

Dopamine Protects Neurons against Glutamate-Induced Excitotoxicity

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Abstract—Parkinson’s disease is associated with neuronal loss in the midbrain and the resulting development of dopamine-deficient states. At the later stages of the disease, increased neuronal death is also observed in other parts of the brain. We hypothesized that dopamine may function as a glutamate antagonist, and dopamine deficiency may increase glutamate-induced excitotoxicity. Using rat hippocampal primary culture and fluorescence microscopy, we show that dopamine reduces the amplitude of calcium response evoked by the activation of NMDA receptors but does not affect calcium signals mediated by AMPA and KA receptors. Voltage-gated calcium channels are also unaffected by dopamine. It was shown that the effect of dopamine depends not only on NMDA receptors, but also on D2-type dopamine receptors and on GABA(A) receptor. Dopamine reduced glutamate-induced mitochondrial depolarization and improved neuronal survival in the presence of toxic levels of glutamate. The data presented suggest a protective role of dopamine against glutamate toxicity.

Keywords: dopamine, glutamate, calcium, excitotoxicity, ionotropic glutamate receptors, neuroprotection

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INTRODUCTION

Dopamine is a neurotransmitter involved in the regulation of various brain functions. The disturbances of dopamine metabolism and signaling lead to the development of neurodegenerative diseases such as Parkinson’s disease, Alzheimer’s disease, schizophrenia. In Parkinson’s disease dopaminergic neurons loss is observed mainly in *substantia nigra* [1].

Dopamine is able to suppress a cytosolic calcium ($[Ca^{2+}]_i$) increase induced by glutamate. Glutamate is the most abundant excitatory neurotransmitter in the brain that becomes toxic and causes pathological conditions when accumulates in high concentrations [2]. During the development of Parkinson’s disease, which is characterized by a dopamine deficiency, it is possible that even low doses of glutamate can lead to neurodegeneration. Glutamate has been found to mediate activation of several subtypes of glutamate receptors, including ionotropic NMDA, AMPA, and KA receptors, which function as ligand-gated ion channels activated by specific agonists, and metabotropic G-protein coupled receptors mGlu1–mGlu8 [3].

Dopaminergic functions are mediated by activation of a family of dopamine receptors, consisting of five types and belonging to two groups: D1-like receptors (D1 and D5) and D2-like receptors (D2, D3,

and D4). D1-like receptors are G_s -coupled serpentine receptors that regulate the release of neurotransmitters such as glutamate, GABA, and acetylcholine. D2-like receptors are coupled with the inhibitory G-protein $G_{i/o}$ and regulate dopamine secretion by neurons in response to changes in extracellular levels of the neurotransmitter [4].

It is known that the interplay between dopaminergic and glutamatergic systems provides the basis for complex neural interactions in the brain. Activation of D1- and D2-like receptors can regulate the function and transport of NMDA receptors. Thus, dopamine receptors interact with NMDA receptors either directly or indirectly, through the activation of protein kinase A, and can influence NMDA responses in neurons [5].

We assumed that dopamine could act against glutamate neurotoxicity because the addition of dopamine to hippocampal cell culture reduces glutamate-induced increase in $[Ca^{2+}]_i$ and mitochondrial depolarization and increases the number of live cells after exposure to toxic glutamate levels. In order to determine the target of dopamine action, we investigated its effect on the amplitude of calcium responses (ACR) caused by agonists of glutamate and dopamine receptors.

MATERIALS AND METHODS

Culture of neurons. The primary culture of the rat hippocampal cells was obtained from the brain of newborn *Sprague Dawley* rat pups (P1–P3). After decapitation the hippocampus was removed and placed in a cold HBSS according to the method described in [6]. Then the tissue was cut into small pieces, incubated in 0.1% trypsin solution for 15 min at 37°C, washed three times with Neurobasal-A medium (Gibco, USA) containing 2% Supplement B27 (Gibco), 1 mM *L*-glutamine (Sigma, USA), 7.5 µg/mL gentamicin (Dalkhimpharm, Russia), dissociated using a pipette until the formation of cell suspension. After that, the cells were placed on round coverslips (25 mm in diameter) covered with polyethyleneimine (Fluka, USA) in 35-mm Petri dishes. After 1 h, 1.5 mL of Neurobasal culture medium was added to the attached cells and they were incubated for 5–12 days at 37°C and 5% CO₂.

