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Protection of neurodegeneration through fetal therapy

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There has been remarkable progress in molecular genetic and biological research into Alzheimer's disease (AD) over the past decade. It has become obvious that the prior role in developing of AD plays ß-amyloid. Molecular genetic analysis and biological research have provided powerful evidence that the formation and aggregation of β-amyloid (Aβ) in the brain, and particularly in hippocampus and cerebral cortex is the central event in the pathogenesis of AD. In considering amyloid cascade hypothesis, it is important to realize that amyloid fibrils themselves do not neccessarily initiate the cascade of events that ultimately leads to neurodegeneration and dementia. The real culprit in AD may be an intermediate aggregates en route to fibril formation, as this is more likely to show neurotoxic properties[10]. It is evident that the neurodegeneration associated with AD involves a complex system of cellular and molecular interactions, including the component of the ß-amyloid aggregation, oxidative damage, inflammatory response, progressive neurotransmitter and growth factor deficits such as nerve growth factor (NGF) and insulin-like growth factor (IGF)[18–20, 22,].

Insulin-like growth factors (IGFs). It is well established that insulin-like family members are critically involved in maintenance of body homeostasis. Insulin-like growth factors (IGF-I and IGF-II) play an important role in the normal development and maintenance of the cellular integrity of the organism, including the central nervous system[6]. Both trophic factors are selectively localized in the brain and their specific receptors are uniquely distributed in various neuroanatomical regions, being especially concentrated in the hippocampal formation [12]. The IGF-I receptor is composed of two α chains where the ligand binds and two ß chains possessing a tyrosine kinase domain [13]. In contrast, the IGF-II receptor is made of a single transmembrane segment containing a binding site for IGF-II and another for mannose-6-phosphate residues. Both receptors bind specifically to their cognate ligands but they can also recognize each other with lower affinity. It has been recently shown that cultured hippocampal neurons are highly enriched with IGF-I and IGF-II receptors each being differentially internalized [7], and serving distinct functions. Earlier studies have shown that IGFs possess neurotrophic activities in the hippocampus [4,12,24], an area severely affected in AD. Interestingly, it was also observed that the levels of IGF-I binding sites are significantly increased in cortical areas of AD brains [5]. It is unclear if these increases in IGF-I receptors represent a protective compensatory mechanism against neuronal losses. Considering the broad actions of IGFs on the maintenance of normal cellular functions and the presence of high levels of IGF receptors in the hippocampus, it was investigated the potential neuroprotective effects of IGFs against АЯ-induced toxicity in rat hippocampal and cortical neurons as the main targets for AD, as well as the places of its synthesis[8]. It has been established that IGF-I is able to protect, and more importantly, to rescue rat hippocampal primary neurons against Aβ-and human amylin-induced toxicity. IGF-II is less potent suggesting the activation of the IGF-I receptor and the related signaling pathway. These unique properties of IGF-I, parallel to its well known involvement in various metabolic pathways, suggest that the development of IGF-I-related mimetics could be a promising strategy toward the treatment of various neurodegenerative diseases including AD [2].

Nerve growth factor (NGF) and neurotrophins. Nerve growth factor (NGF) is widely distributed in basal forebrain cholinergic neurons (BFCNs) and in regions of the central nervous system innervated by the magnocellular BFCNs. NGF promotes the differentiation of BFCNs , ameliorates lesion-induced abnormalities in these cells and reverses atrophy of BFCNs and spatial memory impairments in aged rats. A decline in the integrity of the central cholinergic function in aged human brain has been postulated to be responsible for the neuropathological changes leading to cognitive and mnemonic deficits [3,17,21]. The extensive overlap in signaling pathways regulated by neurotrophins and those likely to be involved in AD degeneration along with the expression of neurotrophin receptors by neurons undergoing degeneration point to neuroprotective applications for neurotrophins. The major role of neurotrophins in synapse stabilization and function along with emerging evidence that synaptic failure is a critical early process in AD further adds to interest in neurotrophins as candidate therapeutic agents. In the context of AD, NGF and brain-derived neurotrophic factor (BDNF) have been of particular interest. Neurotrophins each bind to a dual receptor system, consisting of p75NTR along with one of the tyrosine kinase receptors

(Trk). NGF binds to TrkA and BDNF to TrkB. Neurotrophins receptors are expressed by neuronal populations particularly vulnerable in early stages of AD. p75NTR, TrkA, and TrkB are each expressed by basal forebrain cholinergic neurons, hippocampal pyramidal neurons and layer V cortical neurons [3,21].

