

EXPERIMENTAL ARTICLES

Impairment of Protein Synthesis Is an Early Effect of Amyloid- β in Neurons

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Abstract—Amyloid β -peptides (A β) play a key role in the development of Alzheimer's disease (AD). The response of neurons to the administration of A β in experimental animal models of AD remains poorly studied. We investigated the early effect of A β_{25-35} in neurons of the CA1 field of the hippocampus after intraventricular or intrahippocampal injections. We used various microscopy methods, including light microscopy combined with immunohistochemistry, as well as fluorescent and electron microscopy. The ratio of red and green fluorescence, K_α , after staining of cells with acridine orange (AO) reflects the state of rRNA in ribosomes and correlates with the fraction of active polyribosomes in the total number of ribosomes in the cytoplasm, which allows one to estimate the intensity of protein synthesis. One day after intrahippocampal injection of A β_{25-35} , the K_α value decreased to 55% in morphologically undamaged neurons. Fourteen days after the treatment, most of the neurons exhibited signs of degeneration and contained A β deposits. Fourteen days after intraventricular administration of A β_{25-35} , the K_α value decreased to 35% in morphologically undamaged neurons. In these neurons A β deposits were not observed. Ultrastructural analysis revealed a substantial increase in number of heavy polyribosomes, which was probably a cause of the decreased K_α value. Thus, the early effect of A β on CA1 pyramidal neurons of the hippocampus is the impairment of protein synthesis due to the formation of an increased number of heavy polyribosomes in the cytoplasm. Subsequent neurodegeneration is probably a result of the accumulation of endogenous A β in the cytoplasm.

Keywords: amyloid- β , Alzheimer's disease, pyramidal neurons of the CA1 field of the hippocampus, protein synthesis, polyribosomes, neurodegeneration

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INTRODUCTION

Alzheimer's disease is a severe neurodegenerative disorder associated with progressive memory decline and dementia. Amyloid β -peptides (A β) play an important role in AD pathogenesis [1–5].

Multiple indirect studies have demonstrated that AD is associated with impaired protein synthesis [6–11]. Mann et al. reported substantial impairments in protein synthesis, including the content of cytoplasmic RNA and volumes of nuclei and nucleoli in neurons in the AD brain at various stages of the disease [12]. Ding et al. demonstrated that a decrease in the rate and intensity of protein synthesis is associated with impaired function of ribosomes at the early stage of AD [13].

A decrease in protein synthesis was observed in brain regions such as the hippocampus and frontal cortex, which are involved in cognitive function [13].

Therefore, the study of these structures is especially interesting.

Experimental animal models of AD allow one to study the early effect of A β on cognitive processes and the morphological appearance of neurons [14–22]. After its intraventricular administration, A β may influence different brain structures, including the hippocampus [15–19]. After its intrahippocampal injection, A β affects the hippocampus directly [20, 21]. Controversial data exist on the effects of A β on cognitive processes and the state of neurons after these types of treatment [15, 16, 20–23]. A comparative study of different methods of central A β administration may help to clarify these controversies.

Using fluorescence microscopy with acridine orange (AO), we studied cytoplasmic rRNA in individual pyramidal neurons in the CA1 field of the dorsal rat hippocampus at the initial stage after central administration of aggregated A β . The ratio of the intensities of red and green fluorescence, the K_α coefficient, reflects the state of rRNA in ribosomes and allows estimation of the rate of protein synthesis in individual cells. Previously, we described this method

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in detail [24]. Protein synthesis may decrease after ribosome dissociation or formation of a predominate number of heavy polyribosomes. In order to study this question, we used electron microscopy. Signs of neurodegeneration were studied using staining with cresyl violet while A β deposits were stained immunohistochemically and light microscopy was applied for both methods.

In order to study the direct effects of A β on neurons, we used hippocampal slices, in which electrical neuronal activity was recorded [25].

We demonstrated that the early effect of A β in vivo is an impairment of protein synthesis in the pyramidal neurons of the CA1 field of the hippocampus. This effect is due to the formation of a predominate number of heavy ribosomes. This is followed by neuronal degeneration, probably due to the accumulation of endogenous A β in the cytoplasm.

MATERIALS AND METHODS

Eighty-seven 3-month-old male Wistar rats, which were bred in the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences, were used in the study. The rats were housed with free access to food and water and a natural light/dark cycle. All experiments were performed in accordance with the principles of humanity stated in the directives of the European Community (86/609/EC) and were approved by the Commission on medical ethics of the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences in the guide for studies with experimental animals.

Drugs and Drug Administration

A β_{25-35} ("Sigma," United States) was dissolved in distilled water and aggregated for 24 h at 37°C. Aggregation of A β was confirmed using electron microscopy by the presence of fibrillary aggregates after contrasting with a water solution of uranyl acetate. Control animals were injected with 0.9% NaCl. Surgery was performed using a stereotaxic frame under sterile conditions. The animals were anesthetized with a combination of zoletyl (Virbac Sante animale, France) at a dose of 12 mg/kg and xylazine (Bioveta, Czech Republic) at a dose of 20 mg/kg. Single bilateral administration was performed using the following coordinates: for lateral cerebral ventricles: AP = -0.8 mm, L = 1.5 mm, and H = 3 mm (number of animals, $n = 24$); for the CA1 field of the hippocampus: AP = 4 mm, L = 3 mm, and H = 3 mm [26]. All injections were performed using 5- μ L Hamilton microsyringe (Hamilton, Germany). A β_{25-35} was injected at a concentration of 1.6 nmol/1 μ L. Fifteen μ L of this solution was injected to the ventricle or 1 μ L to the hippocampus. Control animals received

an equal volume of 0.9% NaCl. Sites of injections were identified using 40 μ m paraffin hippocampal sections stained with cresyl violet.

