Design of a hybrid nanostructure based on fullerene C_{60} and biologically active substance for modeling physiological properties of compounds*

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The effects of cognitive-stimulating substance BD-2 of the γ-carboline family and a hybrid compound based on fullerene C_{60} and attached BD-2 on various aspects of the behavior of animals were studied. The synthesized hybrid fullerene compound (HFC) has no side psychostimulating effect characteristic of BD-2 but fully retains the properties of a cognitive-stimulating agent. The design of hybrid compounds based on fullerene C_{60} and pharmacologically active groups can be one of the ways for optimizing therapeutically promising compounds.

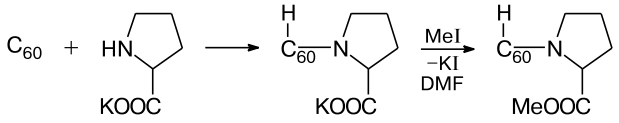
Key words: water-soluble fullerene derivatives, antioxidant activity, glutamate AMPA receptors, cognitive stimulators.

Fullerenes are known to possess unique spatial and electronic structures causing their expressed donor—acceptor and photophysical properties. When addends added to the fullerene spheroid are varied, derivatives of fullerene C_{60} gain amphiphilic and stereospecific membranotropic properties and also exhibit antioxidant and antiviral activity.[1—3] Due to this properties, fullerene C_{60}, on the one hand, is considered in chemical pharmacology as a carrier of functional groups with biological activity and, on the other hand, fullerene itself possesses neuroprotective activity.[4] *N*-(Monohydrofullerenyl)substituted amino acids or peptides,[5] products of equimolar addition of amino acids or peptides to fullerene, compose a special class of organic compounds that can be considered as potential metabolites of a certain subclass of physiologically active substances.

Amino acid fullerene C_{60} derivatives (AFDs) were synthesized as described earlier[5] (Scheme 1).

Fullerenylproline and its derivatives should be emphasized among AFDs. It is of interest to study the influence of the fullerene spheroid on the key role of proline derivatives in the determination of the direction of the polypeptide chain.[6,7] This property of fulleroprolines is related to their biological activity mediate by the inhibition of cysteine and serine proteases.[8] Therefore, design of hybrid fullerene-based nanostructures for therapy of neurodegenerative diseases seems promising. These nanostructures are the products of addition to the fullerene spheroid of two addends, one of which is amino acid providing the solubility of the nanostructures in water, and another is a substance with neuroprotective and/or cognitive-stimulating activity and promising as a therapeutic drug.

Scheme 1



Experimental

All products of the synthesis, except the proline derivative, are amorphous powders with a moderate solubility in water (C = 0.05—0.15 g dL^{-1}) with the formation of associates, whose

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shape and number depend on the hydrophilic—lipophilic balance of the amino acid fullerene derivative, pH, and ionic strength of the solution.[5,9] The content of amino acid in the products was studied by HPLC.[9]

The IR spectra of the synthesized AFDs (KBr pellets) contain intense absorption bands in the ranges 1590—1620 and 1350—1420 cm^{-1} assigned to asymmetric and symmetric stretching vibrations of the carboxyl group (COO^-, $\nu_{as}(COO^-)$, and $\nu_s(COO^-)$).[10] This proves that an ADF molecule includes a zwiterionic form with the ionized carboxy group. In addition, the IR spectra of thus obtained ADFs are characterized by broad absorption bands with a maximum about 3200 cm^{-1}. They possibly belong to stretching vibrations of the N—H bonds of the ammonium group in the zwitterions or of the amino group in the non-ionized amino acid.

A characteristic feature of the IR spectra of ADFs is the presence of three absorption bands: near 1108 (strong intensity), and at 960 and 840 cm^{-1} (medium intensity). These bands are present in the spectra of all ADFs, and their frequencies and intensities depend slightly on the addend structure in the ADF, which makes it possible to assign them to vibrations of bonds of the addend atoms with the fullerene spheroid (C_{60}—N and C_{60}—H). The position of the stretching vibration band of the C_{60}—H bond (hydrofullerenyl) is not quite unambiguous, because after the addition of the hydrogen atom to the spheroid the hybridization of the corresponding carbon atom of fullerene changes from sp^2 to sp^3. Probably, the observed shift of the stretching vibration band $\nu(C_{60}—H)$ to a sufficiently broad range of 2900—2920 cm^{-1} can be due to the change in hybridization.

The analytical data on the structure of the AFDs suggest that they are products of monoaddition of amino acids to fullerene, *viz.*, *N*-(monohydrofullerenyl)amino acids, and the quantum chemical calculations confirm this assumption.[11] It is known that hydrogen in the $C_{60}H$ moiety of the fullerene derivatives has proton mobility[12] caused by the electronegativity of the fullerene spheroid. This results in a high polarization of the C—H bond and predominant directivity of S_E2 electrophilic substitution at this bond, which is confirmed by the quantum chemical calculations.[13]

Substitution of hydrofullerenyl hydrogen significantly extends the possibility of directed synthesis of hybrid AFDs covalently bound to proteins, peptides, chromophores, and others. In this work, the hydrogen atom is replaced by a biologically active substance with neuroprotective activity: 5-benzyl-8-chloro-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole hydrochloride (BD-2).

