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CELL BIOPHYSICS

Fullerenes C_{60}, Antiamyloid Action, the Brain, and Cognitive Processes

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Dedicated to the memory of a remarkable scientist and humanist Levon Mikhailovich Chailakhyan

Abstract—A short review of investigations along a new line: the antiamyloid action of fullerenes C_{60} and correction of disturbed cognitive processes is presented. The prospects for the development of drugs based on fullerenes acting on the key molecular mechanisms at the early stage of Alzheimer's disease are discussed.

Key words: fullerenes C_{60}, antiamyloid action, neuron, memory, Alzheimer's disease, neurodegenerative diseases

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INTRODUCTION

Fullerenes C_{60} are carbon nanoparticles with unique physicochemical and biological properties. Investigation of fullerenes appears as one of the leading directions of nanobiotechnology and nanomedicine. The action of fullerenes on β-amyloids, neurons and cognitive processes is a new problem that throws a bridge from nanotechnology to neuroscience.

Buckminsterfullerene (for short, fullerene C_{60}) consists of 60 atoms of carbon positioned at the vertices of regular hexagons and pentagons forming a symmetrical hollow sphere of less than 1 nm in diameter. The carbon atoms are connected between themselves by conjugated double bonds creating on the entire surface of the molecule a unified system of nonlocalized π-electrons.

Fullerenes were discovered by H. Kroto, R. Smalley and R. Curl in 1985. In 1996 the authors were awarded the Nobel Prize in chemistry. The captivating story of the discovery of this "star molecule" is presented in the Nobel lecture of R. Kroto [1].

Hydrophobicity, spherical shape of the molecule, unusual redox properties allowing attachment of up to six electrons, and low toxicity stimulate the investigation of the biological properties of this surprising molecule [2–4]. One of the main biological properties of fullerene C_{60} is the ability to quench free radicals, to behave as a "sponge of free radicals." Application of this property is prevented by the exceptionally low solubility of C_{60} in water and aggregation of its nanoparticles. Dissolution strongly affects the quenching of reactive oxygen species (ROS), and this is necessary to be taken into attention during characterization of various preparations and evaluation of their action [3, 5].

One of the properties of fullerenes is permeability through model lipid membranes exceeding all other molecules [6, 7].

Recently started was the introduction of nanotechnology into neuroscience, which is rapidly developing [8, 9]. One of the promising directions is the investigation of the mechanisms of the neuroprotector action of fullerenes and the possibility of developing on their basis medications acting on the key molecular mechanisms of neurodegenerative diseases [8, 10].

ANTIAMYLOID ACTION OF FULLERENES

In recent years a strong influence has been disclosed of nanoparticles on aggregation of the amyloid

[1] *Abbreviations*: C60, fullerene; ROS, reactive oxygen species; Ab, amyloid b-peptide; FWS, colloidal water suspension of fullerene; AD, Alzheimer's disease; NMDA, N-methyl-D-aspartate; AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; LTP, long-term potentiation; PS, population spike; PVP, polyvinyl pyrrolidone; AIDS, acquired immune deficiency syndrome; HF, high frequency.

[2] *Editor's Note*: This text is a meticulously prepared equivalent of the original Russian publication with all its factual statements, terminology, phrasing and style, so the reader may more clearly recognize the major problems with this area of scholarly activity.

