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Alzheimer's disease-like pathology-triggered oxidative stress, alterations in monoamines levels, and structural damage of locus coeruleus neurons are partially recovered by a mix of proteoglycans of embryonic genesis

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ABSTRACT

Alzheimer's disease (AD) pathogenesis includes oxidative damage and perturbations of monoamines. However, as many details of these alterations are not known, we have investigated the changes in monoamine levels as well as the free radical oxidation processes (FRO) in the brainstem of rats that were administered *i.c.v.* Aβ (25–35) (rat model of AD-like pathology). The level of oxidative stress was found elevated in the brainstem along with the increased concentrations of monoamines, especially norepinephrine in the locus coeruleus (LC) area of the brainstem. This was accompanied by the substantial structural damage of monoaminergic neurons of LC. In addition, we have tested the ability of proteoglycans of embryonic genesis (PEG) that were shown previously to act as neuroprotectors, to restore the AD-triggered alterations in monoaminergic system and FRO. Indeed, PEG reduced the increased FRO and upregulated monoamines in the brainstem of Aβ (25–35) treated animals. Administration of PEG to control animals led to the increase of the antioxidant capacity as well as the intensity of free radical oxidation processes.

Our study confirms the important role of the brainstem FRO and monoamine shifts in AD development along with the known aggregation of Ab peptide and Tau hyperphosphorylation. We suggest that at the early stages of AD development, with still functional neurons, regulation of monoamine levels via stabilizing FRO processes can be beneficial. Our data demonstrate the regulatory action of PEG on the monoamine disturbances and the level of oxidative stress in the AD damaged structures, suggesting its possible therapeutic application in AD.

1. Introduction

Alzheimer's disease [AD] accounts for 60–70% of all dementia cases (Šimić et al., 2017). The prevalence of AD is anticipated to increase dramatically during the next decade due to the longer life expectancy. Currently, diagnostic criteria for AD (GERAD, Braak and Braak, etc.) mainly focus on cognitive decline as a consequence of preferentially affected subpopulation of pyramidal neurons in high-order regions of neocortex and hippocampus ("Consensus Recommendations for the Postmortem Diagnosis of Alzheimer's Disease," 1997; Simic et al., 2009). This is perhaps the reason why AD is considered as a supratentorial disease. To date, less importance is given to the neural substrate of AD-related "infratentorial" origins of dementia. From this perspective, brainstem, as a reservoir of several nuclei involved in a wide range of processes modulating cognitive functions of the cerebral cortex, can be suggested to be involved in AD pathogenesis (Lee et al., 2004). It is worth to mention that brainstem reticular formation, particularly locus coeruleus (LC), allows the intracellular neurofibrillary tangles (NFTs) to travel along the LC axons reaching the cortical neurons (Giorgi et al., 2017). LC is a powerful source of monoamines and by releasing them through the "bouton en passage" it modulates cognitive functions (Foote, 1990; Murchison et al., 2004; Simic et al., 2009). The most pronounced effect of AD on the norepinephrine system is revealed by the loss of the majority of LC norepinephrine neurons (Šimić et al., 2017). LC is the only noradrenergic nucleus that innervates the cortex (Samuels and Szabadi, 2008). The loss of

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noradrenergic neurons from LC correlates with the increase of extracellular Aβ deposition in mice (Heneka et al., 2010), neurofibrillary abnormalities at the early stages of AD (Grudzien et al., 2007), onset and duration of dementia (Counts and Mufson, 2010). It is conceivable the LC may play an important role in propagating the AD pathogenesis. Therefore, restoring monoaminergic regulation in the LC can be instrumental for developing novel treatment approaches of AD.

Neurotoxicity of Aβ peptide, as well as the tau protein accumulation, are enhanced by oxidative stress (Huang et al., 2016; Lee et al., 2004). Oxidative stress in AD is manifested by the abnormal levels of oxidized proteins, advanced glycation end products, lipid peroxidation end products, modifications of nuclear and mitochondrial DNA (Gella and Durany, 2009), which appear to be the driving force in AD-associated cytoskeletal changes. This, in turn, plays a key role in the irreversible cellular dysfunction and ultimately leads to neuronal death (Perry et al., 2002).

During the last decade, our laboratory has been testing the potential of neuroprotective compounds and their effects on AD-like pathology induced by the Aβ (25–35) peptide (Yenkoyan et al., 2009; 2011; Aghajanov et al., 2014). We have previously shown monoamine level alterations in AD-like pathology and the potential of one of such compounds, proline rich polypeptide-1 (PRP-1) to restore their levels (Yenkoyan et al., 2018).

The aim of this study is to test the effects of another promising neuroprotective compound, a mix of proteoglycans of embryonic genesis (PEG), on the altered levels of the brainstem monoamines, which will also support the monoaminergic hypothesis of AD pathogenesis. We have previously reported that administration of PEG leads to the amelioration of spatial memory and improves neurotransmission via modulation of glutamate and GABA levels in AD target regions (Yenkoyan et al., 2009). In this study, we demonstrate the correlation between the oxidative stress, monoamines and LC neurons damage at AD, as well as the capacity of a mix of proteoglycans of embryonic genesis with the previously demonstrated neuroprotective properties (Yenkoyan et al., 2009) to ameliorate the oxidative stress and modify the brainstem monoamine levels in the rat AD model.