Determination of the intensity of peroxide oxidation of lipid membranes. The intensity of peroxide oxidation of lipids (POL) in the homogenate of the rat cerebral cortex was estimated from the content of malonic dialdehyde (MDA) in 1 g of the tissue.[14] For the estimation of the effect of BD-2 and hybrid compounds based on fullerene C_{60} (HFC) on POL of the homogenate of the rat cerebral cortex, HFC (0.25 mL, 10^{-4} mol L^{-1}) in the K,Na-phosphate buffer was added to the homogenate (2.25 mL) in the same buffer (pH 7.4) to the final concentration of the compound in the sample equal to 10^{-5} mol L^{-1}, and the sample was incubated for 30 min at 37 °C. To the obtained sample, 17% trichloroacetic acid (1 mL) was added, and the solution was centrifuged for 10 min at 3000 *g*. The samples (2 mL of supernatant) were added with 1 mL of 0.8% thiobarbituric acid, and sample was incubated in a water bath for 30 min at 100 °C and cooled to room temperature, and the absorbance was detected at the wavelength 532 nm.

The concentration of MDA was calculated by the formula $K = (A_1 - A_2)/\varepsilon$, where K is the MDA concentration, A_1 and A_2 are the absorbances of the tested sample and buffer solution, respectively, and ε is the molar absorption coefficient equal to $1.56 \cdot 10^5$ L mol^{-1} cm^{-1}. The obtained value was divided by the weight of the tissue (rat cerebral cortex) from which the homogenate was prepared.

Determination of the activity of monoaminoxidases A and B (MAO-A and MAO-B). The enzymatic activity of MAO-A and MAO-B in a suspension of mitochondria of the rat cerebral cortex was determined using a known procedure[15] based on the spectrophotometric determination of the amount of ammonia evolved due to the enzymatic deamination of biogenic amines, serotonin and benzylamine, by membrane-bound enzymes MAO-A and MAO-B. The substrate in the final concentration 10^{-3} mol L^{-1} and 0.2 mL of the studied compounds in a concentration of $5 \cdot 10^{-5}$ mol L^{-1} (final concentration in the sample 10^{-5} mol L^{-1}) were added to a sample of a suspension of mitochondria containing 5 mg of the protein, and the volume was brought to 1 mL with a 0.1 *M* K,Na-phosphate buffer (pH 7.4). The sample were incubated at 37 °C for 1 h, after which the substrate reaction was stopped by the addition of 0.1 mL of 50% trichloroacetic acid, and the sample was centrifuged at 4500 *g* for 20 min. The supernatant was placed in tubes, and a saturated solution of K_2CO_3 (2 mL to each tube) was added to the samples to acidify the medium. The tubes were closed with ground stoppers with glass sticks inside (one droplet of 0.5 *M* sulfuric acid was at the end of the stick), and the tubes were kept at room temperature. In 12 h, the sticks with droplets containing ammonium sulfate (evolved due to the neutralization reaction) were placed in 4.5 mL of distilled water. Nessler´s reagent (0.5 mL) was added to the tubes, and the absorbance of the solutions was detected on a Specord M40 spectrophotometer (Germany) at $\lambda = 430$ nm during 30 min. The concentration of evolved ammonia was determined from the calibration plot. The obtained value corresponded to the activity of the enzyme. To determine the specific activity, this value was divided by the amount of protein in the sample. The amount of protein was determined using the Lowry method.[16]

Effect of HFC and BD-2 on AMPA receptors. The effect of HFC and BD-2 on AMPA receptors (AMPA is α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, a subtype of glutamate receptors of the central nervous system of mammals) was studied by the patch-clamp electrophysiological method.[17] Single isolated Purkinje neurons were isolated from cerebellum of Wistar rats 12—16 days aged using the Kaneda method.[18] The section cuts of the cerebellum 400—600 μm thick were placed in a 10-mL temperature-controlled chamber in a solution (pH 7.42) of the following composition: 150 m*M* NaCl, 5 *mM* KCl, 2 m*M* $CaCl_2$, 2 m*M* $MgSO_4 \cdot 7\,H_2O$, 10 m*M* HEPES buffer, and 15 m*M* glucose and were incubated at 34 °C. After 60 min, the buffer was replaced by a similar solution but containing pronase (2 mg mL^{-1}) and collagenase (1 mg mL^{-1}) and incubated for 70 min at 34 °C. After washing with the starting buffer for 20 min, the section cuts were placed in a Petri dish and separated by the mechanical method using a Pasteur pipet. In the course of neuron isolation, the solutions were continuously purged with 100% O_2 at 34 °C. The studied neurons were placed in the 0.6-mL working chamber in buffer A (pH 7.36) of the following compo-

sition: 150 m*M* NaCl, 5 m*M* KCl, 2.6 m*M* $CaCl_2$, 2 m*M* $MgSO_4 \cdot 7H_2O$, 10 m*M* HEPES buffer, and 15 m*M* glucose.