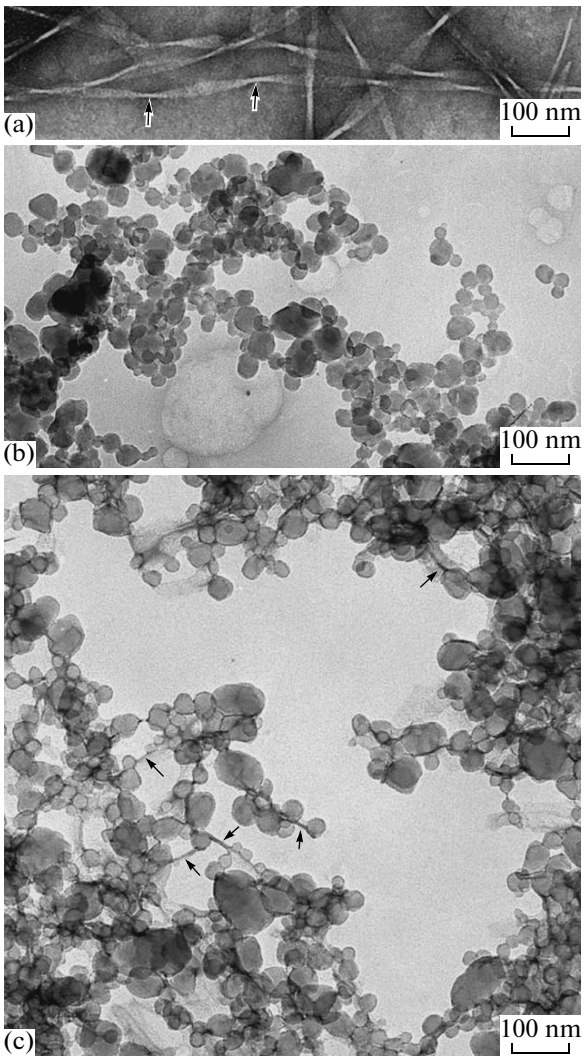

Fig. 1. Electron micrographs of aggregated $A\beta_{25-35}$-peptide, colloidal water solution of fullerene (C_{60} FWS) and $A\beta_{25-35}$-peptide upon addition of C_{60} FWS. $A\beta_{25-35}$-peptide incubated for 24 h at 37°C. Helically twisted ribbon fibrils of 26 nm diameter (shown with arrows) (a). Spherical aggregates of C_{60} FWS of 5–40 nm diameter and their conglomerates (b). Incubation of $A\beta_{25-35}$-peptide with C_{60} FWS at a molar relationship 6:15. All fullerene aggregates are bound with short protofibrils of Aβ-peptide, shown with arrows (c). Scale, 100 nm [16].

β-peptide [11–14]. The first work was performed by Kim and Lee in 2003. The authors showed that in a water solution the 1,2-(dimethoxymethano)fullerene quenched the fluorescence of thioflavin T bound with amyloid β-peptide (1–40) ($A\beta_{1-40}$). The effect was significantly stronger pronounced than in other inhibitors of Aβ aggregation [15]. The authors supposed that the fullerene binding with the hydrophobic region of $A\beta_{1-40}$ (motif KLVFF), precluding aggregation of monomers. Recently we have for the first time by a visual method (with the aid of highly resolving electron microscopy) shown the strong influence of fullerenes C_{60} on amyloidogenesis of Aβ-peptides. In the experiments in vitro addition of fullerene leads to decoration of amyloid fibrils by small spherical aggregates of fullerene. A colloidal water suspension of fullerene (C_{60} FWS) added before formation of mature amyloid fibrils (helically twisted ribbons) of $A\beta_{25-35}$-peptide prevented their formation. The fullerene destroyed as well the peptide-formed fibrils (see Fig. 1) [16], and exerted the same action on fibrils of $A\beta_{1-42}$ [17]. Beside that, it was shown that C_{60} FWS and a polycarboxyl derivative of fullerene, $C_{60}Cl(C_6H_4CH_2COONa)_5$, destroyed mature amyloid fibrils of muscle X-protein and prevented formation of new fibrils [18]. Our data allow one to suggest that amyloid peptides represent the target of fullerene action. It should be underscored that strong antiamyloid action is exerted by small aggregates of fullerene and its water-soluble derivatives. It appears that hydrophobic interactions of fullerenes with amyloid β-peptides and X-protein fibrils lie in the basis of their antiaggregation action. Our data allow one to suggest that amyloid peptides represent the target of fullerene action. Of great interest is the investigation in vivo of the action of fullerenes on β-aminoloids. This work has been just initiated by us.