2. Methods

2.1. Animals

Experiments were performed on 12–14 months old, 60 Sprague-Dawley male rats weighting 220–300 g and housed under a 12:12-h light/dark cycle with water and food available ad libitum. All procedures were performed in accordance with the European Communities Council Directive (86/609/EEC) and was approved by the Ethics committee of Yerevan State Medical University after Mkhitar Heratsi.

2.2. Experimental protocol

Animals were equally divided into four experimental groups. The animals were anesthetized with Nembutal (40 mg/kg), positioned in a stereotaxic frame. A midline sagittal incision was made in the scalp to expose the skull. The holes were drilled over the projection of lateral ventricles using the following coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to the sagittal suture in accordance with the stereotaxic atlas of Paxinos and Watson (2007). In the control group the animals were injected with the vehicle (3 μl double distilled water) into the each of the cerebral ventricles. The first experimental group animals were intracerebroventricularly (*i.c.v.*) injected with Aβ (25–35) (3 μg per 100 g body weight) (AD-like group). The injections of both the sterile double distilled water and Aβ (25–35) were performed at a rate of 1 μl/min using the peristaltic pump. The animals in the second experimental group (PEG-control) were subcutaneously injected with PEG (0.5 mg per 100 g body weight). The animals from the third experimental group (PEG-1, preventive mode) were subcutaneously administered with PEG (0.5 mg per 100 g body weight) seven days prior to the *i.c.v.* Aβ (25–35) injection. The fourth experimental group animals (PEG-2, preventive/treatment mode) were subcutaneously injected with PEG (0.5 mg per 100 g body weight) seven days prior to the *i.c.v.* Aβ (25–35) and on the 30 day after amyloid administration. The control, first, third and fourth experimental groups' animals were decapitated at the 90th day of the experiment; the second experimental group was terminated at the 7th day after PEG injection (the start point of the amyloid exposure for the third and fourth experimental groups) after intraperitoneal (i.p.) administration of 40 mg/kg of Nembutal. The brainstem was dissected in accordance with the atlas of Paxinos and Watson (2007), then frozen in liquid nitrogen, weighed, and homogenized in the specific buffer depending on the assay method.

2.3. Composition of PEG

PEG is the following mix of proteoglycans of embryonic genesis: alpha-fetoprotein, chorionic gonadotrophin, b1-glycoprotein, carcinoembryonic antigen, and carbohydrate antigens Ca-19-9 and Ca-125 (Yenkoyan et al., 2009). 0.5 mg/100 g body weight PEG was administered subcutaneously.

2.4. Preparation of Aβ (25–35) peptide

The aggregation of Aβ (25–35) peptide (Sigma–Aldrich, St. Louis, MO, USA), was performed according to the manufacturer's recommendations (Maurice et al., 1996). Briefly, Aβ (25–35) peptide was dissolved in the sterile double distilled water at a concentration of 1 mg/ml, aliquoted and stored at −20 °C. "Aging" of the peptide was performed by incubation at 37 °C for four days before the surgery. The effect was validated by light microscopic observation, which confirmed the existence of both birefringent fibril-like structures and globular aggregates.

2.5. Biochemical study

2.5.1. Monoamine assay

Centrifugation of the corresponding brain tissues' homogenates was performed for 15 min at 15000 rpm at 4 °C, and the supernatants were filtered through the 0.45-μm filter (MultiChrom). We have assayed the epinephrine, norepinephrine and the metabolites of dopamine (homovanillic acid (HVA)) and serotonin (5-HIIA) using a reverse-phase HPLC assay with electrochemical detection. The system consisted of 150 mm × 3 mm "Nucleosil" 100-5 C18 standard column. The high-density glassy carbon-working electrode was combined with an Ag/AgCl reference electrode. The operating voltage was 850 mV. The mobile phase (flow rate 0.4 ml/min) consisted of a citrate-phosphate buffer, pH 3.2, 0.1 mM Na_2EDTA and 8% acetonitrile.

2.5.2. Assessment of free radical oxidation (FRO)

2.5.2.1. Spontaneous (biochemiluminescence) and UV light-induced chemiluminescence (CL). Spontaneous (biochemiluminescence) and UV light-induced CL reflecting the intensity of lipid peroxidation were detected as described elsewhere (Tarusov, B.N., Polivoda, A.I., 1961; Vladimirov and Proskurnina, 2009). The spontaneous or UV light-induced formation of reactive oxygen species (OH•, D_2•) leads to the free radical oxidation of various biomolecules including lipids. Eventually, the unstable tetroxides decay with a release of a quantum of light. Both, spontaneous and UV light-induced chemiluminescence characterize FRO intensity without or with external interference respectively. In the latter case the ongoing free radical process was recorded in the course of first 2–3 min when the luminescence is the most intense. The following parameters were measured: maximum signal intensity (Imax, pulses/10 s) and intensity of chemiluminescence (S, imp.), and the tangent of the kinetic curve's slope angle, represented as K (Imax/S ratio) reflecting the antioxidant capacity of the cell.