Transmembrane currents are caused by the activation of the AMPA receptors using application of solutions of the agonist of these receptors (kainic acid) by the method of fast superfusion of solutions: the buffer (30 μL) with the agonist (the concentration of the agonist was varied in the range 10^{-4}—10^{-3} mol L^{-1}) was introduced at an interval of 2 min with a constant rate to the buffer washing neurons. The currents were detected using borosilicate microelectrodes (resistance 2.5—5.5 MOhm) filled with a buffer (pH 7.2) of the following composition: 140 m*M* CsCl, 11 m*M* ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*,*N*-tetraacetic acid, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 10 m*M* HEPES buffer, and 5 m*M* adenosine triphosphate.

To study the effect of HFC on the AMPA receptors, buffer A washing neurons was replaced by the same buffer but containing the studied compounds in concentrations of 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} mol L^{-1}.

An EPC-9 instrument (HEKA, Germany) was used for detection. The currents were detected on a computer using the Pulse license program (HEKA). The results were processed using the Pulsefit program (HEKA).

Study of the behavior effects of BD-2 and HFC on laboratory animals with a TruScan system. The purpose of this study is a comparative investigation of the effect of BD-2 and HFC on the motor activity of mice performed in the open field test.

The research model chosen for the solution of the stated problem is highly informative and is excellently suitable for the primary evaluation of psychotropic activity of new chemical compounds. The open field test was modified[19] under the conditions of automated monitoring of motor activity and orientational behavior of animals in the TruScan photosensor system (Coulbourn, USA).

The TruScan system is a square chamber with a base of 30×30 cm made of transparent organic glass with a wall height of 35 cm. The gray-colored floor was conventionally divided into 64 squares and had 16 holes with a diameter of 20 mm. This computerized positioning system allows one to monitor the motion of an animal in three axes. This makes it possible to reveal 62 parameters of the locomotor activity of an animal and to determine activity of a chemical compound immediately for several aspects of the behavior of mice, of which four main aspects were distinguished: motor activity, anxiety, orientational behavior, and awareness reaction.

Prior to the experiment (1 h before), the mice were transferred to the experimental room. The studied substance was administered 1 h before testing. Then, the animals were placed in the far left corner of the TruScan system, where the parameters of vertical and horizontal activity were detected within 3 min. The irradiation intensity of the experimental chamber was 130 lx at the floor level. The parameters of the locomotor function of the mice were estimated according to the following 14 parameters: overall number of motions, motor activity (s), stationary moment (s), motion distance (cm), velocity of motion (cm s^{-1}), distance in perimeter (cm), time in perimeter (s), distance in center (cm), time in center (s), number of visits to center, number of vertical stands, time of stands (s), number of studied holes, and time of burrowing reflex (s). Such a wide set of parameters makes it possible to completely evaluate the parameters of motor activity of mice that were stated in this work.

The psychotropic activity of the studied substance is presented in the diagrams as a percentage of deviation (*D*) from the parameters of the control group, which was calculated by the formula $D = (M_1 - M_2)/M_1 \cdot 100\%$, where *M* is the parameter averaged over the group (M_1 is experiment, and M_2 is control).

The studied substances were administered in the dose range from 0.001 to 0.1 mg kg^{-1} intragastrically based on 0.1 mL of the solution per 10 g of the body weight.

The random data processing was performed using the Statistica 6.0 standard program, and the reliability level was determined by the Student *t*-criterion.

Investigation of the effect of BD-2 and C_{60}—Pro—BD-2 on the memory of animals in the test "recognition of new localization of known object." The test of recognition of an object is based on the fact that both rats and mice spontaneously investigate a new object or a new localization of an object. This test was first applied to rats.[20] Further, independent researchers showed that this test is also appropriate for testing recognition memory of mice.[21]

The experimental system was an observation chamber of white non-transparent organic glass 48×38×30 cm in size. Brown-colored glass bottles with a diameter of 2.7 cm and a height of 5.5 cm served as objects of the study. Two—three minutes before the animal was placed in the chamber, the chamber and investigation objects were weared through with 85% ethanol. Animals were always placed at the center of the chamber.

Familiarization with the behavior chamber. On the first day, the mice were transferred to the research room and acclimatized for 20—30 min. Then, each animal was placed for 10 min in an empty, pre-treated with ethanol behavior chamber for familiarization. Then, the animal was caged and brought to vivarium.

Training. On the next day, the same mice were brought to the research room and acclimatized for 20—30 min, and then a solution of the studied substance was injected intragastrically. One hour after administration, the animal was placed in the behavior chamber, on the bottom of which two same objects (glass bottles) for recognition were placed along the diagonal at a distance of 14.5 cm from the corners. The duration of training for each animal was 15 min. In 15 min, the animal was caged and returned to vivarium.

Testing was carried out in 48 h after training. For this purpose, the animal after acclimatization was placed in the chamber for 1 min for repeated familiarization. The animal was removed in 1 min, and one object was put on the floor of the chamber in the localization known for the animal and another object was put in a new localization. The awareness time of each object (separately for 10 min) was detected using two electronic stopwatches (with the accuracy to 0.1 s). The behavior of the animals was observed using a mirror. The positive reaction of testing was considered to be the purposeful approach of the animal nose to the object at a distance of 2 cm or a direct touch of the object with the nose.