Morphological and histochemical studies were performed 1 and 14 days after intrahippocampal treatment or 14 and 30 days after intraventricular treatment. Three groups of rats were studied for each type of treatment: 1, intact control, $n = 6$ and 9, respectively; 2, administration of 0.9% NaCl, $n = 6$ and 9, respectively; 3, administration of A β_{25-35} , $n = 6$ and 9, respectively.

Surviving Slices of Hippocampus

Experiments were performed with 300- μ m hippocampal slices from the rats, $n = 12$. Population spikes were recorded in pyramidal neurons of the CA1 field after electrical stimulation of Schaffer collaterals. The recording was started 2 h 40 min after the beginning of the experiment in accordance with the previously described method [27]. The control slices were placed into a perfusion medium consisting of Krebs-Ringer bicarbonate buffer (number of animals, $n = 6$). Experimental slices were perfused with 260 nM A β_{25-35} [28], $n = 6$. Perfusion was performed for 20 min.

Preparation of Tissue Samples

After decapitation, some of brain samples and a portion of the slices were fixed using Carnoy's fixative consisting of ethanol : chloroform : acetic acid at a ratio of 6 : 3 : 1, respectively. The other part of the samples was fixed with 4% formaldehyde in phosphate buffer. After dehydration, the samples were paraffin embedded. Blocks were prepared and 7- μ m sections were cut using a microtome (Leica, Germany). The sections were used for light and fluorescent microscopy and immunohistochemistry.

Fluorescent Microscopy

In order to study the RNA state in neuronal cytoplasm, we used staining with AO (Fluka Chemia AG, Buchs, Switzerland) under conditions that were optimal for the interaction of the stain and RNA. After deparaffinization and rehydration, the sections were washed in a citrate-phosphate buffer at pH 4.2 for 4 min and stained with 3×10^{-4} M AO dissolved in the same buffer for 10 min in accordance with the previously described method [24].

AO monomers form complexes with double-stranded RNA fragments, which fluoresce at 530 nm (I_{530}), whereas AO dimers bind to single-stranded RNA and fluoresce at 640 nm (I_{640}). Fluorescence in neuronal cytoplasm was measured using a DMF-2 microfluorometer equipped with a registration probe (6.5 μ m in diameter) and at $\times 85$ magnification [11].

Measurement and processing of the data were performed using Microfluor software. We studied 100 pyramidal neurons of the CA1 field of the hippocampus for each animal.

For analysis, we calculated the coefficient $K_\alpha = (I_{640})/(I_{530})$, which is a ratio of the intensities of red and green fluorescence.

The rRNA represents more than 80% of the total cytoplasmic RNA, therefore, K_α reflects the state of rRNA in ribosomes. In our previous special study, we compared K_α and the ultrastructural traits of neurons with various levels of functional activity and different numbers of active polyribosomes in the cytoplasm [24]. We found that in the neurons, where most of the polyribosomes were dissociated, the K_α value was low, and K_α increased when the number of actively functioning polyribosomes was elevated. In the culture of neuroblastoma cells treated with cycloheximide, an inhibitor of protein synthesis, we compared AO fluorescence and incorporation of labeled amino acids, which reflects the rate of protein synthesis. We found a positive correlation between the K_α value and incorporation of labeled amino acids. This study demonstrated that AO fluorescence not only reflects the presence of polyribosomes in the cytoplasm but functional activity of the type of ribosomes that predominates in the cytoplasm of pyramidal neurons at the moment.

Light Microscopy

Degeneration of neurons was studied using Nissl stained hippocampal sections. Staining was performed with cresyl violet (Sigma-Aldrich, St. Louis, United States) and studied using an Axio Imager M1 microscope (Carl Zeiss, Germany).

Immunohistochemistry

Immunohistochemical study was performed according to the standard method [21]. In order to improve staining for A β , the sections were treated with 70% formic acid for 4 min. Endogenous peroxidase activity was quenched by incubation in 0.5% H₂O₂ dissolved in ethanol and 5% normal goat serum. Then, the sections were incubated with primary rabbit polyclonal anti-A β_{1-40} antibody (1 : 400, Sigma) at +4° for 15 h. Primary antibodies were detected with anti-rabbit IgG (1 : 800, Sigma), which was followed by Strept-ABComplex/HRP (Sigma) in accordance with the manufacturer's protocol. Immunohistochemical reaction was visualized using 3,3'-diaminobenzidine (DAB; Sigma, United States). Substitution of primary antibody with buffer was used as a negative control. The sections were studied using an Axio Imager M1 microscope (Carl Zeiss, Germany).

Electron Microscopy

For ultrastructural analysis, tissue was prepared using the routine procedure. After decapitation, 0.5–1- μ m-thick sections of the dorsal hippocampus were fixed in 2.5% glutaraldehyde dissolved in 0.1 M Na-cacodilate buffer (pH 7.2–7.4) for 2–4 h and postfixed in 1% OsO₄ in the same buffer for 1–2 h. After dehydration in ethanol, the sections were embedded in Epon-Araldite (Agar Scientific Inc., United Kingdom). Serial ultrathin sections of pyramidal neurons of the CA1 hippocampal field were cut using a Leica UM6 microtome with diamond knife. The sections were stained with uranyl acetate and lead citrate on grids covered with pioloform. The sections were studied using a JEOL 1200EX electron microscope (Institute of Bioorganic Chemistry, Russian Academy of Sciences, Pushchino, Russia).

Morphometric Analysis of Polyribosomes

Counting of polyribosomes using transmission electron microscopy (TEM) has some limitations because a group of ribosomes in the depth of an ultrathin section may not represent the whole number of ribosomes of a single polyribosome. The most appropriate method for estimation of the ribosomal composition of polyribosome is high resolution electron tomography; however, this method also has some drawbacks, such as the presence of electron-dense RNP granules, which are very similar to ribosomal subunits. The only method to count the number of ribosomes in polyribosomes using TEM is probably measurement of the length of polysome spread on flat cisterns of the endoplasmic reticulum (ER) or the external surface of the nuclear membrane.

