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Cytokine System Reactivity of the Rats' Brain at Intrabulbar Injection of β-Amyloid Aggregates

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Original Research Article

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Abstract: Neuroinflammation, mediated by the reactivity of the system of cytokines, accompanies the flow of amyloidosis in Alzheimer's disease, alongside with disturbances in the work of central departments of the olfaction analyzer. Therefore, it is advisable to determine the reactivity of the cytokine system in the brain sections of elderly rats (neocortex, hippocampus, olfaction bulbs, hypothalamus, caudate nuclei) under the conditions of the bulbar effect of β-amyloid peptide aggregates. The content of tumor necrosis factor α, interleukin-6 and interleukin-10 in the brain sections of the rats having the age of 18 months (n = 24) that are being studied was determined 5 days after the intrabulbar injection of the suspension of β-amyloid peptide 40 (Amyloid β Protein Fragment 1-40, Sigma-Aldrich, USA) in the bidistilate or the solvent itself. It has been shown that the initial level of activity of the cytokine system (intact animals of the control group, n = 8) in the brain sections that are tangent to the olfaction analyzer (neocortex, hippocampus and olfactory bulbs) is probably lower in comparison to other parts (hypothalamus and caudate nucleus) of elderly rats. Intraubulbar injection of β-amyloid peptide 40 aggregates to elderly rats (experimental group, n = 8) increases the activity of the cytokine system in the brain structures distant from the spot of injection (neocortex and hippocampus) responsible for memory mechanisms. It has been determined that the reactivity of the cytokine system in the neocortex, hippocampus and hypothalamus is higher not only in response to the specific toxic effects of the β-amyloid peptide 40 aggregates (experimental group, n = 8), but also due to surgical intervention with the injection of solvent alone (comparison group, n = 8). It has been generalized that the low content of pro- and anti-inflammatory cytokines in phylogenetically younger parts of the brain (neocortex, hippocampus) of intact animals corresponded to increased reactivity of the cytokine system under the influence of exogenous β-amyloid peptide 40.
Keywords: β-amyloid peptide, cytokines, Alzheimer's disease, rat, neuroinflammation, neocortex, hippocampus, olfactory bulb.

INTRODUCTION

At present, evidence is provided that the β-amyloid peptide (Aβ) aggregates are ordered oligomers and the causes, rather than amyloidogenic pathology outcome, in particular - Alzheimer's disease (AD) [1, 2]. For the first time, the description of AD was made by Alois Alzheimer in 1907, although other doctors also made similar and quite complete assessments of neuropathological events occurring in the brain with AD [3-5]. It has been shown that neuroinflammation is one of the early biomarkers of AD leading to increased neurodegenerative pathology and amyloidosis [6-8]. Aβ-oligomers interact with microglial and astrocytic pattern recognition receptors that activate the innate immunity. The process involves the secretion of cytokines, chemokines, and the formation of active forms of oxygen, the surplus of which leads to a dysregulated immune response that promotes neurodegeneration. The mechanisms with the help of which a neuroinflammatory reaction can affect the formation of Aβ and its aggregation become key, the therapeutic intervention may slow the progression of AD [9]. Nevertheless, the reactivity of the congenital (peptide) immunity, in particular the system of cytokines, may have its specificity in different sections of the brain according to the age norm and under the toxic effect of Aβ oligomers.

It is known that another early biological marker of AD is anosmia (loss of olfaction) [10-12], which is associated with a violation of central mechanisms for processing olfactory information, namely in olfactory bulbs and periformal cerebral cortex. It should be noted that olfactory centers have

numerous connections with the hippocampus, the limbic system and the individual nuclei of medulla oblongated marrow nerves. Therefore, it has been advisable to determine the cytokine response in the brain parts of animals involved in the olfactory analyzer (neocortex, hippocampus, olfactory bulbs) in comparison with other (hypothalamus, caudate nuclei) in the localization of Aβ aggregates in the olfactory bulb.

The purpose of the study was to determine the reactivity of the cytokines system in the brain parts of rats under the conditions of the bulbar effect of the β-amyloid peptide aggregates.

MATERIALS AND METHODS

The study was performed on 24 elderly male rats (18 months age) weighing 450-520 g. All the animals were staying under the controlled 12-hour light-dark cycle and standard feeding for rodents and tap water. Experimental protocols were conducted in accordance with the General Ethical Principles of Animal Experiments [25], the European Convention for the Protection of Vertebrate Animals used in experiments and other scientific purposes [26], internationally recognized principles for the use and care of laboratory animals, as specified in United States guidelines [13].