The influence of nanoparticles on β-amyloids causes great interest. Thus in work [11] during conduction of experiments in vitro it was shown that polyethylene glycol phospholipid nanomycelles destroyed fibrils of $A\beta_{1-42}$. In distinction from this, nanoparticles of titanium dioxide strengthened the aggregation of $A\beta_{1-42}$, causing amyloidogenic action [12]. The authors suggested that certain nanoparticles can be an etiological factor of the spontaneous form of Alzheimer's disease (AD). The causes of the spontaneous form of AD are unknown. The supposition about the role of certain nanoparticles in the etiology of AD is the subject of further investigations.

Interaction of proteins and nanoparticles is a highly specific process depending on the properties of the surface of proteins and nanoparticles. Of critical significance is the curvature of the nanoparticle surface. This hypothesis explains the mechanism of the different influence of nanoparticles on the formation of Aβ fibrils [13, 14].

INFLUENCE OF FULLERENES ON BRAIN NEURONS

What action do fullerenes exert on brain neurons? On a culture of rat embryo brain neurons, polyhydroxylated fullerenes fullerenols ($C_{60}(OH)_{18}$) suppressed the binding of subtypes of ionotropic glutamate receptors: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate ones and lowered the level of intracellular calcium [19]. In sections of rat hippocampus a colloidal water suspension of fullerene C_{60} (C_{60} FWS) in which both separate molecules and associates thereof were present [20], at a low concentration (7×10^{-6}, $7 \times$

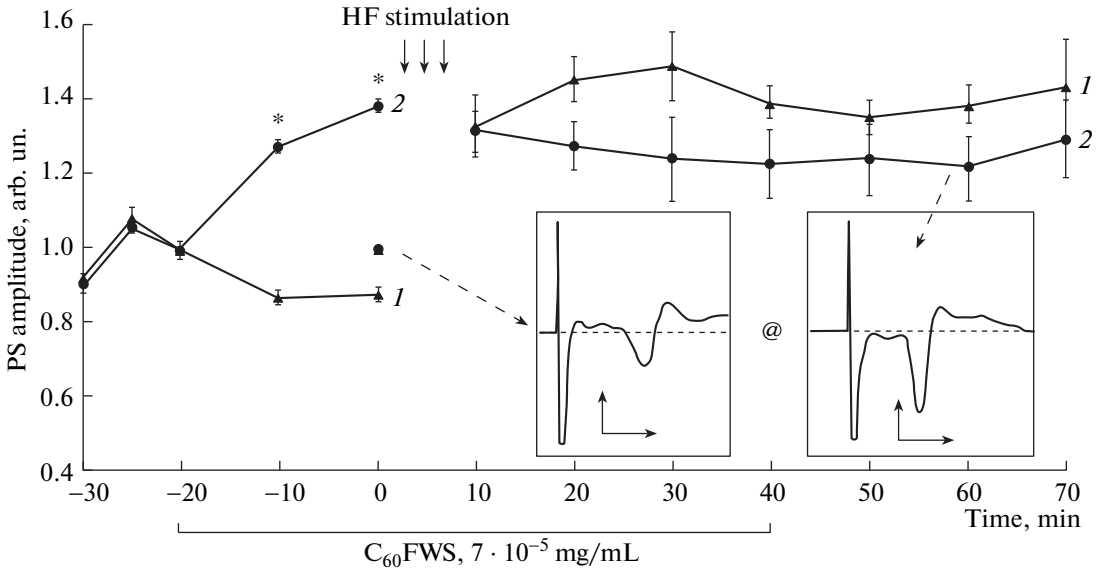

Fig. 2. Dynamics of the change in the amplitude of the population spike (PS) (normalized values) in control (*1*) and under the action of C_{60} FWS (*2*). Shown are the mean value ± standard error ($n = 4$). The insets present examples of PS before and after high-frequency (HF) stimulation. The line shows the time of C_{60} FWS introduction. *Significant difference from control before and after HF stimulation, $p < 0.05$ [21].