Consequently, there is a widespread interest in NGF, as well as IGF and neurotropnins as potential therapeutic agents in neurodegenerative disorders connected with aging, such as Alzheimer's disease.

On the other hand, for the last decade the interest to the fetal therapy of the neurodegenerative diseases has significantly increased. It has been established that transplantation of hippocampal stem cells improves memory and behavioral functions [11], inhibits neuronal cell loss at the model of scrapie(1); dopaminergic neurons cloned on stem cells proceed an expressed effect in Parkinson's mice[9]. Unfortunately, the mechanism of the protective effect of embryonic factors is still unknown [5,23].

The goal of our investigation was to show the possible neuroprotective effect of a humoral component of fetal therapy, particularly a new proteoglycan of embryonic genesis (PEG) created by prof. L. Mkrtchyan in Aβ-induced neurodegeneration(experimental model of AD).

Materials and Methods

It was investigated the regulatory role of PEG on the level of NGF & IGF in the cerebral cortex and hippocampus. These experiments were accompanied by morphological studies in the same organs. The experimental model of AD was created in rats by intracerebroventricular injection of aggregated Aβ(fragment 25-35).

Commercially available Aβ(25–35) was purchased from Sigma-Aldrich (USA) and aggregated according to Maurice et al. (16). In brief, the peptide was dissolved in sterile bidistilled water at concentration of 1 mg/ml, aliquoted into tubes and stored at –18^{0}C. It was "aged" by incubation at 37^{0}C for 4 days before the surgery. Light microscopic observation demonstrated the existence of both birefringent fibril-like structures and globular aggregates.

The amyloid-beta induced neurodegeneration was made by the following way: animals under chloral hydrate anesthesia (325 mg/kg) were positioned in a stereotaxic frame and a midline sagittal incision was made in the scalp. Holes were drilled in the skull over the lateral ventricles using the following coordinates: 0.8mm posterior to bregma; 1.5mm lateral to the sagittal suture. All injections were made using a 10μl Hamilton syringe equipped with a 26S-gauge needle. The needle of the microsyringe was put 3.8mm beneath the surface of the brain. Animals were injected with 3.0μl sterile bidistilled water (vehicle-treated) or 3.0μl aggregated Aβ(25–35) solution (3μg) into each cerebral lateral ventricle at a rate of 1μl/min.

Fifty young adult male rats, weighing 230–290 g at the beginning of the experiment were housed five per cage at 12:12 h light/dark cycle (08.00–20.00 h) and fed ad libitum. The animals were divided into 4 groups: the control group consisted of vehicle treated animals; the 1st experimental group was intracerebroventricularly injected with aggregated Aβ; the 2nd experimental group was subcutaneously injected with PEG (1mg/200g) only; the 3rd experimental group was subcutaneously administered with PEG 7 days before Aβ injection and on the 25th day after it. Supernatants obtained from rat internal organs (cerebral cortex, hippocampus) underwent ELISA. The analysis of the growth factors was performed with ChemikineTM Sandich ELISA (USA) kit and IGF-I was determined with Diagnostic Systems Laboratories, Inc. EIA (USA) kit. IGF-I concentration was displayed in nmol/ml and NGF content in pg/ml. The animals were killed on the 85th day after Aβ injection. All efforts were made to minimize the animals' suffering and to reduce the number of animals used. The statistic calculation of the obtained results was done by the known method of Student's variation statistics.

To reveal the histological changes the slices were taken from the cerebral cortex and hippocampus The slices were coloured by Ca2+-dependent acid phosphatase method modified by I. Meliksetyan [25].