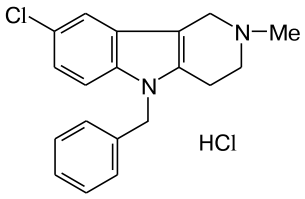
Experimental animals were male mice of the C57Bl/6 line. Prior to experiments, the animals were placed in a fortnight quarantine. The mice were kept under standard vivarium conditions with the free access to food and water and 12-h light cycle (8.00—20.00). At the moment of experiment, the weight of the mouse body was 22—24 g. Each experimental group, including the group of control animals, contained 10 animals.

Random processing of results. Since significant oscillations of the research time is observed between animals, we calculated the percentage of the research time for each mouse by the formula $t_1/(t_1 + t_2) \cdot 100\%$, where t_1 is the research time of the object in the new localization, and t_2 is the research time of the

object in the known localization. The overall time spent to the study of two objects was accepted to be 100%. The further processing of the results were performed by the Student method using the *t*-test.

Results and Discussion

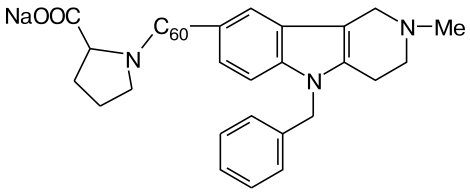
Antihistaminic drug dimebon is of considerable interest for researchers in the recent time in the area of neuropharmacology. Dimebon manifested a strong therapeutical effect at the second stage of clinical trials for people suffering from Alzheimer disease.[22] In spite of the fact that at the third phase of trials the clinical effect of dimebon was aligned by an unusually high therapeutical placebo effect,[23] the question about the possibility of the therapeutical application of this drug again became urgent after the discovery in 2012 of the ability of the drug to stimulate autophagy and neurogenesis.[24–26] Several highly active dimebon analogs of good pharmacological challenges have recently been obtained. In particular, the benzyl analogs, among which is BD-2, exhibit the neurotropic properties and were proposed as a basis for the design of promising neuroprotective agents.[27]



BD-2

The design of hybrid drugs based on similar compounds using various nanostructures is of significant interest for the optimization of pharmacological properties of these compounds.

In terms of this work, we studied some aspects of molecular mechanisms of neuroprotective, in particular, cognitive stimulating action of the hybrid fullerene nanostructure obtained by the addition of two addends to the fullerene spheroid. One of the addends was model amino acid proline (Pro) to impart solubility to the derivative, and compound BD-2 served as the second addend.



HFC

We have previously shown[28] that the hybrid structures based on fullerene C_{60} with the attached NO_2 groups or antioxidant moieties manifest the cognitive stimulating effect both *in vitro* and *in vivo*. The efficiency of the fullerene derivatives was considerably higher than that of the attached biologically active groups; however, the HFC almost were not toxic. In this work, neuroprotector BD-2 synthesized using a known procedure[29,30] was chosen as a biologically active compound (modulator of cognitive stimulating activity).

It is known that the important mechanisms of accomplishment of neuroprotective properties of the compounds are their ability to manifest the antioxidant activity and affect the catalytic properties of one of the key enzymes of monoaminoergic neurotransmission, monoaminooxidase.[3,31] Therefore, in this work, we also comparatively evaluated the influence of BD-2 and HFC on the peroxide oxidation of lipids and catalytic activity of MAO-A and MAO-B.

Antioxidant activity of HFC and BD-2 was estimated from a change in the MDA content in the homogenate of the rat brain (Fig. 1). It is seen that HFC C_{60}—Pro—BD-2 decreases the MDA concentration. Both compounds BD-2 and C_{60}—Pro have antioxidant activity; however, the HFC is more efficient inhibitor of POL.

Effect of the HFC and BD-2 on the catalytic activity of MAO-A and MAO-B. The results of studying the influence of the studied compounds on the catalytic activity of MAO-A and MAO-B are presented in Fig. 2. It was found that the addition of the HFC increases the catalytic activity of MAO-A, whereas compound BD-2, unlike the HFC, inhibits the enzyme. Since in the central nervous systems the main physiological function of MAO-A is the deamination of such neuromediator amines as noradrenaline and serotonin, the change in the activity of MAO-A in the presence of these drugs indicates an opposite effect of them on the processes of nervous excitation transfer.[32]

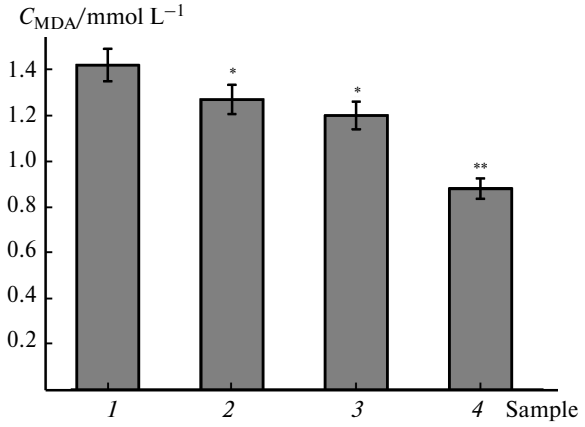


Fig. 1. Content of dimalonic aldehyde in the rat brain homogenate under the action of the studied compounds in a concentration of $2 \cdot 10^{-5}$ mol L^{-1}: *1*, control; *2*, BD-2; *3*, C_{60}—Pro; and *4*, C_{60}—Pro—BD-2. Signs * and ** mark the results at $p < 0.05$ and $p < 0.01$ relative to the control, respectively.