10^{-5} mg/mL) significantly raised the activity of pyramidal neurons, the basic cell elements of the hippocampus, without disturbing the development of long-term potentiation (LTP) (see Fig. 2). At a higher concentration (7×10^{-3} mg/mL) the fullerene did not influence the activity of pyramidal neurons and suppressed LTP [21]. The genesis of the population spike (PS) evoked by stimulation of glutamatergic synaptic inputs from Schaffer collaterals depends both on activation of postsynaptic AMPA receptors of glutamate and on potential-dependent Na^+ channels of pyramidal neurons. A factor of initiation of the development of LTP of synaptic transmission in the CA1 field of the hippocampus is the activation of NMDA receptors [22]. We suppose that nonmodified fullerene exerts another action than polyhydroxylated fullerenes and at low concentration, without influencing the activity of NMDA receptors, is capable of either selectively raising the efficiency of transmission of the synaptic signal mediated by AMPA receptors or enhancing activation of potential-dependent Na^+ channels of postsynaptic pyramidal neurons. Further investigations of this question are required.

ROS and glutamate excitotoxicity represent the leading factors of the pathogenesis of many grave and widespread brain diseases: neurodegenerative diseases, brain circulation disorders, epilepsy [23]. In hippocampal sections, hydrogen peroxide and cumene hydroperoxide reversibly suppressed the amplitude of the PS of pyramidal neurons in the CA1 field. Introduction of fullerenol at low concentration (0.1 mM) prevented the damaging action of ROS. Fullerenol restituted the synaptic conductivity at a concentration an order of magnitude lower than deferoxamine, an iron chelator [24]. On a culture of cortical neurons it was shown that carboxyfullerenes lowered the excitotoxicity caused by stimulation of NMDA and AMPA receptors and suppressed apoptosis caused by $A\beta_{1-42}$ [10]. On a culture of phenochromocytoma neurons, fullerenol at a concentration of 0.1–1.0 μM decreased the level of free calcium in the cytosol elevated by $A\beta_{25-35}$, a neurotoxic fragment of $A\beta_{1-42}$ [25].

Thus, in experiments in vitro fullerene elevated the activity of pyramidal neurons—the basic cell elements of the hippocampus. On a culture of neurons and hippocampal sections the fullerene derivatives (fullerenol and carboxyfullerene) exhibited antioxidant action and lowered the neurotoxic action of β-amyloids. One of the cell targets of fullerene action appear to be the ionotropic glutamate receptors.

INFLUENCE OF FULLERENES ON THE BRAIN AND DISORDERS OF MEMORY

Nanoparticles during nasal respiration, bypassing the hematoencephalic barrier, through the olfactory nerves penetrate into the brain [26]. Nanoparticles interact with amyloid proteins, glutamate ionotropic receptors and neuronal membrane. Therefore it is of great interest to investigate the influence of fullerenes introduced into the brain on the behavior and cognitive processes in the norm and on the models of brain pathology. A single intraventricular (i/v) administration of carboxyfullerene at a high concentration

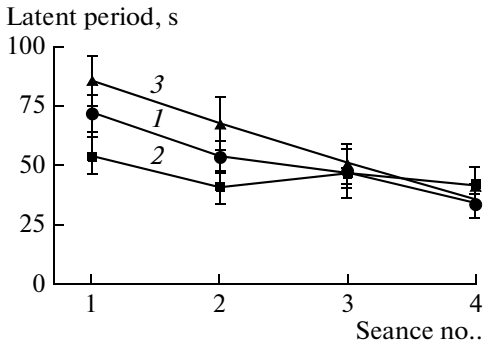

Fig. 3. Influence of microinjection of C_{60} FWS into lateral ventricles of the brain on cognitive processes (rapid formation of spatial memory at random position of invisible target) [16]. Latent period, the time of solving a probabilistic spatial problem. Fullerene at concentration of 3.6 and 7.2 nmol/20 μL/ventricle (curves *1* and *2* corespectively) did not disturb spatial learning and solving the probabilistic spatial problem; control, 0.9% solution of NaCl 20 μL/ventricle (curve *3*). Five tests in each shance.