Results and Discussion

The results of immunoenzyme analysis have demonstrated that the PEG in a dose 1mg significantly increases the level of NGF and IGF both in hippocampus and cerebral cortex of normal animals (Fig.1–4). It is worth to be noted that the changes in the level of IGF in cerebral cortex and particularly in hippocampus (about 4 times more in comparison with the norm) have been more sensitive than NGF changes. In our opinion the results of immunoenzyme analysis of NGF and IGF are of certain interest. The obtained results testify that PEG has a modulatory effect on different brain structures which can lead to the neuroprotection. As shown in Fig.2, the level of NGF decreased in the cerebral cortex and increased in the hippocampus under intracerebroventricular injection of Aβ. This data closely correlated with the morphological changes in mentioned structures, which shows different stage of degeneration of neurons in cerebral cortex and hippocampus. The level of IGF both in cerebral cortex and hippocampus significantly increased (about 6 times in cerebral cortex and 8 times in hippocampus in the comparison with control level). In our opinion such changes of the growth factors can testify to the process of neuronal survival (both recovery and/or apoptosis) in β-amyloid-induced neurodegeneration. Administration of PEG in-

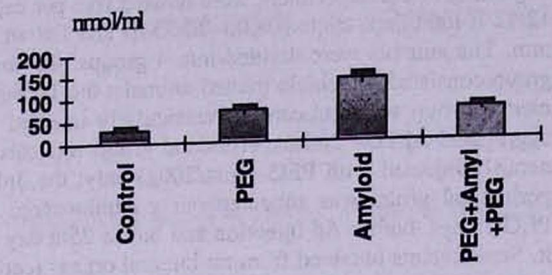

Fig. 1. The level of IGF in parietal cortex of control and experimental animals control and experimental animals

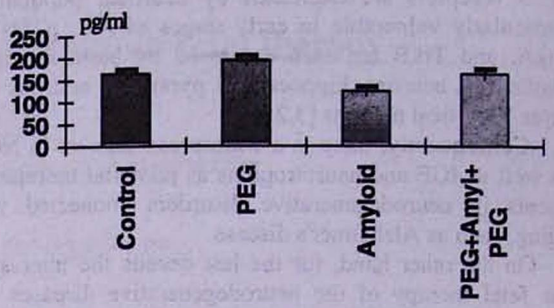

Fig. 2. The level of IGF in parietal cortex of control and experimental animals control and experimental animals

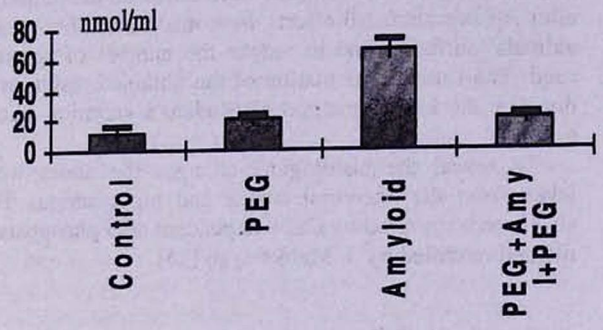

Fig. 3. The level of IGF in hippocampus of control and experimental animals

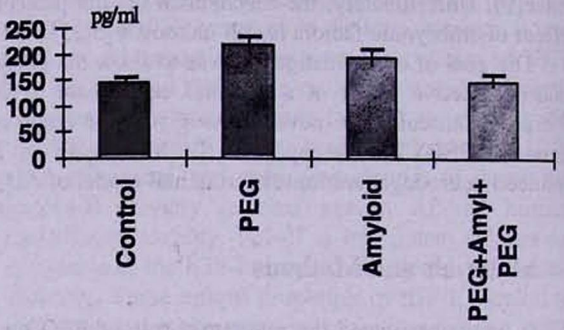

Fig. 4. The level of IGF in hipcontrol of control and experimental animals

Fig. 5. Parietal cortex, norm. Magnified x 1000

Fig. 6. Parietal cortex, A-ß. Magnified x 1000

Fig. 7. Parietal cortex, PEG+A-ß. Magnified x 1000

creased the level of NGF in the cerebral cortex and hippocampus(Fig.2,4). The obtained results testify also to normalization of the IGF level in cerebral cortex and hippocampus(Fig.1,3). On the basis of the data obtained it can be concluded that embryonic proteoglican plays a regulatory role in conditions of neuronal pathology.