2.5.2.2. The evaluation of Fe^{2+}-induced CL. Fe^{2+}-induced CL was measured as described elsewhere (Vladimirov et al., 1980). Briefly, the brain tissue supernatants were exposed to 100 μM Fe^{2+} salt ($FeSO_4$), and the kinetics of the process was followed by recording the fast flash of CL that was induced immediately after addition of Fe^{2+}, latent period (LP) during which CL is suppressed, slow flash of CL (SF) (see Fig. 6). Additionally, the "time-to-pea" (tau value) was measured as the representation of LP. The kinetics of CL in the presence of Fe^{2+} ion was measured at 25 °C using Lum-5773 portable chemiluminometer (Vladimirov et al., 1980). The characteristic kinetic curve of CL in for the chain lipid oxidation reaction initiated by Fe^{2+} salts is shown in Fig. 6C (a) (Sharov, V. S., Driomina, E. S., Vladimirov, 1996) (Driomina et al., 1993).

2.6. Morphological study

2.6.1. Ca^{2+}-dependent acidic phosphatase activity

The brains were perfusion-fixed for detecting Ca^{2+}-dependent acidic phosphatase activity. This method is the modification of Nissl staining and Golgi silver impregnation (Meliksetyan, 2007; Yenkoyan et al., 2011). It allows to enhance the visual resolution of the cell structures. For this purpose, the isolated brains were formalin fixed in the 5% buffered neutral formalin for 24–48 h at 4 °C. The formalin fixative solution was prepared in 0.1 M phosphate buffer (pH 7.4), and contained 0.3% $CaCl_2$ and 15% sucrose. The 40–50 μm thick frontal brainstem free-flow frozen slices were cut based on the previously described coordinates (Bruyn, 1988). The slices were washed in distilled water and transferred into incubation mixture, containing 0.4% lead acetate, 1 M acetate buffer (pH 5.6), 2% sodium glycerophosphate for 2–3 h at 37 °C. The slices were thereafter washed in distilled water, transferred to 3% sodium sulfide solution, rewashed in distilled water and embedded into the Canada balsam.

2.6.2. Luminescent assessment of adrenergic granules

The method is the modification of the sucrose-potassium phosphate-glyoxylic acid (SPG) histofluorescence based on the condensation of glyoxylic acid with monoamines (de la Torre, 1980) that improves the visualization of monoaminergic neurons. The slices of the frontal brainstem LC nucleus were produced using cryostat based on the previously described coordinates (Bruyn, 1988).

2.7. Statistical analysis

Statistical analyses were performed using SPSS software package (v. 23). The normal distribution of the data was tested using Kolmogorov test. T-test was used to compare the normally distributed variables. Comparisons between groups were performed using one-way ANOVA in the normally distributed samples. The differences in the monoamine levels were determined using one-way ANOVA. Tukey post hoc test was applied for normally distributed data. *P* values below 0.05 were considered as statistically significant.

3. Results

3.1. Morphological study. Changes in LC neurons

Histological examination of the control group revealed pigmentation at the top of the pons, medially from the cerebral nucleus of the trigeminal nerve, which were part of LC (Fig. 1A). Despite the high staining intensity of LC neurons, the bright nucleus could be relatively easy discerned at high magnifications (Fig. 1B). The cells had amoeboid shape and were closely positioned. The granular precipitate looked homogeneously in the perinuclear zone. Separate precipitate clusters were shifted to the cell periphery. Strictly ordered dark pellets were seen across the surface of the neuron, similar to synaptosomes, which implied their abundance in the LC neurons. In addition, excessive amounts of precipitate granules were identified in the intercellular space.

Under amyloid exposure (Aβ (25–35) administration), the LC neurons lost their typical structure, swelled, and nuclei translocated (Fig. 1C). In the majority of neuronal nuclei, we have observed central chromatolysis and a gradual reduction of the granular precipitate in the perikaryon of the cell, similar to the Nissl-degeneration (Fig. 3C).

In PEG-1 group rats, one could notice a tendency of neural recovery in LC (Fig. 1D and E), although certain eccentrically disposed and disproportionately swelled nuclei were also observed. The granulation was clearly visible within neurons. In the neighboring neurons subjected to central chromatolysis, different types of precipitate granules were observed (Fig. 1 E). It is important to note that the nuclei of neurons had a central location (Fig. 1D and E) after injection of PEG in both PEG-1 and PEG-2 modes.

3.2. Luminescent assessment of adrenergic granules from LC

To prove the quantitative changes of NE in brainstem we stained corresponding brain sections of LC, the principal site of brain NE synthesis, with glyoxylic acid, condensation of which with monoamines produces bioluminescence (de la Torre, 1980). After *i.c.v.* administration of Aβ (25–35), we observe significantly increased luminescence arising from the adrenergic formations of LC (Fig. 2B). The intensity of luminescence in brainstem cells in PEG-1 group is certainly stronger as compared to the control group (Fig. 2C). Interestingly, the injection of PEG produces the pattern of luminescence similar to the one observed in the amyloid group, however, less intense, reflecting apparently an increased synthesis of NE (Fig. 2 D). Altogether, these data confirm the trend of NE changes as revealed by HPLC assay.