The experiments were carried out in Hanks solution (HBSS) containing (in mM): 138 NaCl, 1.3 CaCl₂, 0.4 MgSO₄, 0.5 MgCl₂, 5.3 KCl, 0.45 KH₂PO₄, 4 NaHCO₃, 0.3 Na₂HPO₄, 10 glucose, 20 HEPES (pH 7.3) at a temperature of 27–28°C. The experiments with NMDA were carried out in Mg²⁺-free medium.

Measurements of the intracellular calcium concentration ([Ca²⁺]_i). Changes in [Ca²⁺]_i in neurons were assessed by the fluorescence intensity of a ratiometric Ca²⁺-sensitive probe Fura-2 [7]. The cell culture was loaded with 5 µM Fura-2AM (Invitrogen, USA) for 60 min followed by washing. [Ca²⁺]_i was detected using an imaging system based on Leica DMI6000B inverted motorized microscope (Leica Microsystems, Germany) equipped with a Leica HC PL APO 20×/0.7 IMM objective. A set of FU2 light filters (excitation: 340 ± 6 and 380 ± 10 nm, emission: 510 ± 20 nm) was used to excite and detect Fura-2 fluorescence. We also used a Cell Observer (Carl Zeiss, Germany) imaging system based on Axiovert 200M inverted microscope equipped with a Plan Neofluar 10×/0.3. Fura-2 fluorescence was excited and recorded using a Filter set 21HE (excitation: 340 ± 6 and 380 ± 10 nm, emission: 510 ± 20 nm). Images were obtained at a frequency of 1 frame per 3 s. The two-channel time lapse images were processed in ImageJ using the Time Series Analyzer plugin. The amplitude of calcium responses (ACR) in individual cells was estimated as the ratio of Fura-2 fluorescence signals upon excitation at 340 and 380 nm. In the experiment, calcium signals from 100–200 neurons in the field of view of the microscope was measured.

For the experiment, a coverslip with a cell culture was mounted in a measuring chamber and kept in 0.5 mL HBSS. Additions of reagents and washing out were carried out using a perfusion system consisting of a feeding tip connected to the tanks with solutions and a tip connected to a water jet pump and providing a

constant level of the medium in the measuring chamber. The tips were located on opposite sides of the chamber. To change the bathing solution, the perfusion system was turned on for 8 s, that provided a tenfold (5 mL) replacement of the working solution in the chamber. Washing was carried out in the same way. Control experiments with an optically dense dye (Trypan blue) showed that under these conditions a complete (more than 98%) replacement of the solution in the measuring chamber occurred. This protocol for changing solutions is the result of optimization and ensures that cells do not respond to mechanical stress when the medium is changed. Nevertheless, to assess a possible reaction of the cells, a control washing with 5 mL of HBSS was carried out at the beginning of each experiment to prove the absence of [Ca²⁺]_i changes during the medium replacement.

To compare the results of experiments performed on different days and on different cultures, to normalize the signals in the experimental scheme, and to functionally separate neurons and glial cells in culture, a standard depolarizing solution of KCl was added to the cells. In this study, we used a technique to assess the modulating effect of various compounds on the activity of glutamate ionotropic NMDA, AMPA, and KA receptors [8], which is based on the fact that several short-term (20–30 s) additions of these receptor agonists at 10–15 min intervals induce calcium signals of the same amplitude. This makes it possible to use the signal in response to the first addition as a control, and to test the effect of a certain compound on the second signal (Fig. 1a). The ACRs were quantified relative to the initial ACR value before the addition. In the case of activation of KA receptors, experiments were carried out in the presence of an inhibitor of AMPA receptors, 30 µM GYKI-52466 (Tocris, UK), as kainic acid (KA, Sigma) is a nonselective agonist of KA and AMPA receptors; also, an inhibitor of desensitization of KA receptors, 200 µM concanavalin A (ConA, Sigma), was added in these experiments. The selective agonist of AMPA receptors, 5-fluorowillardiine (FW, Santa Cruz Biotechnology, USA), and the inhibitor of AMPA receptor desensitization, 5 µM cyclothiazide (CTZ, Tocris), were used to activate AMP-receptors (Fig. 1b) [8].

Measurement of mitochondrial membrane potential.