The rats were randomly assigned to 3 groups. Control group (n = 8) included intact animals. Experimental group (n = 8) - rats being 5 days after the injection of a suspension of $A\beta_{40}$ aggregates into the olfactory bulb (experimental model of AD); Comparison group (n = 8) - rats being 5 days after the injection of the solvent (H_2O) into the olfactory bulb. The volume of the solutions was 10 μl per animal, the speed of injection through the needle of the chromatographic syringe was 0.03 μl/sec, and the injection duration equaled 5 minutes. Stereotaxic operations were performed under general anesthesia of rats using intraperitoneal injections of thiopental, 50 mg/kg per body weight. A solution of the β-amyloid peptide 40 (Amyloid β Protein Fragment 1-40, Sigma-Aldrich, USA) in the bidistillate was aggregated for 24 hours at 37°C. Large $A\beta_{40}$ conglomerates were dispersed with the help of ultrasound and sterilized immediately before the injection. The stereotactic coordinates of the area of the left olfactory bulb were determined based on the brain card of Ya.Buresh [14], which corresponds to the distance from the intersection point of the sagittal suture with the bregma (zero point): distally (-6) mm, laterally - 0.5 mm and to the deep - 2.5 mm. The animals were decapitated. In the cold, neocortex, hippocampus, hypothalamus, olfactory bulbs and caudate nuclei were isolated, the latter were frozen and stored prior to the ELISA analysis of cytokines at -40°C. The tissues of the brain sections being studied were homogenized in Tris buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.5), centrifuged at 14,000 rpm for 5 minutes, then they collected a supernatant. Samples of supernatants of the above-mentioned brain sections of rats were used in order to determine cytokines according to the instructions of the Rat ELISA Kits Invitrogen BCM DIAGNOSTICS, USA tumor necrosis factor-α (TNFα), interleukin-6 (IL-6) and interleukin-10 (IL- 10). OD was read by a microplate analyzer GBG Stat FAX 2100 (USA) at 450 nm with a wavelength correction at 630 nm. Data of ELISA (μg/l) were counted in view of the total protein in grams. The figure shows the data presented as a percentage of the performance of Control group intact animals. The total protein content was determined by Lowry's method [15]. The obtained results were processed statistically, mean values and standard deviations were calculated. Statistical analysis of the differences was performed using Student's *t*-test, values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Table-1 shows the data on the baseline cytokine content (TNFα, IL-6 and IL-10) in the studied rat brain sections (Control group intact animals). The probable lower overall level of both pro- (TNFα, IL-6) and anti-inflammatory (IL-10) cytokines catches the eye as regards the neocortex, hippocampus and olfactory bulbs in comparison to the hypothalamus and caudate nuclei of the rat brain, with the exception of IL-6 in the hippocampus and olfactory bulbs. Such unexpected at first glance results contradict the information that with coming of age, the new brain cortex and hippocampus suffer from excessive activity of the cytokine system, even without amyloidosis due to the forced hyperactivity of their neurons [16-17]. That is, according to our data, the initial level of activity of the cytokine system in the rat brain sections that are relevant to the olfactory analyzer (neocortex, hippocampus and olfactory bulbs) is probably lower in comparison to other parts (hypothalamus and caudate nuclei). Why then is exactly the olfaction primarily affected by age-related amyloidosis, and only then cognitive deficits, depression and dementia are involved?

Figure-1 presents the results regarding the reactivity of the cytokine system in response to the toxic effect of exogenous $A\beta_{40}$ aggregates in 5 days after their intrabulbic injection. Diagram A gives data on the overall effect of $A\beta_{40}$ as a percentage of the initial level (Table-1), in Diagram B - non-specific solvent effect (H_2O) alongside with surgical intervention. The injection of the $A\beta_{40}$ aggregates induced a significant increase in the cytokine system in almost all of the studied brain sections of rats in the Experimental group: the content of TNFα, IL-6 and IL-10 increased in comparison to intact animals primarily

in the direct administration line, olfactory bulbs, as well as in hippocampus; proinflammatory TNFα and IL-6 in neocortex and hypothalamus and TNFα in caudate nuclei. Instead, the effect of intrabulbic injection of the solvent itself (alongside with surgical intervention) in the Comparison group affected the probable increase in the concentration of all three cytokines only in hippocampus; TNFα and IL-6 in neocortex and hypothalamus and TNFα in olfactory bulbs and caudate nuclei.