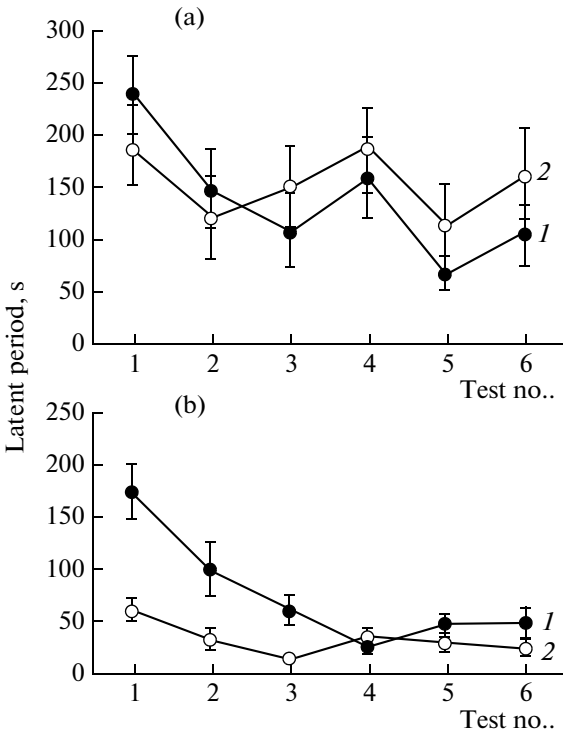

Fig. 4. Influence of bilateral intrahippocampal microinjection of PVP (a) and fullerene (C_{60}/PVP) (b) on spatial memory disturbed by intraventricular introduction of cycloheximide, inhibitor of protein translation. Experiments performed in a Morris aquatic labyrinth. Training conducted in one seance of 5 min duration consisting of six tests (curve *1*). Checking the preservation of information was performed by repeated learning after 24 h (curve *2*). Cycloheximide (200 μg/20 μL/ventricle) disturbed the preservation of information (nor shown). It is seen that PVP did not prevent amnesia caused by cycloheximide (a), C_{60}/PVP at a concentration of 1.7 μg/1 μL/hemisphere completely abolished it (b) [29].

(4.5 mM in 20 μL) did not cause a disturbance in rat behavior. An increase of the concentration by three times led to convulsions and death [27]. Introduction of fullerene into lateral ventricles of the brain increased the locomotor activity and elevated the rate of turnover of neuromodulators (serotonin and dopamine) in brain structures. In distinction from this, intravenous administration of fullerene did not cause such action. This is explained by that the fullerene poorly penetrates through the blood–brain barrier [28]. We showed that a single administration of fullerene into brain ventricles and hippocampus did not disturb cognitive processes (Fig. 3) [16, 17]. On the basis of these data it can be concluded that a single introduction of fullerenes into the brain does not cause acute neurotoxic action. However, this is only the very beginning of investigations. It is important to investigate in detail how the brain is influenced by chronic administration of various compounds of fullerenes. We have for the first time studied the influence of fullerene on the disturbance of memory in animals caused by deep suppression of protein synthesis in the brain. Suppression of protein synthesis is a classical model of disturbance of formation of long-term memory [31]. The hippocampus plays a key role in memorizing events, facts, space and time [32]. We found that intrahippocampal microinjection of a complex of C_{60} with polyvinyl pyrrolidone (C_{60}/PVP) prevented the disturbance of spatial memory in rats caused by a high concentration of cycloheximide, a blocker of protein translation (Fig. 4) [29, 30]. The mechanism of this effect is unknown. According to a computer model, fullerene can absorb cycloheximide, decrease the suppression of protein synthesis and as a result of this prevent memory disturbances [33]. However, other explanations are also possible. We have planned conduction of experimental investigations of the mechanisms of this interesting effect.