For registration of mitochondrial potential, cells were loaded for 10 min with a fluorescent voltage sensitive probe 20 µM Rhodamine 123 (Rh123, Sigma). Then the cells were washed twice with HBSS and used in the experiment. The measurements were performed using a Cell Observer (Carl Zeiss) imaging system based on an Axiovert 200M inverted microscope equipped with a Plan Neofluar 10×/0.3 objective. Rh123 fluorescence was excited and recorded using Filter set 44 (excitation: 490 ± 6 nm, emission: 550 ± 20 nm). The experiments used a staining protocol based on dye concentration-dependent fluorescence quenching in

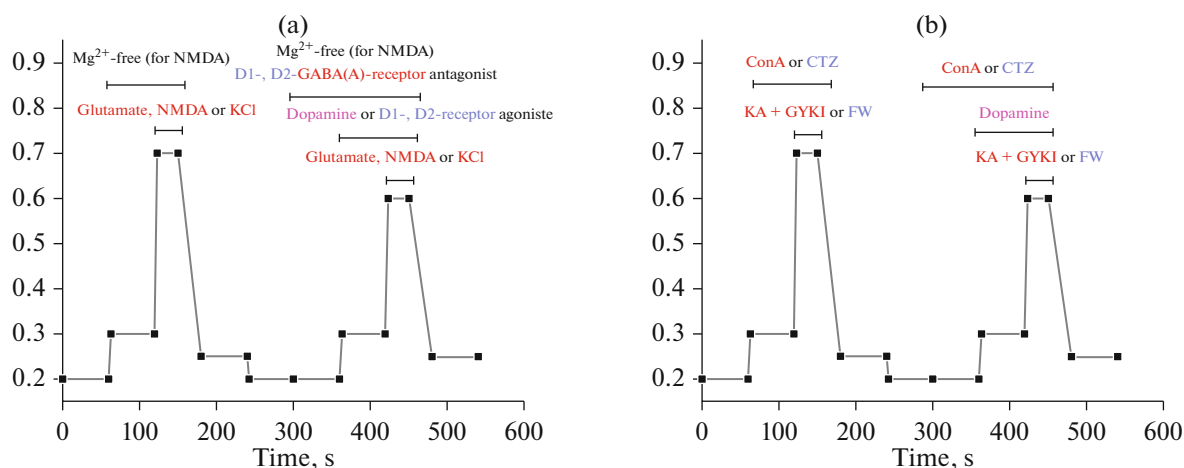


Fig. 1. Experimental scheme to determine dopamine effect on the ACR caused by agonists of glutamate receptors and KCl, based on a previously developed method [8]. (a) For glutamate, NMDA, and KCl; (b) for KA and AMPA.

energized mitochondria; an increase in Rh123 fluorescence is observed upon depolarization of mitochondrial membranes [9]. Images were obtained at a frequency of 1 frame per 5 s, and processed in ImageJ using the Time Series Analyzer plugin. The signals from 100–200 neurons in the field of view of the microscope were measured in the experiment. To normalize the signals in the experimental scheme, 2 μ M FCCP (Sigma) was added to achieve a complete mitochondrial depolarization.

Cell viability assessment. The number of living cells in the neuroglial culture was assessed using double staining with fluorescent dyes Hoechst 33342 (2 μ g/mL, Sigma) and Propidium Iodide (PI, 2 μ g/mL, Sigma) for 10 min, followed by washing with HBSS. Dye fluorescence was assessed using an imaging system based on a Leica DMI6000B inverted motorized microscope (Leica Microsystems) equipped with a Leica HC PL APO 20 \times /0.7 IMM objective. A set of DAPI light filters (excitation: 340 \pm 6 nm, emission: 470 \pm 20 nm) was used for excitation and registration of Hoechst fluorescence; for PI, a set of Texas Red light filters (excitation: 575 \pm 10 nm, emission: 624 \pm 10 nm). Cells were counted in two channels in the ImageJ using the Cell Counter plugin. The Hoechst dye enters cells and binds to DNA, which allows evaluating the nuclear morphology. In contrast, PI dye penetrates through the plasma membranes of damaged or fixed cells only. Colocalization of Hoechst and PI in cells indicated a violation of the barrier function of the plasma membrane, and such cells were considered dead; cells with fragmented nuclei and bright fluorescence in the blue (Hoechst) channel, were counted as apoptotic. The data were presented as the percentage of dead and apoptotic cells from the total number of cells in the field of view. The fluorescent signals from 300–600 cultured neuroglial cells were measured in the experiment.

Processing of the results. For plotting and statistical processing, the OriginPro2019 and GraphPadPrizm8

were employed, one-way ANOVA (post hoc Turkey test) was used for parametric analysis. The graphs show representative curves, the number of cells in one experiment (N) was 100–140, the number of experiments of the same type (n), 3–4. Data in bars are presented as mean \pm standard deviation of mean, differences were considered statistically significant at $p < 0.05$.