For counting the ribosome number, we applied conventional transmission microscopy of flat ER cisterns and nuclear membrane (Fig. 4a) located at a depth of relatively thick 100-nm or thicker to some extent ultrathin sections. At least 25 microphotographs of the perinuclear area of the cytoplasm of pyramidal neurons of the CA1 field of the hippocampus from each of control rats ($n = 3$) or rats taken 14 days after intraventricular injection of A β ($n = 3$) were captured at $\times 12K$ magnification. Negatives were scanned at 2400 dpi resolution using with an Epson Perfection V700 Photo scanner and the number of ribosomes in polyribosomes was counted using IGL Trace software (<http://synapses.clm.utexas.edu>).

Polyribosomes located on the border of the surface of cut ER cistern were excluded from the counting of ribosomes in polyribosomes. In Fig. 4, these polyribosomes are indicated as “ex,” whereas the polyribosomes included in the count are indicated as “in.” We also did not include all polyribosomes consisting of 3–4 ribosomes in the count. We included

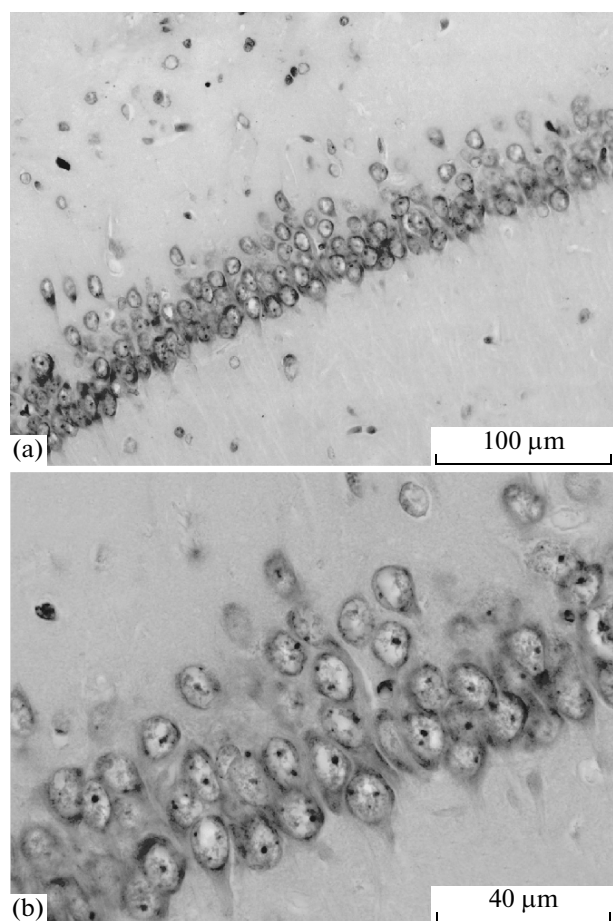


Fig. 1. Microphotographs of the CA1 field of the rat hippocampus of control (intact) animal. Cresyl violet staining.

only polyribosomes that formed curved chains like those formed by ER-bound heavy polyribosomes. On average, in each control rat, 142 ± 16 ribosomes were

counted in polyribosomes, whereas after A β treatment, 197 ± 82 ribosomes were counted; the lowest number of counted polysomes was 107.

Statistical Analysis

The data are presented as means and standard deviations. Fluorescence intensity was compared using the Student's *t* test and the differences were considered as significant at $p < 0.01$. Analysis of variances (ANOVA) and the Wilcoxon's test were applied to the data on the number of ribosomes in polyribosomes with the levels of significance of $p < 0.01$ and $p < 0.05$, respectively. For the analysis we used Stadia 8.0 software (<http://statsoft.msu.ru>). We minimized the number of animals used in each group ($n = 3$), hence, for analysis at the level of significance $p < 0.05$ (95%), the values of Wilcoxon's statistics were compared with tabulated critical values of the smallest rank sum (table).

RESULTS

Intraventricular Administration

Pyramidal cells, the predominate type of cell in the CA1 field of the hippocampus [31], stained with cresyl violet had a homogenously stained cytoplasm with clearly seen nucleolus, plasma, and nuclear membranes (Fig. 1a, b).

Fourteen days after the administration of A β_{25-35} , the morphological appearance of most pyramidal neurons stained with cresyl violet in the entire CA1 field was similar to the control (Fig. 2a, 2b). However, K_a decreased significantly to 36% of the control value (Fig. 3a).

Thirty days after the administration of A β_{25-35} , dark and degenerating neurons appeared together with

The distribution of ribosomes in the cytoplasm of pyramidal neurons of the CA1 field of the hippocampus in control and experimental rats 14 days after intraventricular administration of A β_{25-35} (see Fig. 4) and paired comparison of ribosome content in different fractions of polyribosomes. Arrows indicate an increase $\uparrow$ and a decrease $\downarrow$ in the % maximum/% minimum ratio

Ribosomes in polysomes	Control, % of total number of ribosomes, mean $\pm$ S.D., $n = 3$	Experiment, % of total number of ribosomes, mean $\pm$ S.D., $n = 3$	Ratio, % maximum/% minimum	Sum of Wilcoxon's ranks, calculated
3–5	10.5 ± 2.3	2.6 ± 2.6	4.5* $\downarrow$	15
6–7	25.9 ± 5.4	9.4 ± 5.7	2.8* $\downarrow$	15
8–9	33.1 ± 3.4	30.1 ± 4.8	1.1	12
10–11	17.5 ± 4.2	25.8 ± 1.5	1.5* $\uparrow$	6
12–13	7.1 ± 2.7	15.0 ± 4.8	2.1* $\uparrow$	6
14–15	3.3 ± 0.4	9.2 ± 4.6	2.8* $\uparrow$	6
16–19	1.4 ± 2.5	6.8 ± 4.2	4.7 $\uparrow$	7
20–25	1.2 ± 0.4	1.2 ± 0.6	1.0	10.5
3–7	36.4 ± 7.7	11.9 ± 8.3	3.1* $\downarrow$	15
10–19	29.3 ± 9.7	56.8 ± 12.3	1.9* $\uparrow$	6

