrolases or in the case of their complete blockade. However, if a certain deficiency of hydrolases appears and taken-up organelles are not completely disrupted, multilamellar structures are formed (Nunomura, Miagishi, 1993; Hariri et al., 2000). Enzymes in autophagolysosomes appear not only as a result of fusion with lysosomes, but also at fusion with transport vesicles from GC (Ushiyama et al., 2008). Protein synthesis blocking naturally leads to the cessation of their transport from GC, and some deficiency of hydrolases appears in autophagolysosomes (Lawrence, Brown, 1993). This is what happens in hibernator's neurons: by the middle of bout the prepared lysosomes are already utilized, whereas there is no source of new portions of enzymes. As a result, autophagolysosomes are converted into LF granules (Fig. 1b), while LF granules can form large aggregates. In the case of aging and the development of pathological processes in the brain, LF granules remain until the end of life (Boellaard, Sñhlote, 1986; Sulzer et al., 2008), whereas during hibernation, between bouts, they are rarely revealed in neurons; rather, they are either liquidated or utilized somehow.

Multilamellar structures are more than just a form of lipid accumulation during the disintegration of organelles (Le-Beux et al., 1969, Feldman et al., 1981; Schmitz, Muller, 1991). Apparently, they also can play a significant role in fast ER formation. Cell membranes differ in their lipid and protein composition (Komissarchik, 1975). The probability of the formation of multilamellar membrane structures increases during the disassembly of membranes of namely intracellular organelles in autophagolysosomes (Lajoie et al., 2005). The composition of membranes in turn predetermines the shape of cellular structures (Voeltz, Prinz, 2007; Shibata et al., 2009). In neurons of torpor ground squirrels, the multilamellar structures in LF granules are built predominantly from remnants of similar intracellular membrane structures, that enable their easier transformation into ER.

The reassembly of the GC during the arousal of hibernators is not unique at all; in fact, it is the usual process (Mistel, 2001; Glick, 2002). For instance, during mitosis, the GC is also disintegrated into vesicles, as in neurons of the cooled ground squirrels (Fig. 2). After the completion of cell division, vesicles are merged and the GC structure is restored (Persico, et al., 2009). The assembly of the ER has many differences from the assembly of the GC; i.e., the former is

a unique form of cellular structure renewal. The usual formation of the ER *de novo* goes from the outer membrane of the nuclear envelope (Pannese, 1968), while in neurons of hibernators, the assembly of the ER occurs in special structures (autophagolysosomes and LF granules) from fragments that are stored and semidigested there.

Hibernators demonstrate significant protection of their organs and tissues from diverse stress effects during cooling that support the cell survival in these animals (Carey et al., 2003; Drew et al., 2007; Storey, Storey, 2007). This is particularly true for brain cells.

At least a part of neurons can realize the unique mechanism of cellular adaptation and the economic renewal of membrane structures of the cytoplasm via their assembly from fragments, rather than by new synthesis. In nerve cells whose fast activation is critically important for normal arousal from hibernation, both the removal of damaged and assembly of new structures of saved elements are provided by autophagy.

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