RESULTS

Dopamine suppresses the glutamate-induced [Ca²⁺]_i increase in neurons. It has been shown previously that dopamine is able to suppress the [Ca²⁺]_i increase that occurs in response to low doses of glutamate (5 μ M) [10]. In this study, we tested whether dopamine could protect cells from the action of higher concentrations of glutamate, 50 and 100 μ M, which are known to be toxic. We assessed the ability of dopamine to influence the ACR to glutamate using a previously developed method for assessing the modulating effect of various compounds on the activity of ionotropic glutamate receptors—NMDA, AMPA, and KA receptors [8]. We utilized a protocol providing repetitive in shape and amplitude [Ca²⁺]_i increase in response to selective and short-term activation of certain glutamatergic receptor. It was shown that dopamine at a concentration of 50 μ M is able to reduce the ACR to the second addition of 10 μ M glutamate by 19%. Thus, the response to the addition of glutamate in the presence of dopamine was 81 \pm 13% as compared to the response to the first addition of glutamate (in Figs. 2a, 2b the amplitude of the first response to glutamate was taken as 100%). In the case of high doses of glutamate (100 and 50 μ M), the glutamate-induced calcium response also decreased by 28% (72 \pm 22%) in the presence of dopamine (Figs. 2b, 2c). This effect may lead to the prevention of glutamate-induced calcium overload of cells and thus be neuroprotective under conditions of excitotoxicity.