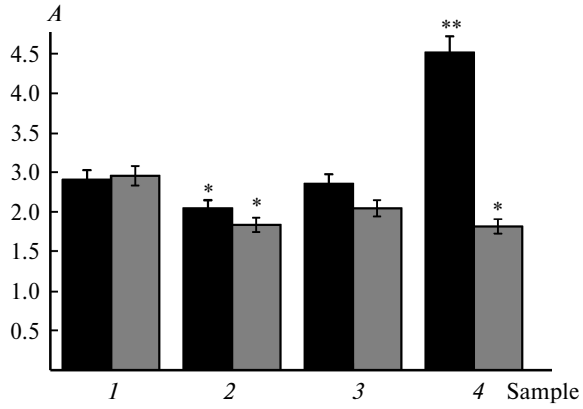


Fig. 2. Catalytic activity (A/(mmole of NH_3) (mg of protein)$^{-1}$) of MAO-A (dark columns) and MAO-B (light columns) in mitochondria of the rat brain under the action of the studied compounds in a concentration of $2 \cdot 10^{-5}$ mol L^{-1}: *1*, control; *2*, BD-2; *3*, C_{60}—Pro; and *4*, C_{60}—Pro—BD-2. Signs * and ** mark the results at $p < 0.05$ and $p < 0.01$ relative to the control, respectively.

As known, MAO-B is the key enzyme of dopamine receptors, and its inhibition makes it possible to prolong effects of synaptic dopamine, which substantiates the use of inhibition of MAO-B in therapy of neurodegenerative diseases.[33] As can be seen from Fig. 2, the hybrid structure C_{60}—Pro—BD-2 inhibits MAO-B. It can be assumed that this effect is achieved predominantly due to pharmacophore BD-2, because the inhibition effect of the HFC coincides with the effect of compound BD-2 on MAO-B. In this case, the hybrid structure activates MAO-A and exerts no psychostimulating effect on animals, unlike BD-2 inhibiting MAO-A.

Effect of HFC and BD-2 on AMPA receptors. The experiments were carried out on single Purkinje neurons isolated from the rat cerebellum ($n = 5$). The application of kainic acid induced the generation of incoming transmembrane currents caused by the activation of the AMPA receptors. The addition of compound BD-2 or the C_{60}—Pro—BD-2 hybrid structure to the neuron-washing solution induced the concentration-dependent change in the amplitude of currents compared to the control, whereas the addition of C_{60}—Pro exerted no effect on the amplitude. Experiments with each concentration was repeated three times. The experimental results are shown in Fig. 3. The study showed that the action of the HFC on the amplitude of currents of the AMPA receptors does not almost differ from the effect of BD-2 itself.

Effect of the HFC and BD-2 on the motor activity. The injection of substance BD-2 results in a reliable elongation of the way passed by the mice within 3 min of testing, in other words, exerts a stimulating effect on the animals. The injection of the HFC exerts no similar stimulating effect, unlike BD-2 (Fig. 4).

Anxiety level of mice. The experimental open field model makes it possible to evaluate the degree of anxiety of