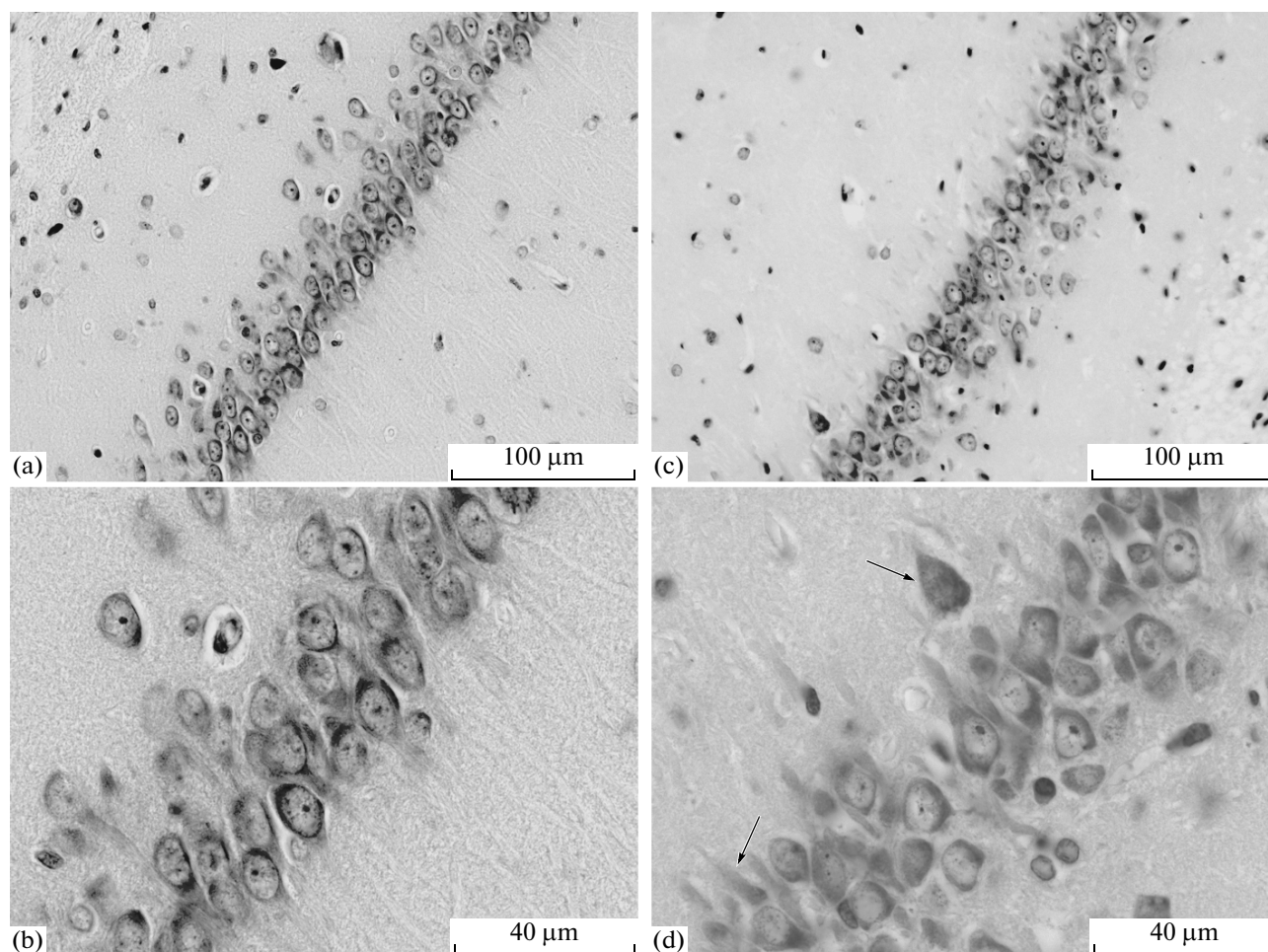


Fig. 2. Microphotographs of the CA1 field of the rat hippocampus after injection of 1.6 mM $A\beta_{25-35}$ into the lateral ventricles. a, b, 14 and c, d, 30 days after the treatment. Arrows indicate dark and degenerating neurons. Cresyl violet staining.

morphologically intact cells in the CA1 hippocampal field (Fig. 2c, 2d). The K_{α} value was 56% of the control level.

Administration of physiological saline did not influence K_{α} and the values of this index 14 and 30 days after the treatment were similar to those observed in the control, i.e. intact, animals.

Ultrastructural study of the cytoplasm in pyramidal neurons of the CA1 field revealed that the number of polyribosomes that contained 3–7 ribosomes was three times higher, whereas the number of polyribosomes containing 10–19 ribosomes was two times lower in the control as compared to the experimental group (Fig. 4, table). We did not observe any immunohistochemically positive $A\beta$ deposits in neuronal cytoplasm at all the time points studied.

Intrahippocampal Administration

Injection of $A\beta_{25-35}$ directly to the hippocampus allows one to study the effect of the substance on hip-

pocampal neurons; however, local traumatic damage due to microinjection should be taken into account.

One day after intrahippocampal administration, cresyl violet staining showed a few damaged neurons at the site of injection in the CA1 field in all groups of animals subjected to surgery (Fig. 5a, b).