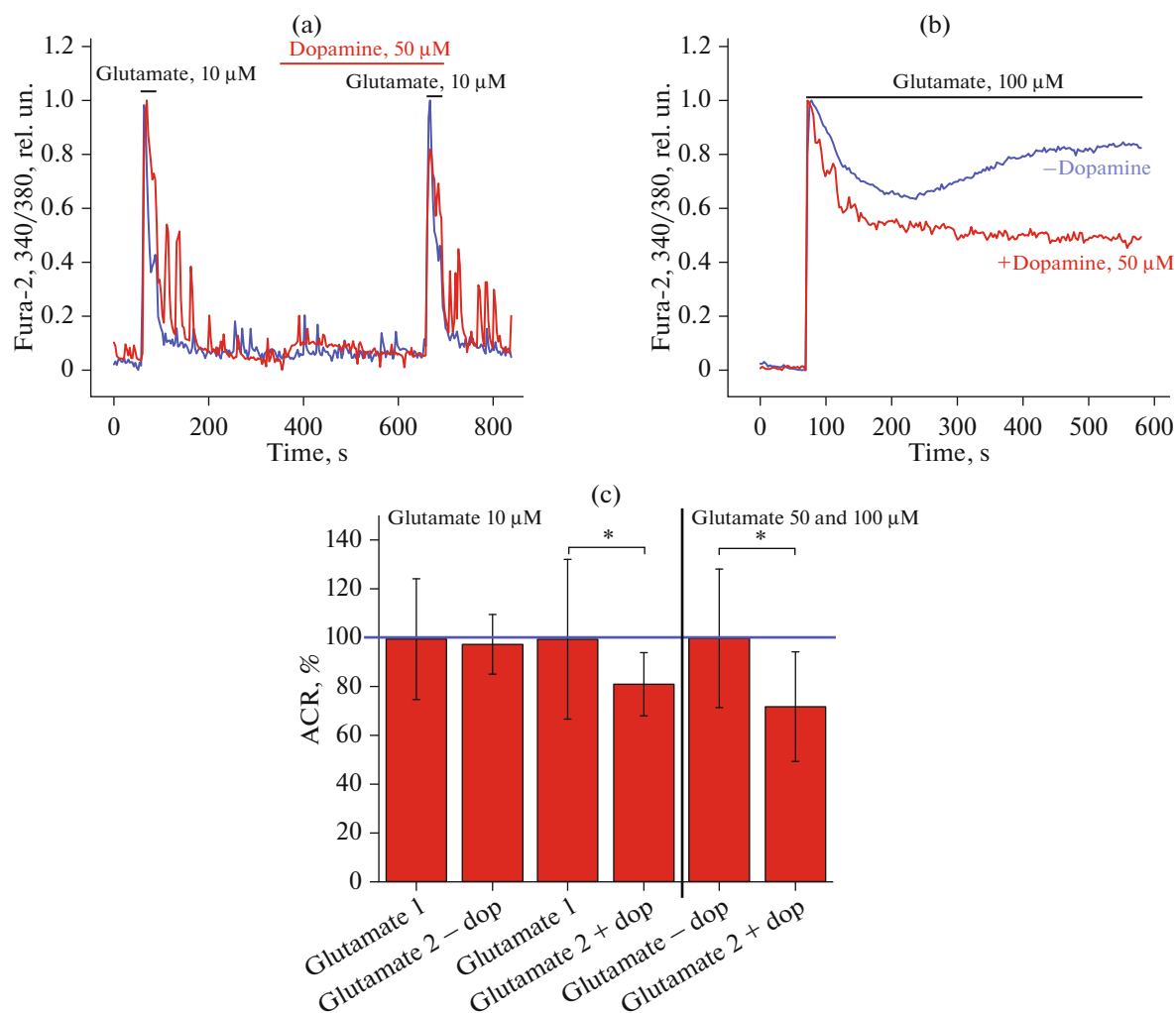


Fig. 2. Dopamine effect on the amplitude of the Ca^{2+} signal in response to glutamate in neurons. (a) Repetitive calcium signals in neurons to short-term $10 \mu\text{M}$ glutamate applications (*blue curve*) and the effect of $50 \mu\text{M}$ dopamine on the cell response to glutamate application (*red curve*); normalized relative to the first glutamate application. (b) Calcium signal in response to $100 \mu\text{M}$ glutamate in the presence of $50 \mu\text{M}$ dopamine (*red curve*) and in the absence of dopamine (*blue curve*); normalized to response to glutamate. (c) ACR to 10 and 50 – $100 \mu\text{M}$ glutamate application in the absence (–dop) and in the presence of $50 \mu\text{M}$ dopamine (+dop). In the case of $10 \mu\text{M}$ glutamate, responses to the second application glutamate (Glutamate 2) are presented relative to the responses to the first application (Glutamate 1). In the case of high doses of glutamate (50 – $100 \mu\text{M}$), the calcium response to glutamate together with dopamine (Glutamate + dop) is presented relative to the response to glutamate without dopamine (Glutamate – dop). * $p < 0.05$.

In order to identify which receptor subtype is involved in this effect, we tested how dopamine affects the amplitude of calcium signals caused by selective activation of ionotropic glutamate receptors, NMDA, AMPA, and KA receptors. At the same time, NMDA receptor is permeable to Ca^{2+} itself, and the activation of AMPA and KA receptors is mainly mediated by the activation of voltage-gated calcium channels [11]. Dopamine has been shown to suppress the activity of NMDA receptors without affecting the activity of KA and AMPA receptors. Here we show that dopamine at a concentration of $10 \mu\text{M}$ suppresses the calcium signal induced by the low doses of NMDA (5 and $10 \mu\text{M}$) by 23% ($77 \pm 23\%$ relative to the first control response,

Figs. 3a, 5b). In the presence of NMDA in high concentrations (50 and $100 \mu\text{M}$), dopamine suppresses the ACR to this agonist by 15% (Fig. 3b).

It has been shown that dopamine does not reduce calcium signal induced by the KA-receptor agonist kainic acid (in the presence of AMPA-receptor antagonist GYKI-52466, KA+GYKI). The ACR to the second application was $101 \pm 22\%$ of the first signal (Figs. 4a, 5b). Also, dopamine does not affect the activity of AMPA receptors, since the dopamine application did not change the ACR to fluorowillardiine (FW), an AMPA-receptor selective agonist ($98 \pm 13\%$) (Figs. 4b, 5b). To prevent desensitization of KA and AMPA receptors, these experiments were performed