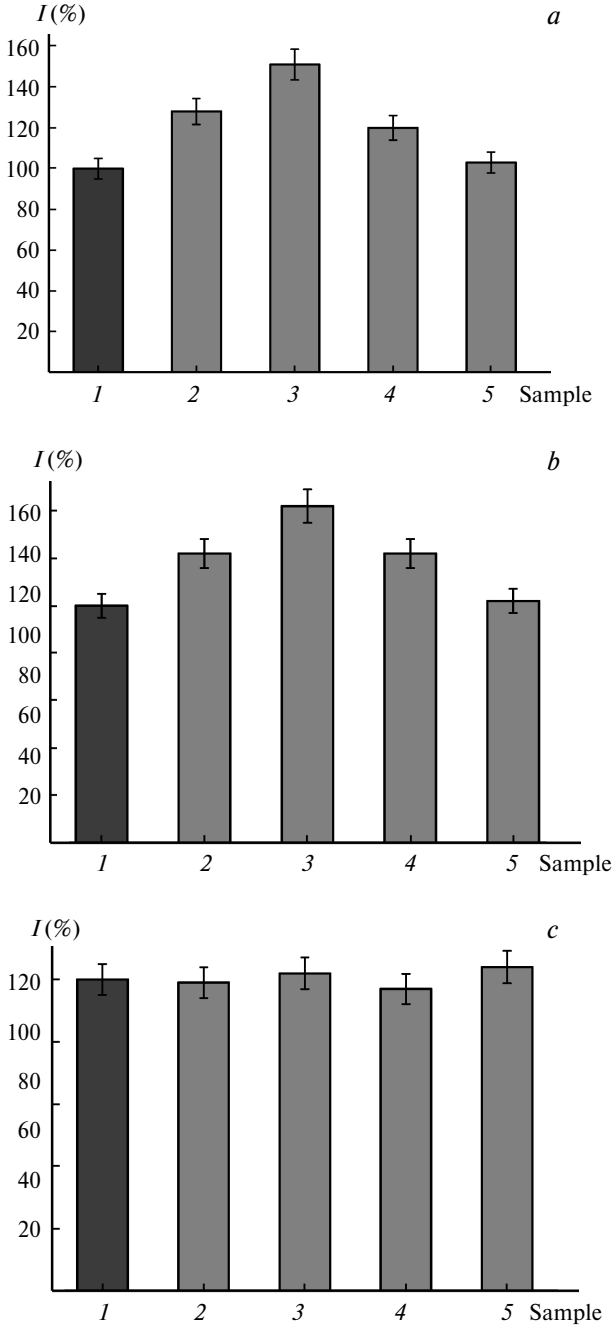


Fig. 3. Effect of BD-2 (*a*), C_{60}—Pro—BD-2 (*b*), and C_{60}—Pro (*c*) on the amplitude of the kainate-induced currents (I (%)) of the AMPA receptors of the Purkinje cells of the rat cerebellum in various concentrations: *1*, control; *2*, 10^{-9}; *3*, 10^{-8}; *4*, 10^{-7}; and *5*, 10^{-6} mol L^{-1}.

animals by the time spent by them at the conventional center of the behavior chamber. Compound BD-2 in doses of 0.1 and 0.05 mg kg^{-1} reliably elongates the time of residence at the center by 68 and 93%, respectively. This behavior of the mice can indicate a decrease in the anxiety level.[34] No reliable deviations of this parameter were ob-

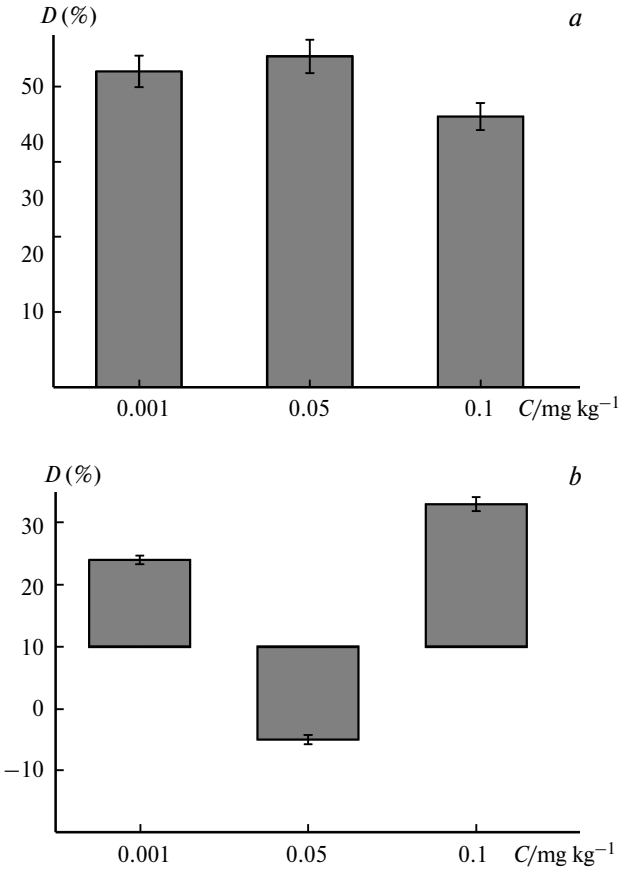


Fig. 4. Effect of BD-2 (*a*) and C_{60}—Pro—BD-2 (*b*) on the motor activity of mice (expressed by the distance passed by the animals (deviation from control, *D*).

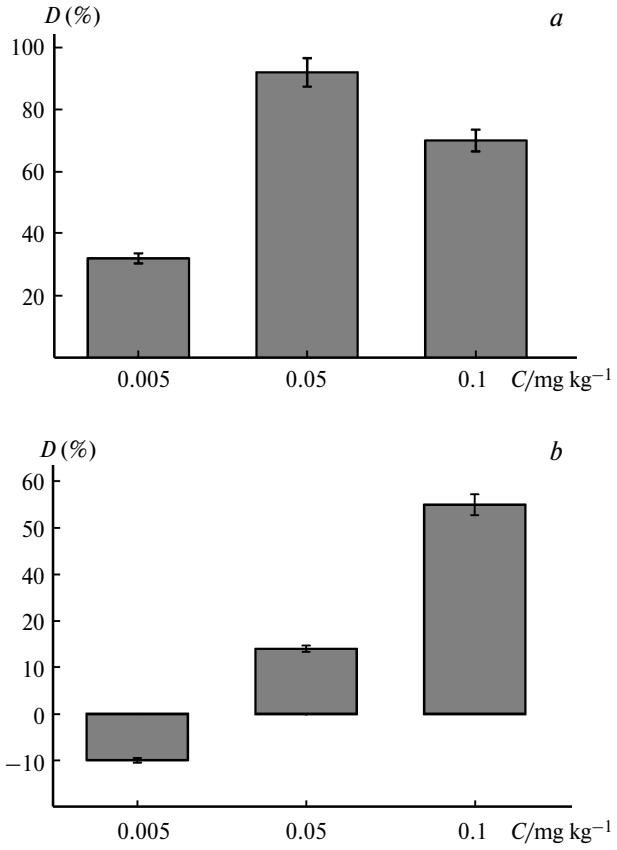


Fig. 5. Effect of BD-2 (*a*) and C_{60}—Pro—BD-2 (*b*) on the awareness level of mice (deviation from control, *D*).