The specificity of the pro-inflammatory effects of $A\beta_{40}$ aggregates is evidenced by the data of Diagram C, which shows cytokine response in the studied brain sections of rats, with the exception of the consequences of surgical intervention (with intrabulbic injection of solvent - bidistilled water). Consequently, unlike the cerebral or hypocampal effects of the β-amyloid peptide aggregates [18, 19], the most specific $A\beta_{40}$-induced inflammation was not detected in the injection line of $A\beta_{40}$-aggregates - olfactory bulbs. A specific increase in the level of proinflammatory cytokines (TNFα, IL-6) and the absence of changes in the concentration of anti-inflammatory IL-10 was observed in neocortex, hippocampus and hypothalamus. Transsynaptic disturbances and activation of astroglia and microglia in remote locations of $A\beta_{25\text{-}35}$ were also detected in the administration of the oligopeptide in the almond-like complex or the hippocampus [20-21], the loss of neurons and the presence of diffuse deposits of amyloid in neocortex under intravenous cerebrovascular injection of $A\beta_{25\text{-}35}$ to the ventricles the brain [22]. However, as our study in caudate nuclei showed, there were not any specific changes of the cytokine activity in response to the bulbar effect of the $A\beta_{40}$ aggregates.

It is necessary to generalize that in neocortex, hippocampus and hypothalamus, the reactivity of the cytokine system was the greatest not only due to the specific toxic effect of $A\beta_{40}$ aggregates (Fig-C), but also due to surgical intervention (Fig-B). That is, the generally low content of pro- and anti-inflammatory cytokines in these brain sections is offset by the increased reactivity of the cytokine system. Olfactory bulbs have displayed low levels of cytokines in intact animals and a low degree of their activation under the influence of exogenous aggregates of the β-amyloid peptide. This gives the opportunity to assume: neuroinflammation damage to the olfactory analyzer in amyloidosis primarily concerns high-reactivity and new brain sections – neocortex and hippocampus, but not olfactory bulbs. Nevertheless, our data show that olfactory bulbs play a startup role in the spread of neuroinflammation in brain structures, which are responsible for cognitive abilities (neocortex and hippocampus), which can serve as a mechanism of memory damage. In general, it should be noted about essential and vital deeper neuronal bonds existing between olfactory bulbs and neocortex and hippocampus. The Data of Almeida R. F. and other collaborators show an increase in levels of proinflammatory cytokines (IL-1, TNFα, IL-6) and depletion of IL-10 in hippocampus, which weakly correlated with the degree of neuroinflammatory process in neocortex, in mice with bilateral bilobectomy olfactories [23]. Some authors have demonstrated by a similar model of AD that olfactory bulbs protect the neurons of neocortex and hippocampus from toxic aggregates of β-amyloid peptides at the morphological level and prevent memory loss [24].

Thus, the specific reactivity of the cytokine system of the brain sections of rats discovered while examining highlights the functional mechanisms of their interaction.

Competing interests

Authors have declared that no competing interests exist.

Table-1: Comparative analysis of the content of cytokines in the brain sections of intact rats

Brain sections Cytokine	Neocortex	Hippocampus	Olfactory bulb	Hypothalamus	Caudate nucleus
TNF α (ng/g protein)	56.5±3.2[a,b]	74.9±5.9[a,b]	66.3±6.5[a,b]	94.0±11.4	110.5±15.9
Interleukin-6 (ng/g protein)	51.7±3.8[a,b]	67.0±5.7	62.2±5.7[a]	76.6±8.6	74.7±6.7
Interleukin-10 (ng/g protein)	181.3±26.8[a,b]	200.9±13.5[a,b]	178.9±13.5[a,b]	259.7±20.2	241.3±17.8
Values are expressed as mean ± SEM (n= 8 rats/ group), [a] – $p \leq 0.05$ when compared with the indicators of cytokines in the hypothalamus; [b] – $p \leq 0.05$ when compared with those in the caudate nucleus					