3.3. Biochemical study. Changes in the monoamine levels of brainstem

The morphological changes observed in the brainstem of the animals from amyloid group were accompanied by higher levels of epinephrine, norepinephrine and lower levels of HVA as compared to the controls (Fig. 3). Administration of PEG in the amyloid group led to the drastic decrease of epinephrine and norepinephrine levels to the nearly control levels observed in PEG-1 group and to a lesser extent in the PEG-2 group. A similar trend was also detected for HVA (increase to the control levels). Administration of neither amyloid nor PEG in PEG-1 mode had any significant impact on 5-HIAA levels.

3.4. FRO and antioxidant capacity

3.4.1. The evaluation of spontaneous (biochemiluminescence) and UV light-induced chemiluminescent intensity of lipid peroxidation

In order to evaluate the levels of oxidative stress in the brainstem cells in AD brain and potential protective effect of PEG, we performed chemiluminescent analysis, which is typically used to study chain free radical lipid peroxidation in biological membranes (Vladimirov et al., 2011).

Data, as shown in Figs. 4 and 5 indicate that compared with controls, amyloid damage led to a significant increase in both spontaneous (Fig. 4) and UV-induced chemiluminescence (Fig. 5A) along with the reduction of Imax/S ratio, which reflects the antioxidant state of the cell. Thus, it is obvious that *i.c.v.* administration of Aβ25-35 leads to a noticeable activation of the FRO and reduction of the antioxidant state of the brainstem neurons. After injection of PEG to sham-operated animals, a certain tendency to the increase of the spontaneous and UV-induced chemiluminescence was also observed (Fig. 5B). At the same time, the antioxidant potential of brainstem cells was increased almost 10-fold. Administration of PEG in both preventive and therapeutic modes led to the pronounced drop of chemiluminescence indicators, which were significantly increased in the amyloid group. Furthermore, not to be missed, a single injection of PEG was more effective than the

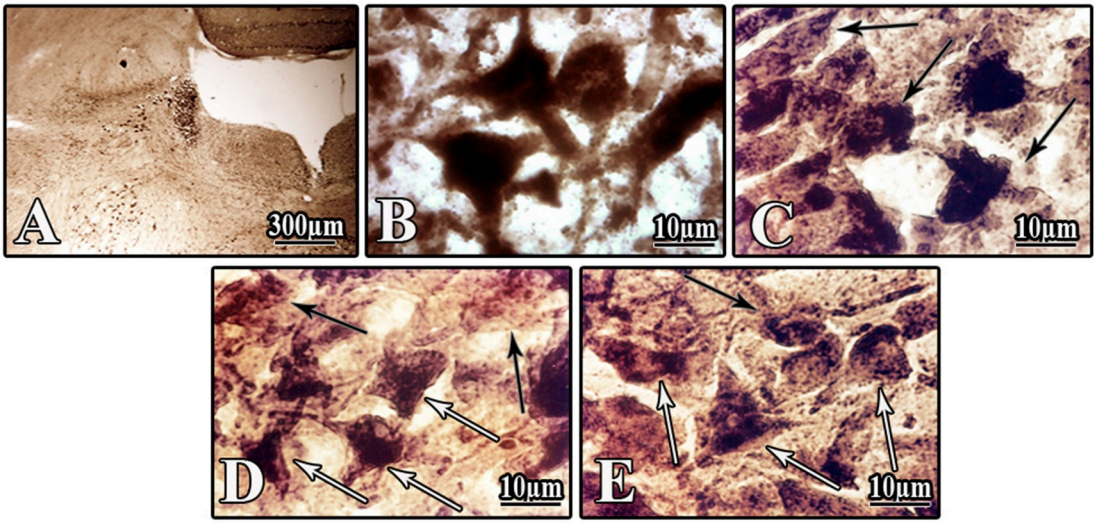

Fig. 1. Frontal slices of LC neurons of intact rat brain (A, B), after bilateral *i.c.v.* injection of Aβ25–35 (C), PEG-1 (D) and PEG-2 (E). Damaged and swelled neurons with the eccentrically disposed nuclei (C, indicated by black arrows) are shown in amyloid group. Survival of LC neurons with preserved soma and apical axons and appearance of precipitating granules in their perikaryon (indicated by white arrows) is shown after injection of PEG in single (C) and double (D) modes. Magnification: × 25 (A); × 1000 (B–E).

double one.

3.4.2. The evaluation of Fe^{2+}-induced CL intensity of lipid peroxidation

Amyloid exposure increased the fast flash (FF) and slow flash (SF) values, and significantly decreased the "time-to-peak" (tau). Administration of PEG to the sham-operated animals also increased FF and SF values, but the tau was significantly longer in comparison with the amyloid group, as well as with the all other investigated groups (Fig. 6A and B). Administration of PEG in both PEG-1 and PEG-2 modes decreased FF and SF values, as well as significantly increased the tau in comparison with amyloid group. Comparative changes are schematically shown in Fig. 6C(b).