One day after injection of $A\beta_{25-35}$, neuronal staining with AO revealed a decrease in the K_{α} value to 55% of the control level in all neurons of the CA1 field studied (Fig. 3b). Injection of physiological saline resulted in a decrease in the K_{α} value to 86%.

In the sections stained with cresyl violet 14 days after injection of $A\beta_{25-35}$ to the hippocampus, we observed that neurons of the CA1 field exhibited substantial swelling and vacuolization of the cytoplasm; many neurons were diffuse, amorphous, dark, or even completely destroyed with clear signs of necrosis (Fig. 5c, 5d). A similar picture of degeneration of neurons in the CA1 field of the hippocampus was observed after hypoxia or ischemia [31–33]. Administration of

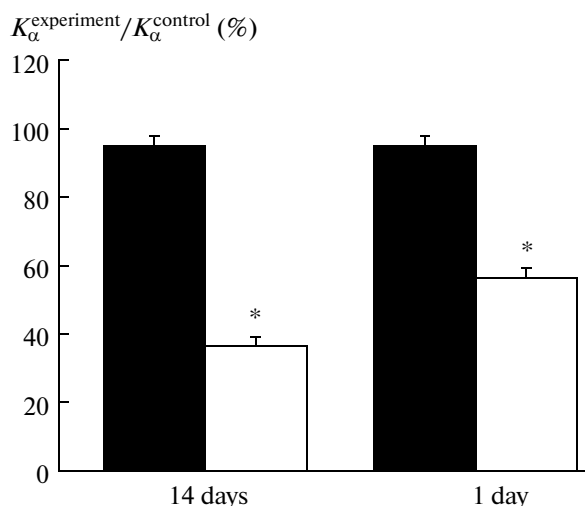


Fig. 3. Changes in the ratio $K_{\alpha}^{\text{experiment}}/K_{\alpha}^{\text{control}}$ (%) which correlates with changes in protein synthesis in pyramidal neurons of the CA1 field of the hippocampus. Data are presented as mean $\pm$ S.D. 300–400 cells from three to four animals were studied in each time point. *, significant differences between control and experimental groups, $p < 0.001$. Black bar, control; white bar, experiment.

NaCl did not induce any substantial changes as compared to the control group.

We observed immunohistochemically positive diffuse deposits of A β in the cytoplasm of CA1 neurons 14 days after the injection. These deposits were detected using antibodies against A β_{1-40} . The deposits were absent on the first day after the treatment (Fig. 6).

Surviving Slices of Hippocampus

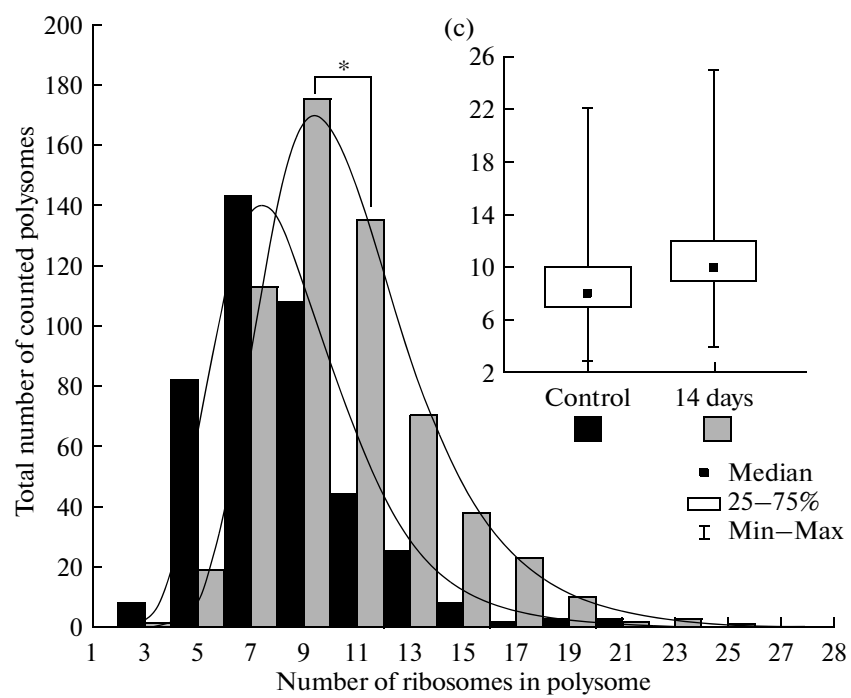
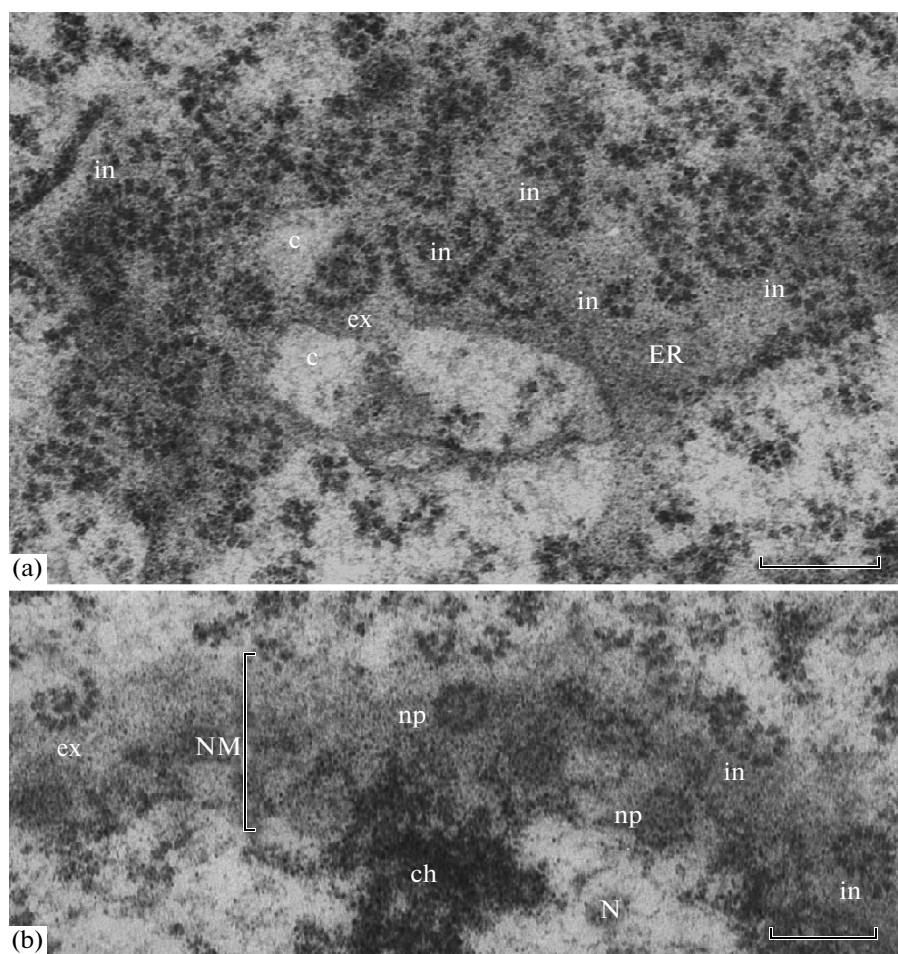
Incubation of slices with A β_{25-35} for 20 min resulted in the appearance of the clear degeneration of pyramidal neurons of the CA1 field, which was observed in the sections stained with cresyl violet (Fig. 7b, d). In the cytoplasm of these cells, we observed diffuse deposits of A β , which were detected immunohistochemically with antibodies against A β_{1-40} . We did not find any signs of degeneration or diffuse A β deposits in the control slices (Fig. 7a, 7e).