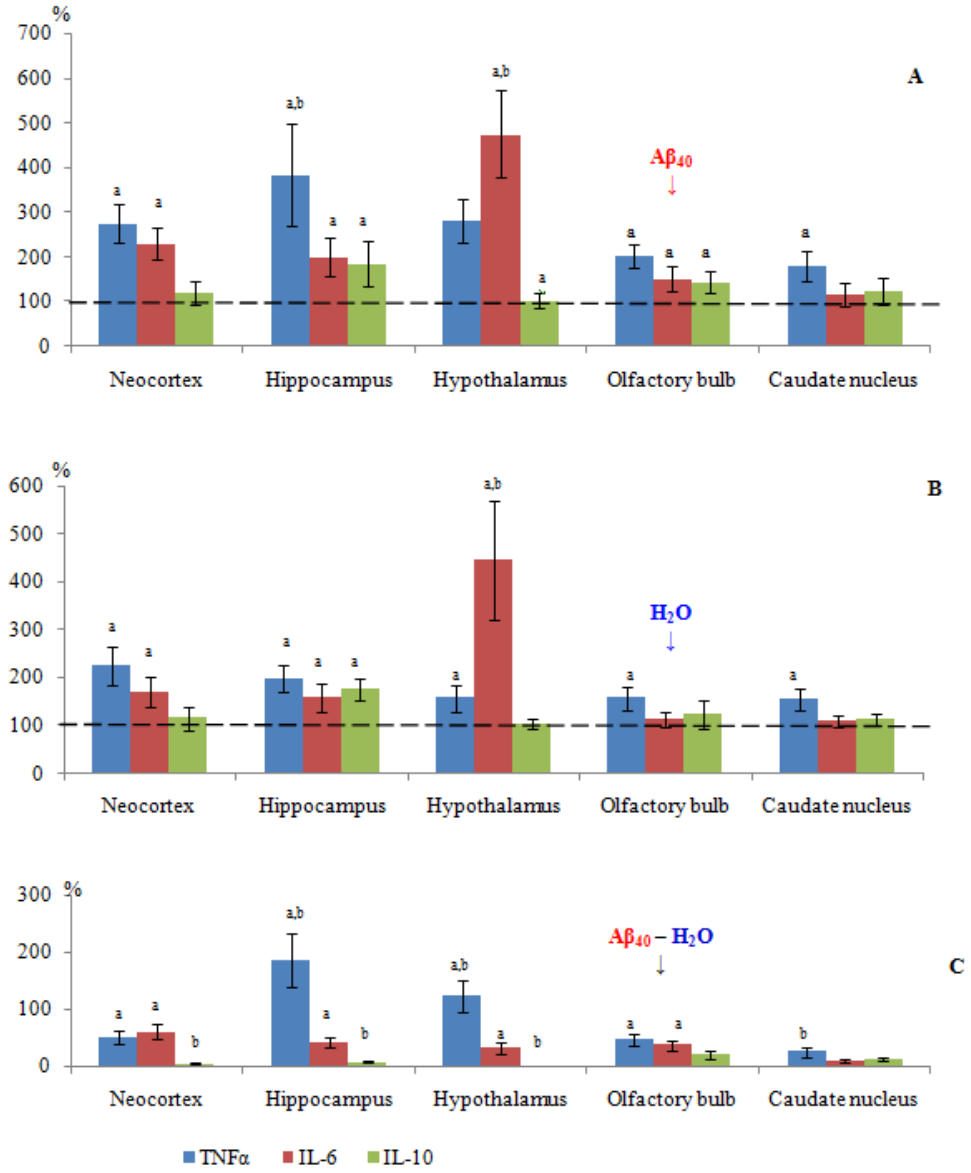

Fig-1: Reactivity of the system of cytokines in the brain sections of male rats 18 months of age 5 days after the intrabulbular administration of the Aβ$_{40}$ aggregates (A); H_2O (B) and the specific anti-inflammatory effect of Aβ$_{40}$, as the difference between these two factors (C).

Data are presented as a percentage of baseline intact animals (Table-1). [a] – $p \leq 0.05$ when compared with the corresponding indicators of the reactivity of the system of cytokines in intact animals (100 %); [b] – $p \leq 0.05$ when compared with the indicators of reactivity of the system of cytokines in olfactory bulbs

CONCLUSIONS

- The initial level of the activity of the cytokine system in the brain sections that are relevant to the olfactory analyzer (neocortex, hippocampus and olfactory bulbs) of elderly rats is significantly lower in comparison to other sections (hypothalamus and caudate nuclei).
- Intrabulbar injection of Aβ$_{40}$ to elderly rats increases the activity of the cytokine system in the brain structures remote from the injection spot (neocortex and hippocampus) relevant to the memory mechanisms.
- The reactivity of the cytokine system in neocortex, hippocampus and hypothalamus is higher not only in response to the specific toxic effect of Aβ$_{40}$ aggregates, but also under the conditions of surgical intervention with a bulbar injection of solvent alone.
- The generally low content of pro- and anti-inflammatory cytokines in the phylogenetically younger brain sections (neocortex, hippocampus) was consistent with the increased reactivity of the cytokine system under the influence of the exogenous β-amyloid peptide 40.

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