4. Discussion

Our previous data established that the administration of Aβ(25–35) leads to a typical degeneration in cortical area and hippocampus of rats accompanied by a strong increase of monoamine levels (Yenkoyan et al., 2018, 2009). Similar results including activation of FRO were shown also in the current study. We suggest that the aggregated beta-amyloid 25–35 induces oxidative stress in brainstem leading to partial damage of LC neurons and provokes the compensatory increase of NE. In turn, NE is capable of protecting neurons from Aβ-induced damage (Chalermpalanupap et al., 2013). Pretreatment of neurons with NE before amyloid exposure increases neuronal GSH level and upregulates expression and activation of peroxisome proliferator-activated receptors (PPARs) (Klotz et al., 2003; Madrigal et al., 2007). Interestingly, quantitative PCR analyses did not show any significant NE-dependent increase in either catalase or SOD mRNA levels (Madrigal et al., 2007), but PPARs activation leads to the stimulation of the expression of antioxidant enzymes (Girnun et al., 2002; Inoue et al., 2001). In addition, there are data that NE reduces the production of reactive oxygen species, serving as the first line of defense against oxidative stress (Dimić et al., 2017; Smythies, 2000) Neuroprotective effect of NE was also shown on the transgenic mouse model of AD, where the precursor of NE, L-threo-3,4-dihydroxyphenylserine (L-DOPS) improved memory and reduced Aβ plaque pathology (Kalinin et al., 2012), and the use of α2 adrenoblocker mesedin had a neuroprotective effect (Melkonyan et al., 2017).

Therefore, we hypothesize that the following circuit is activated during the amyloid-induced neurodegeneration in the brainstem: Aβ25-35 → FRO increase → mild neuronal damage → NE level increase → neuroprotection or at certain circumstances damage (*see also graphical abstract*). Moreover, we believe that in case of the mild damage of LC neurons, the treatments targeting increased levels of NE will be beneficial, however, if there is a strong loss of LC neurons there is no reason to increase the amount of NE. Probably, this is the reason why many NE targeting drugs were found not efficient in clinical trials

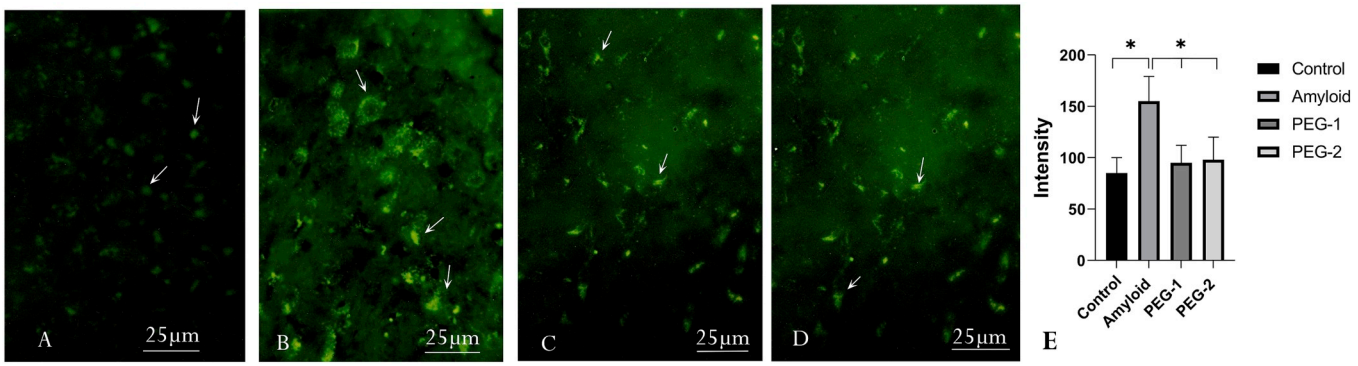

Fig. 2. Luminescence of adrenergic granules in the brainstem (area LC) of the control animals (A), after *i.c.v.* administration of Aβ (25–35) (B), PEG-1 (C), and PEG-2 (D). Magnification ×400 (A–D). E – Intensity of adrenergic granules converted to grayscale. *$p < 0.05$.