served upon the injection of the studied HFC in doses similar to those of BD-2 (Fig. 5).

Awareness and orientational behavior. The orientational behavior as a parameter of emotional state was estimated by the number of vertical stands of mice. The administration of BD-2 in doses of 0.1 and 0.05 mg kg^{-1} increases the number of vertical stands. Unlike the orientational behavior, the awareness reaction, which appears as the number of studied holes in the floor of the research chamber, remains unchanged under the action of compound BD-2. After HFC administration, no changes occurred in the awareness reaction and orientational behavior of the mice (Fig. 6).

Effect of the HFC and BD-2 on the memory of animals. In the behavior experiments, we studied the effect of compounds BD-2, C_{60}—Pro, and HFC C_{60}—Pro—BD-2 in doses of 0.001, 0.05, and 0.1 mg kg^{-1}. It was shown that for testing in 48 h after training the control group mice studied the object for 48.1±7.9% time in the known localization and for 51.9±7.9% time in the new localization (p = 0.17); *i.e.*, they do not remember where the objects were localized during training. Unlike the control, the animals administered by compounds BD-2 and HFC in doses of 0.01 and 0.001 mg kg^{-1} spent reliably more time to the investigation of the object in the new localization. This indicates that the both substances almost equivalently improve the memory of animals, and a dose of 0.001 mg kg^{-1} showed a higher reliability of distinctions from the control than a dose of 0.01 mg kg^{-1}. The administration of C_{60}—Pro to the animals exerted no effect of the memory of the animals (Fig. 7).

The obtained results allow us to draw some preliminary conclusions. The addition of C_{60}—Pro to BD-2 does not affect, most likely, its interaction with the AMPA receptors, since the potentiating effect of BD-2 and HFC C_{60}—Pro—BD-2 on the kainate-activated currents is almost the same and C_{60}—Pro does not affect the current amplitude in the whole studied concentration range. It can be assumed that the effect of the HFC on the memory of animals in the behavioral experiments remains unchanged compared to that of BD-2 due to the unchanged character of the current amplitude. These results are well consistent with the established fact that the potentiation of the AMPA receptors improves the memory.[32] At the same time, some other aspects of behavior change under the action of the HFC on which compound BD-2 exerted an effect. For example, the psychostimulating effect, which

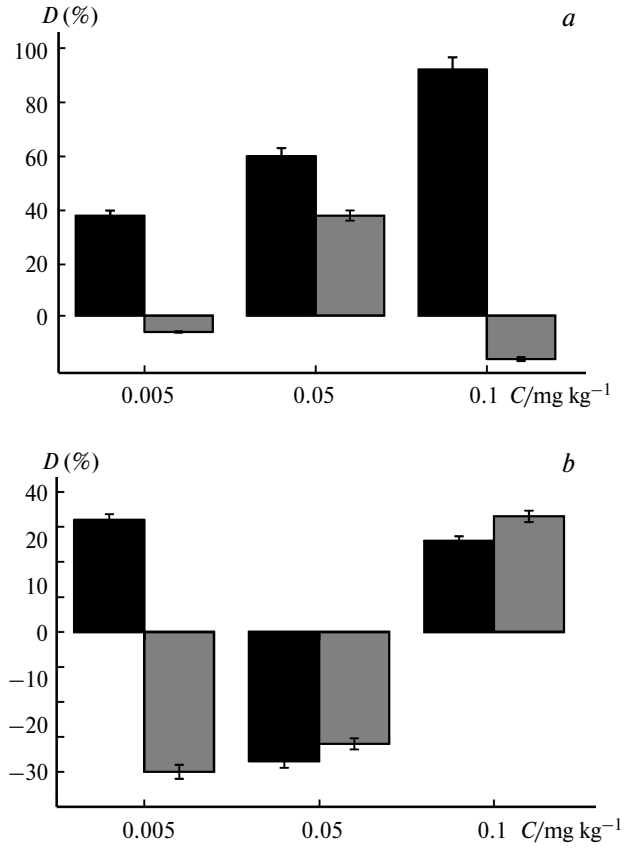


Fig. 6. Effect of BD-2 (*a*) and C_{60}—Pro—BD-2 (*b*) on the awareness and orientational behavior of mice (deviation from control, *D*). Dark columns show the number of vertical stands, and light columns designate the number of studied holes.