DISCUSSION

We revealed changes of individual pyramidal neurons in the CA1 field of the dorsal hippocampus during the early stage after central administration of aggregated A β_{25-35} .

One day after intrahippocampal administration of A β_{25-35} , we observed a substantial decrease in the K_{α} value in most of the morphologically intact cells as compared to the control. Similar changes were found 14 days after intraventricular administration. Thus, modification of K_{α} was not directly related to neurodegeneration. In a previous study, we demonstrated that K_{α} is an index of the rate of protein synthesis and correlates with the content of translating polyribosomes [24]. Electron microscopy allowed us to reveal a predominant number of heavy polyribosomes in the cytoplasm of neurons of the hippocampal CA1 field after intraventricular administration of A β_{25-35} . Thus, the predominance of heavy polyribosomes is the cause of decreased activity of protein synthesis in the cells we studied.

Fig. 4. Representative fragments of flat ER cistern and external nuclear membrane with polyribosomes located on them and used for ribosome count. a, flat ER cistern dotted with well-recognized polyribosomes; b, tangential section of nuclear membrane with polyribosomes. Not all polyribosomes were used for count of ribosomes. “in” and “ex,” ribosomes included into the count or excluded from the count, respectively, located at the border of cut ER cistern. ER, endoplasmic reticulum; c, cytoplasm; NM, nuclear membrane; np, nuclear pore; ch, chromatin. Scale: 200 nm. C, dependence of distribution of polyribosomes on the number of ribosomes that compose them. In order to illustrate the shift in the distribution, curves of functions of extreme values are added. Insertion, a graph of the properties of these two distributions: median, minimum and maximum values, and the upper and lower quartiles for the control group and group 14 days after intraventricular treatment with A β . After administration of A β the number of ribosomes in polyribosomes (10.9 ± 3.2 , $n = 591$) was significantly higher as compared to that in the control group (8.7 ± 2.9 , $n = 427$). *, $p < 0.01$ according to one-way ANOVA.