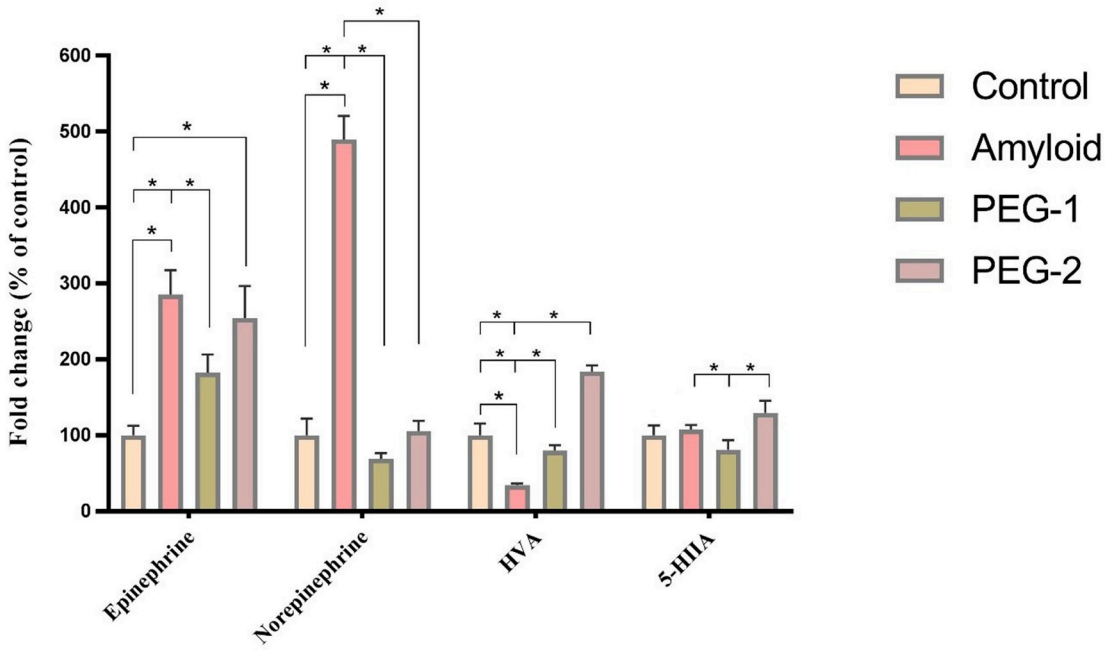

Fig. 3. The levels of epinephrine, norepinephrine, HVA and 5-HIAA in the control, amyloid, PEG-1, and PEG-2 groups in the brainstem measured by HPLC assay (see Methods). Data are shown as the mean percentage relative to control ± S.E.M, n = 10, *p < 0.05.

(Chalermpalanupap et al., 2013) Interestingly, most of the patients enrolled in these trials were at the advanced stages of AD (Chalermpalanupap et al., 2013).

Injection of PEG both in preventive (PEG-1) and preventive/treatment (PEG-2) modes correspondingly prevented and stabilized the rise of monoamines in the brainstem. At the same time, the serotonin levels remained constant, which indicates that PEG does not act via the serotonin pathway in the brainstem.

When comparing the effects of different variants of PEG injections, it is noteworthy that the pattern of monoamine changes in brainstem remained essentially stable with few variations depending on the number of injections. In particular, the concentrations of epinephrine and NE were lower in PEG groups as compared to the amyloid group; however, compared to a single administration, the concentrations remained at a relatively high level after double injection. In contrast to a single dose, after two administrations the concentration of HVA significantly exceeded the control value. Therefore, it can be suggested that after a double injection of PEG, the breakdown of dopamine has been intensified.

Summarizing the effects of PEG on monoamines, we conclude that both single and double injections of PEG stabilize the level of monoamines; however, in contrast to a single injection, it seems that the double administration of embryonic proteoglycans has a tendency of monoamine upregulation.

After injection of PEG to sham-operated animals, there was a similar tendency to increase of the parameters of CL, which is likely due to the activation of metabolic processes under the influence of PEG (Yenkoyan et al., 2009). Importantly, after the introduction of PEG, the antioxidant potential of the brainstem cells increased almost 10-fold. It appears that the upregulation of FRO in course of the amyloid-induced neurodegeneration can be recovered by PEG. It is supported by the pronounced drop of both spontaneous and induced CL as observed after PEG injection to amyloid rats. It is interesting that a single injection of PEG was more effective than double injection. The drop of the antioxidant