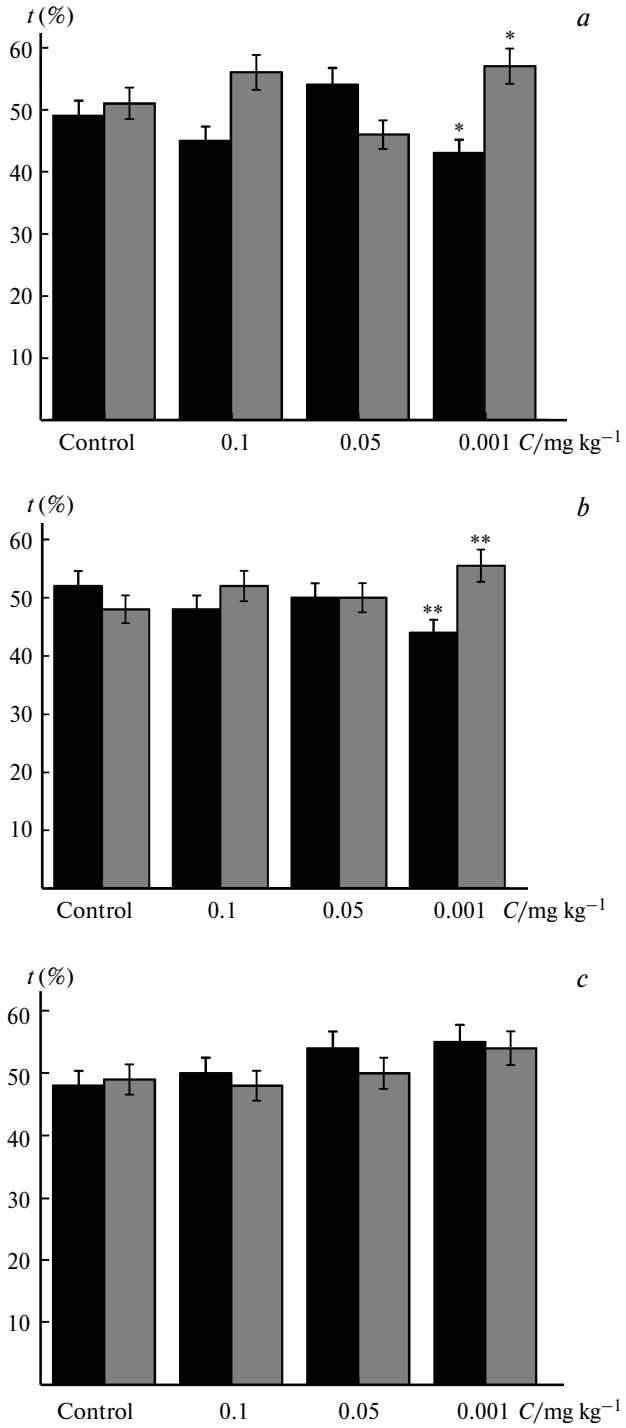


Fig. 7. Effect of BD-2 (*a*), C_{60}—Pro—BD-2 (*b*), and C_{60}—Pro (*c*) on the spatial memory of mice. Dark columns designate the known localization, light columns designate the new localization, and *t* is awareness time. Signs * and ** mark the results at $p < 0.05$ and $p < 0.01$ relative to the control, respectively.

was manifested as an increase in the motor activity and was not induced by C_{60}—Pro—BD-2, disappeared (see Fig. 4). The influence of BD-2 on the emotion state of animals was the enhancement of the orientational behavior (determined by the number of stands executed by the animals), but this effect was absent for the HFC. The awareness behavior remained unchanged under the action of both BD-2 itself and HFC (see Fig. 6).

A series of other parameters also changed. The administration of BD-2 resulted in an elongation of the time spent by the mice at the center of the chamber, indicating a decrease in the anxiety level of the animals. Compound C_{60}—Pro—BD-2 already has no these properties, although a tendency to decreasing anxiety retained for the HFC at the highest of the studied doses (0.1 mg kg^{-1}); however, this effect already has no reliability level (see Fig. 5).

The obtained results demonstrated that some new properties that were not characteristic of BD-2 appeared upon the action on the behavior of the animals of the hybrid structure based on fullerene and cognitive stimulating substance BD-2. First, the psychostimulating effect inherent in BD-2 and appeared as the enhancement of both the locomotor activity and emotional (orientational) behavior (which can be considered, in this case, as an undesirable side effect of BD-2) disappeared. Second, the anti-anxiety effect of the hybrid structure decreased compared to that

of BD-2. The most substantial fact is that the main cognitive stimulating properties of the starting compound retained against the background of these changes.

Thus, the inclusion of γ-carboline derivative with the cognitive stimulating properties into a complex with modified fullerene C_{60}—Pro made it possible to obtain the hybrid structure (HFC) retaining high cognitive stimulating properties and to avoid the side psychostimulating effect of this substance. It is likely related to an increase in the activity of monoaminoxidase A upon the addition of the hybrid compound. The effect of the HFC on the AMPA receptors of the brain is nearly identical to the effect of the starting compound BD-2. The approach proposed in this work for the modification of the structure of the starting pharmacophore (BD-2) by its attachment to modified fullerene substantially improved the pharmacological profile of the pharmacophore.

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