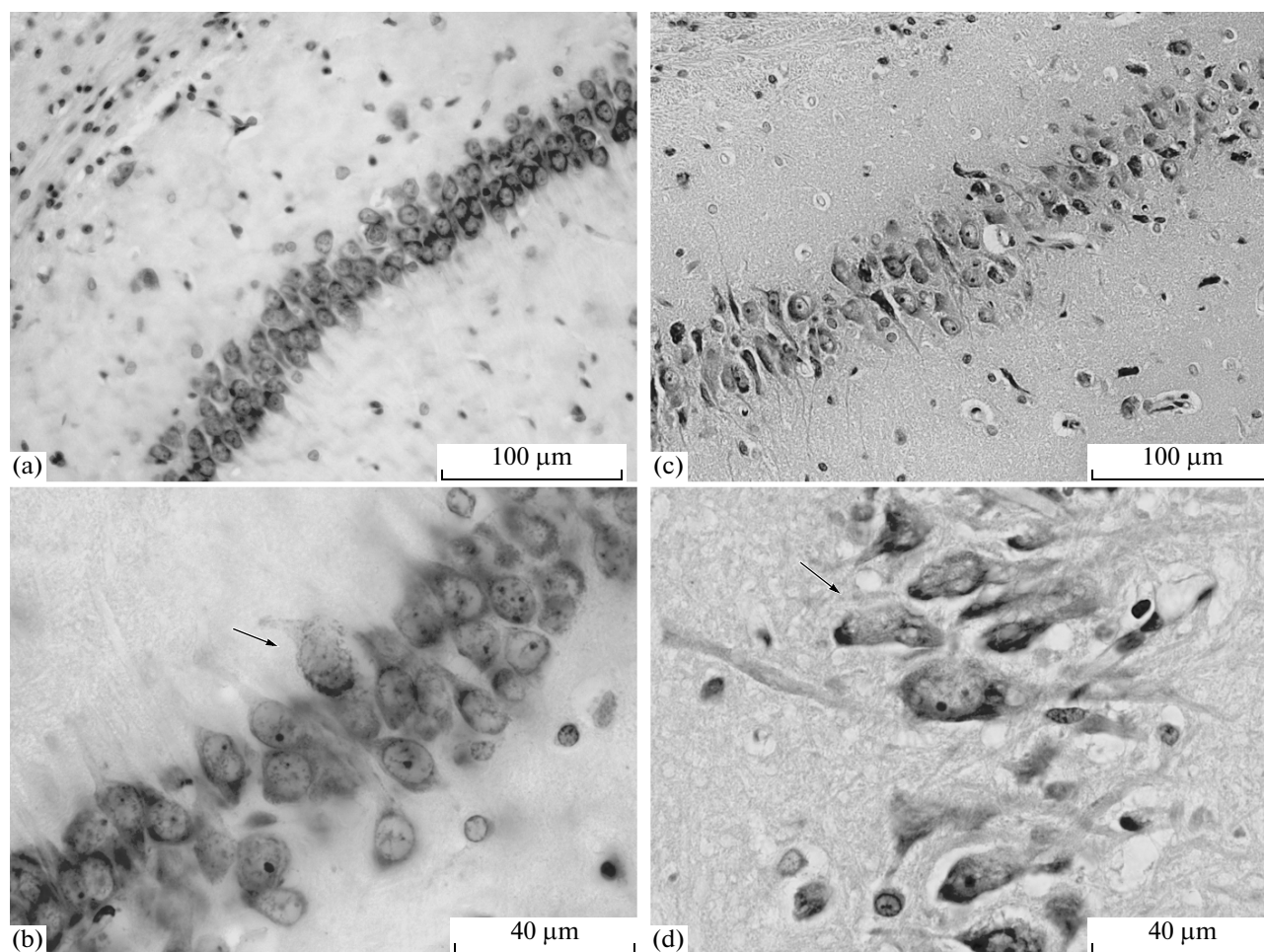


Fig. 5. Microphotographs of the CA1 field of the rat hippocampus after injection of 1.6 mM A β_{25-35} into the hippocampus. a, b, 1 and c, d, 14 days after the treatment. Arrows indicate dark and degenerating neurons. Cresyl violet staining.

Our data are in accordance with the results by Shan et al. who demonstrated a decrease in the rate of protein synthesis in morphologically intact cells 1 day after the application of A β to primary neuronal cultures. The authors supposed that the formation of a predominant number of heavy polyribosomes caused this decrease [34].

On the basis of these data, we hypothesized that the decrease in protein synthesis, which was observed 1 day after intrahippocampal injection of A β , was due to the formation of predominant number of heavy polyribosomes in close similarity with the effect that was found 14 days after intraventricular A β administration.

Ding et al. revealed that at the early stages of AD polyribosomal complexes in homogenates from different brain structures exhibited an impaired ability to synthesize protein. The authors supposed that oxidative stress may inhibit protein synthesis. Impairment of

protein synthesis was considered as a principal factor of the onset and development of AD [13].

Experiments in primary neuronal cultures revealed that A β induced oxidative stress in cells in several hours [34–37]. Reactive oxygen radicals damage all components of protein synthesis including mRNA, ribosomes, tRNA, and chromatin, which results in the formation of heavy polyribosomes [34, 38].

For in vitro experiments, oxidative stress elevated expression of β - and γ -secretases, which increased production endogenous A β [35–37].

Two processes induced by oxidative stress, such as impairment of protein synthesis and activation of the production of endogenous A β are probably separated in time. Thus, 1 day after intrahippocampal A β administration, in morphologically intact neurons, protein synthesis decreased, whereas A β deposits in the cytoplasm were not detected. Fourteen days after the treatment, the deposits were observed in the cyto-

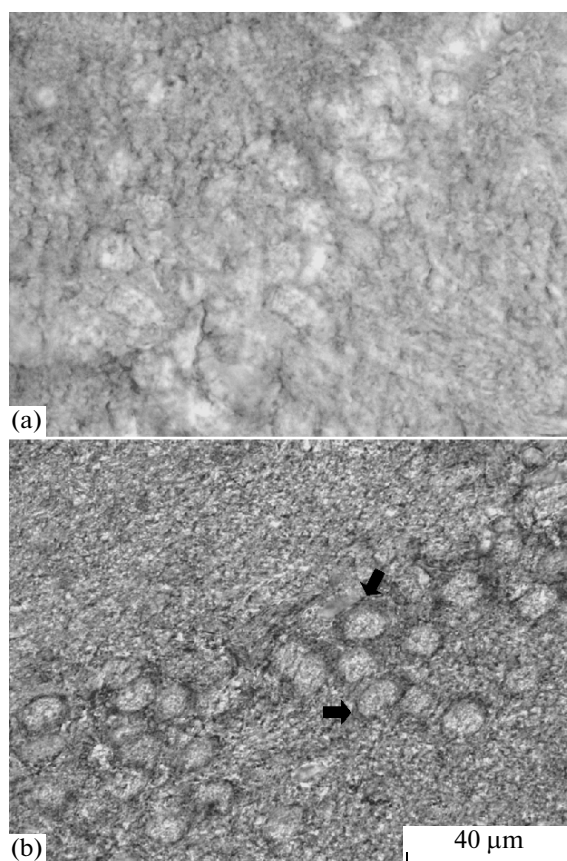


Fig. 6. Microphotographs of the rat CA1 field. Amyloid deposits were detected using antibodies against $A\beta_{1-40}$. (a) control; (b) 14 days after intrahippocampal injection of 1.6 mM $A\beta_{25-35}$. Thick arrows, diffuse amyloid deposits in the cytoplasm of neurons.

plasm of degenerating cells. $A\beta$ deposits were also observed in undamaged neurons of hippocampal slices. The accumulation of $A\beta$ in neurons is probably a result of the penetration of exogenous $A\beta$ owing to damage of cellular membrane or the formation of endogenous $A\beta$ [16]. However, in our study, endogenous $A\beta$ probably accumulates in the neuronal cytoplasm because the animals were injected with A_{25-35} , whereas cytoplasmic $A\beta$ deposits were identified with antibodies against $A\beta_{1-40}$.