According to our preliminary data, intraventricular introduction of C_{60} FWS restituted protein synthesis in the pyramidal neurons of the hippocampus in 20% of rats and weakened the disturbance of spatial memory caused by introduction of $A\beta_{25-35}$ [17].

The investigation of the influence of nanoparticles on the disturbance of cognitive processes has recently found further development. Chronic peroral administration of an antioxidant carboxyfullerene, which acts as a mimetic of superoxide dismutase, significantly weakened the oxidative stress, prevented the disturbance of spatial memory in old mice and increased their life duration [34].

We suppose than in the nearest years the investigation of fullerene action on behavior and cognitive processes will find great development [35].

DEVELOPMENT OF FULLERENE-BASED DRUGS FOR THERAPY OF THE EARLY STAGE OF ALZHEIMER'S DISEASE

Alzheimer's disease is a primary neurodegenerative disease of people of advanced and old age. It afflicts more than 24 million people in the world. This disease is characterized by steady deterioration of memory up to complete disintegration of intelligence and psychic activity. The neurotoxic action of soluble $A\beta_{42/43}$ oligomers and their fibrils leads toward death the synapses and neurons in the hippocampus, neocortex and other parts of the brain [36, 37]. Modern drugs temporarily improve the memory and weaken the dementia. However, there are no means that can stop or cause a reverse development of the destructive neurodegenerative process. Development of antiamyloid drugs represents one of the most active directions in the therapy of Alzheimer's disease [36, 37].

A promising field of investigations has appeared—development on the basis of nanotechnology of drugs for treating neurodegenerative diseases [10, 16, 17, 39, 40, 43]. An important problem is penetration of nanoparticles through the blood–brain barrier. Recently synthesis has been realized for carbon nanomaterials penetrating the blood–brain barrier. Clinical trials of these compounds are conducted [39].

In the literature it is customary to explain the neuroprotector action of fullerene by its ability to quench oxygen radicals and cause antioxidant action [5, 8, 10, 40]. International pharmaceutical companies such as C. Sixty and Merck Co., using fullerenes, develop antioxidants for therapy of neurodegenerative diseases, including AD [40]. However fullerenes are molecules of multipurpose action, and this significantly expands their possible application in medicine [41]. Our data have allowed a suggestion that β-amyloids and amyloid proteins represent a molecular target of the action of fullerenes C_{60}. Investigations of the antiamyloid action of fullerenes may lead to development of a new direction in AD therapy [11–18]. Owing to the combination of antioxidant and antiaggregation activity, fullerenes may prove helpful also in the development of neuroprotective drugs for therapy of neurodegenerative diseases.

Of interest is one more kind of fullerene activity. Derivatives of fullerenes are inhibitors of the aspartyl protease of the AIDS virus [2, 4]. The β- and γ-secretases, as a result of the activity of which $A\beta_{42/43}$ is formed, belong to the group of aspartyl proteases, apparently universal for various cellular systems and organisms [37]. The question of whether fullerenes inhibit γ-secretase remains open.

CONCLUSIONS

The interdisciplinary investigation of the action of fullerenes on molecular and cellular mechanisms of neurodegenerative diseases and disturbance of cognitive processes is a new fundamental problem of neuroscience, nanobiotechnology and nanomedicine. Further study of the antiamyloid ability of fullerenes will make a substantial contribution into the understanding of the mechanisms of their neuroprotector action and influence on the disturbances of cognitive processes. These investigations present great interest for constructing nanodrugs for therapy of the early stage of AD and other neurodegenerative diseases. It is principally important that in Russia conditions be created for investigation of the neuro- and psychotropic activity of fullerenes and development of therapy of neurodegenerative diseases on their basis.

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