Fig. 8. Hippocampus, norm. Magnified x 1000

Fig. 9. Hippocampus, A-ß. Magnified x1000

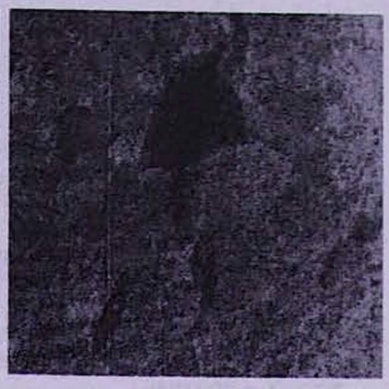

Fig.10. Hippocampus, PEG+A-ß. Magnified x1000

The morphological data testify that in Aβ neurodegeneration it is observed swelling of big cerebral cortex neurons. The cytoplasm of changed neurons is in condition of chromatolisis. Dystrophied neurons fit to smaller or irregular-shaped cells (satellites) (Fig.5–7). There appear cells-shadows. A similar picture is observed in hippocampal cells(Fig.8–10). As a result of PEG injection in neurodegeneration expression of pathomorpholical and hystochemical changes in cerebral cortex and hippocampus were loosely revealed. In these slices the picture was about the same as that in the control ones.

In summary, our findings confirm the suggestion of a potential therapeutic benefit of embryonal therapy in neurodegenerative diseases, and more specifically in AD-related amyloidosis.

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Նեյրոդեգեներացիայի կանխարգելումը սաղմնային թերապիայի միջոցով

Կ. Բ. Ենկոյան

Տվյալ աշխատանքում հետազոտվել են գլխուղեղի նեյրոնների «վարքը», ինչպես նեյրոդեգեներացիայի պայմաններում, այնպես էլ նրա կանխարգելումը սաղմնային սպիտակուցներով: Այդ նպատակով որոշվել է ածի ինսուլինանման գործոնի (ԱԻԳ) և նյարդի ածի գործոնի (ՆԱԳ) քանակը գլխուղեղի կեղևում և հիպոկամպում β-ամիլոիդով հարուցված նեյրոդեգեներացիայի պայմաններում, ինչպես նաև նրա հնարավոր կանխարգելումը սաղմնային ծագում ունեցող պրոտեոգլիկանային կոմպլեքսի (ՍԾՊԿ) ներարկման պայմաններում: Նեյրոդեգեներացիան ուղեկցվում է ԱԻԳ-ի կտրուկ ածով ինչպես հիպոկամպում, այնպես էլ կեղևում, ՆԱԳ-ի քանակի ածով միայն կեղևում, իսկ հիպոկամպում նկատվում է հակառակ երևույթը: ՍԾՊԿ-ի ենթամաշկային ներարկումը նպաստում է ածի գործոնների քանակների կարգավորմանը, ինչը համահունչ է մորֆոլոգիական փոփոխություններին:

Предотвращение нейродегенерации с помощью фетальной терапии

К.Б. Енкоян

В работе рассмотрены биохимические и морфологические проявления «поведения» нейронов головного мозга в условиях нейродегенерации, а также возможность ее предотвращения фетальными белками. Изучена реакция инсулиноподобного фактора роста-I (ИФР- I) и фактора роста нерва(ФРН) в коре и гиппокампе головного мозга крыс в условиях Я-амилоидной нейродегенерации и ее предотвращение с помощью протеогликанного комплекса эмбрионального генеза (ПЭГ). При нейродегенерации как в нейронах коры, так и гиппокампа наблюдался резкий выброс ИФР-I, уровень ФРН в коре также повысился, тогда как в гиппокампе, наоборот, понизился. Введение ПЭГ приводит к стабилизации ростковых факторов. Все вышеуказанные биохимические изменения полностью коррелируют с морфологическими проявлениями, наблюдаемыми со стороны нейронов коры и гиппокампа.

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