We believe that central administration of $A\beta$ induces the following sequence of events: oxidative stress, increased expression of β - and γ -secretases, and accumulation of deposits of endogenous $A\beta$.

Our data revealed the differences in the time courses of modification of neurons after intraventricular and intrahippocampal administrations of $A\beta$. This is supported by the different rate of penetration of $A\beta$ into neurons after these two types of injections 14 days after the treatment. After intrahippocampal injection, we observed necrosis in most of the neurons and the

presence of deposits in them, whereas after intraventricular administration, we revealed only the maximum decrease in the rate of protein synthesis in morphologically intact neurons, which was similar to the decrease observed 1 day after intrahippocampal injection. It is noteworthy that substantial neurodegeneration was observed only 3 months after the intraventricular administration of $A\beta$ [19].

Thus, damage to pyramidal hippocampal neurons after different types of central $A\beta$ injections has common features but differs in the time course of progression.

Cytological techniques thus allowed us to reveal that an early event that is induced by $A\beta$ in pyramidal neurons of the CA1 field of the dorsal hippocampus is impairment of protein synthesis caused by the formation of a predominant number of heavy polyribosomes. This is followed by neuronal degeneration, which is a result of the accumulation of endogenous $A\beta$ in the cytoplasm.

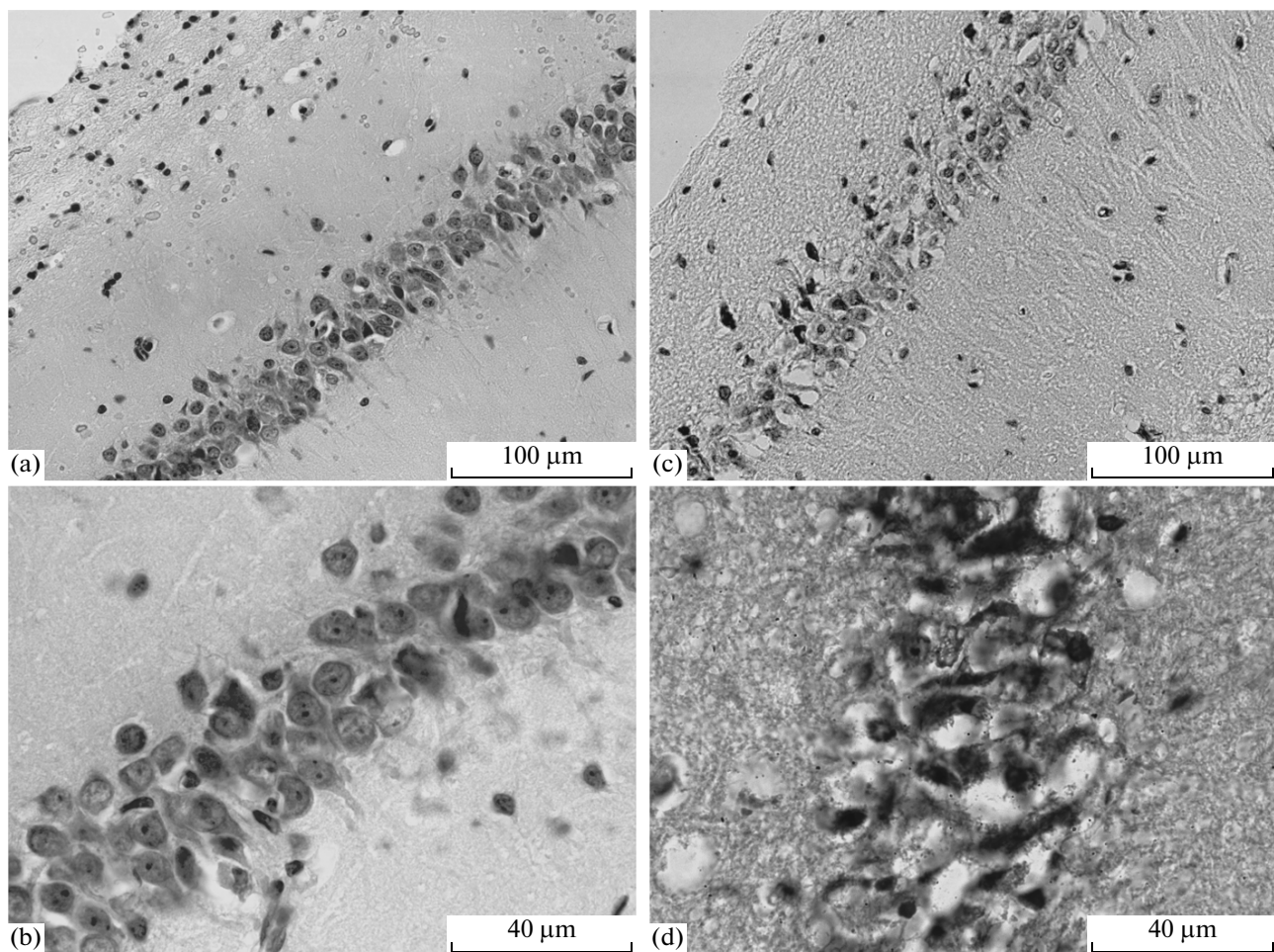


Fig. 7. Microphotographs of the CA1 field of hippocampal slices incubated in: Krebs-Ringer medium (a, b); 200 nM A β _{25–35} (c, d). Cresyl violet staining.

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