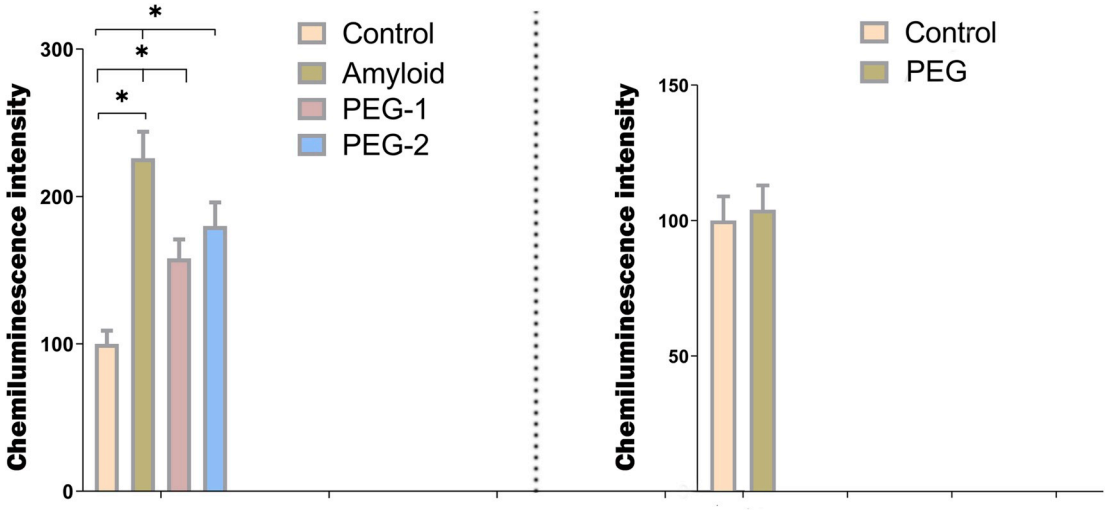

Fig. 4. The parameters of spontaneous chemiluminescence in the control, PEG, amyloid, PEG-1 and PEG -2 treated groups of the brainstem. *p < 0.05.

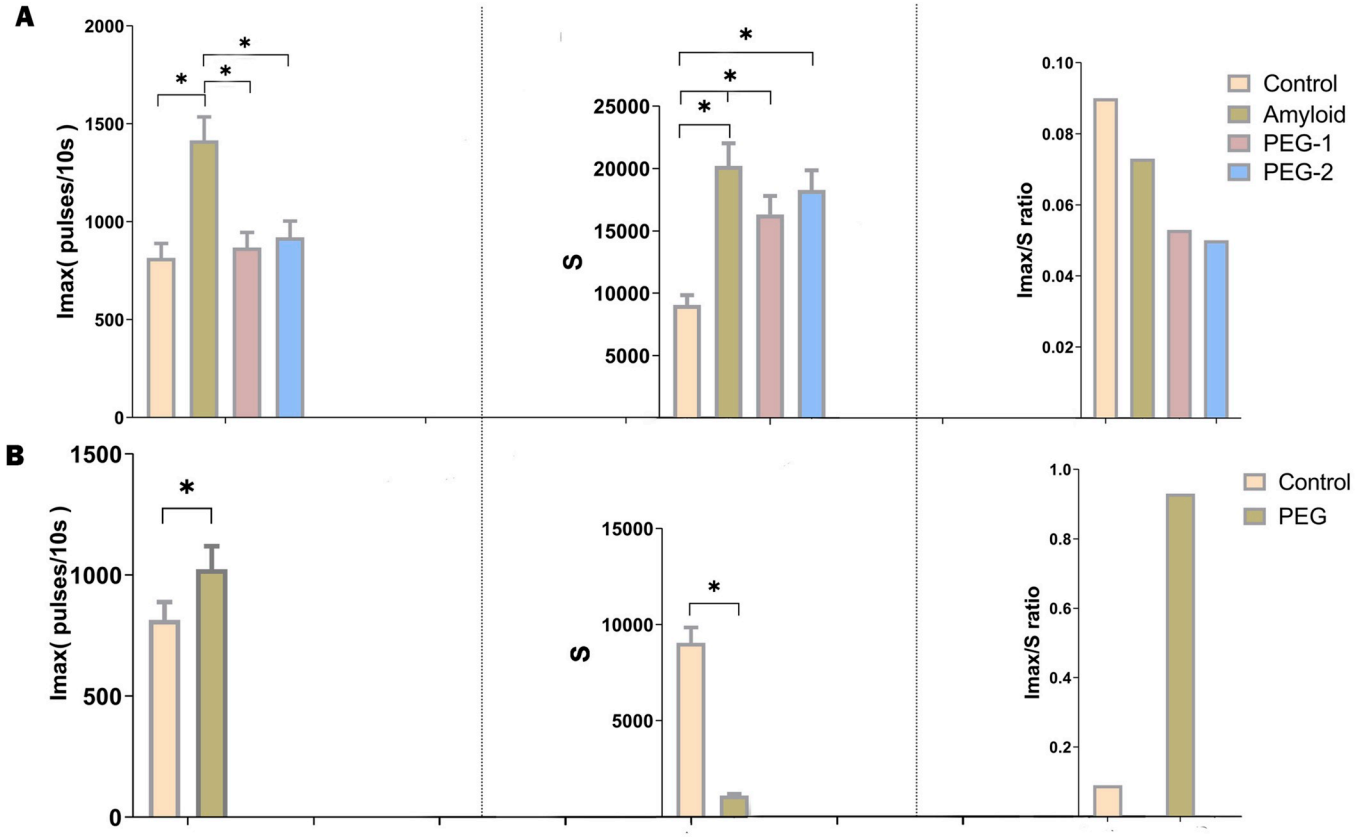

Fig. 5. The parameters of UV-induced chemiluminescence in the control, PEG, amyloid, PEG-1 and PEG -2 treated groups of the brainstem. *p < 0.05.

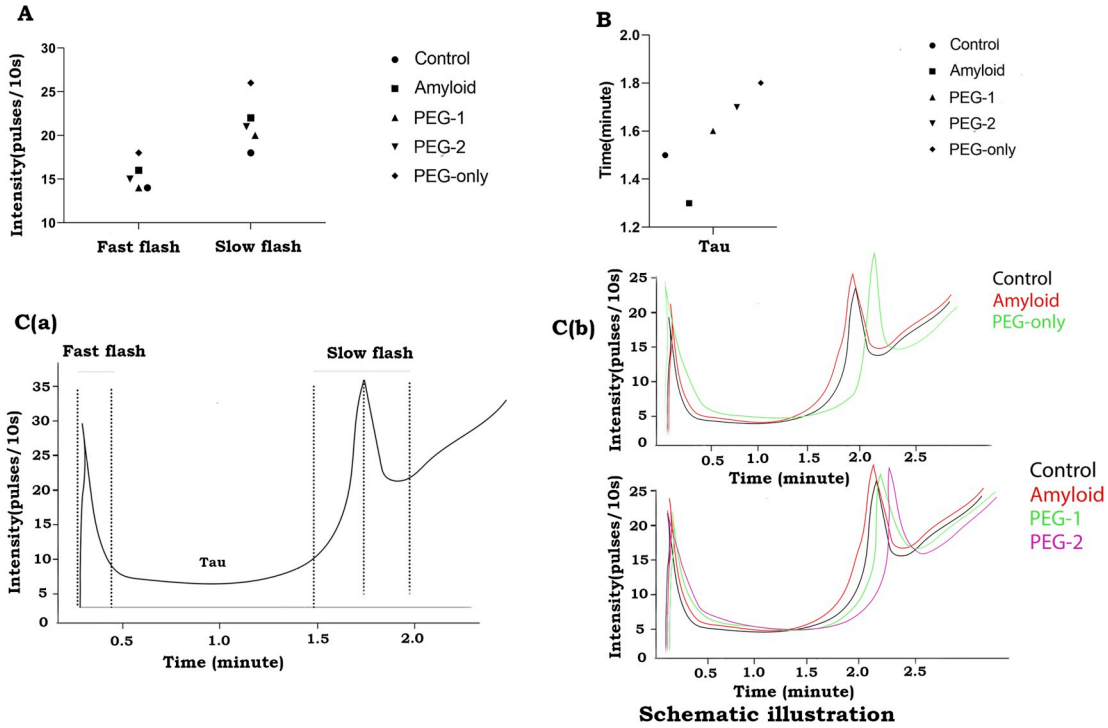

Fig. 6. Fe^{2+}-induced chemiluminescence in the brainstem. Fast flash and slow flash (A), tau ("time of peak") (B) values in the of the experimental groups. C (a), characteristic kinetic curve of CL for the chain lipid oxidation reaction initiated by Fe^{2+} salts including a series of successive stages: fast flash, tau and slow flash. C (b), schematic illustration of the difference in kinetic curves of Fe^{2+}-induced CL between the experimental groups.

coefficient in the PEG-1 and PEG-2 groups compared with the amyloid group seems to be due to the involvement of antioxidants and/or the increase of antioxidant capacity. The same trend was observed after the administration of hypothalamic PRP-1 (Yenkoyan et al., 2018). Thus, it can be suggested that in the model of Aβ25-35-induced neurodegeneration, the introduction of PEG leads to the alleviation of oxidative damage in the neurons of the brainstem and, possibly, prevents their demise. This phenomenon is supported by morphological studies, which have clearly shown survival of brainstem neurons after PEG administration. We propose that PEG via preventing amyloid-induced oxidative damage of LC neurons also prevents the increase of NE, rather there is "no need of NE increase" in that case. We suggest that under PEG administration in brainstem the following circuit works: PEG → increase of antioxidant capacity → FRO prevention → prevention of NE increase (*see also graphical abstract*).

Interestingly, our findings show a correlation between the increase in monoamine concentrations and intensity of FRO and corresponding stabilization of these parameters after PEG injection. Again, the single injection of PEG was more effective for the recovery of monoamines and oxidative stress. Two-fold injection of PEG stabilized the monoamine levels, but had a tendency to increase the monoamine concentrations. In addition, it appears that PEG alone can substantially increase the antioxidant capacity of LC neurons and induce the increase of monoamines in brainstem.

Several limitations of this study should also be noted. We have inferred the antioxidant properties of PEG based on the kinetics of chemiluminescence, which gives us an integral but not very detailed image of the antioxidative defense system: it is difficult or simply impossible to identify the individual antioxidant enzymes or non-enzymatic factors involved. In addition, the molecular mechanisms of PEG neuroprotection, the role of its individual components still remain to be investigated.

5. Conclusion

Data presented in the current study are consistent with our previous findings regarding involvement of LC monoaminergic system in the development of AD-like neurodegeneration. Here we have also tested a novel agent, embryonic proteoglycans and found that they can restore the altered levels of monoamines, decrease the levels of FRO, thus having a potential neuroprotective effect. We suggest that such drugs capable of increasing the NE levels can be especially beneficial at the early stages of AD development with still functional neurons.

Further research is needed to detail how these changes interfere with cortical and hippocampal changes in context of monoamine shifts and demonstrate the protective effect of PEG on the AD target structures.

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