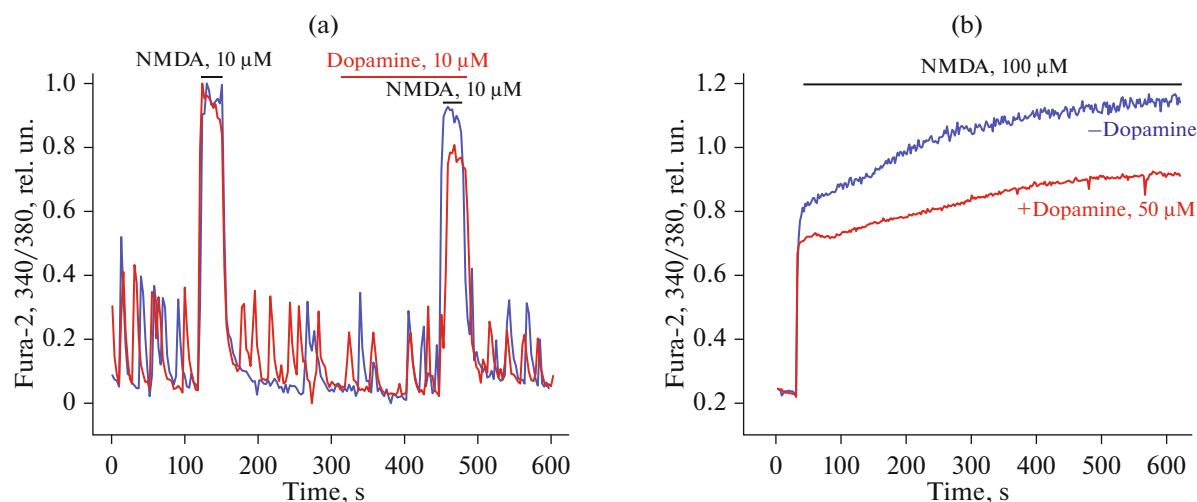


Fig. 3. Dopamine effect on the ACR induced by NMDA in neurons. (a) Calcium signal in response to 10 μM NMDA application in the absence (*blue curve*) and in the presence (*red curve*) of 50 μM dopamine; normalized relative to the first NMDA application. (b) Calcium signal in response to 100 μM NMDA application in the absence (*blue curve*) and in the presence (*red curve*) of 50 μM dopamine.

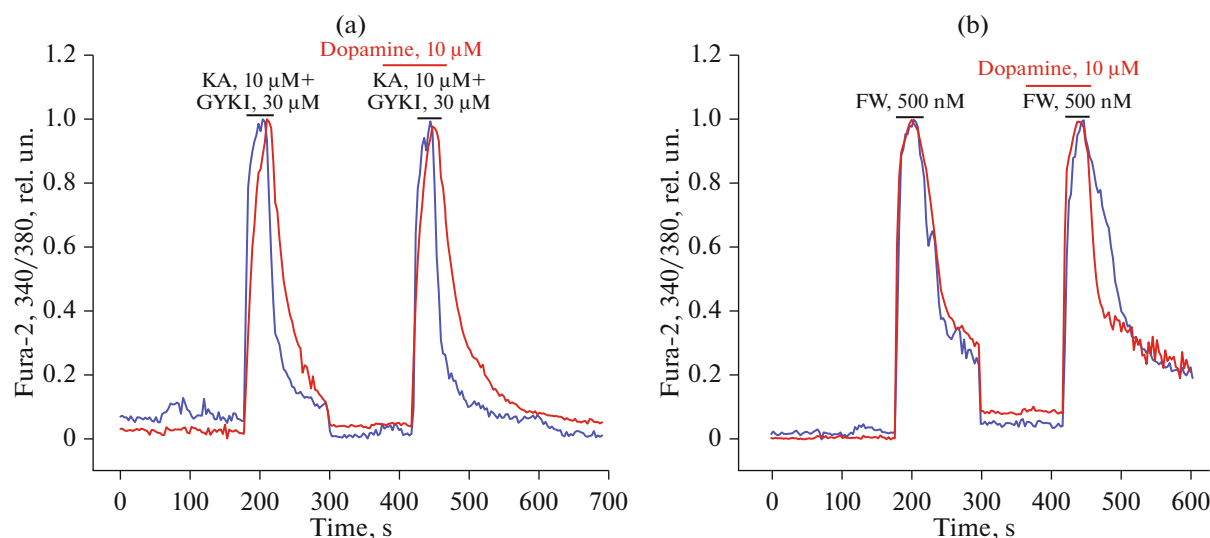


Fig. 4. Dopamine effect on the ACR caused by the activation of KA and AMPA receptors in neurons. (a) Calcium signal in response to co-application of 10 μM kainic acid (KA) and 30 μM GYKI in the absence (*blue curve*) and in the presence (*red curve*) of 50 μM dopamine; normalized relative to the first application of KA+GYKI. (b) Calcium signal in response to application of 500 nM fluorowillardiine (FW) in the absence (*blue curve*) and in the presence (*red curve*) of 50 μM dopamine; normalized relative to the first application of FW.

in the presence of 200 $\mu\text{g}/\text{mL}$ concanavalin A and 5 μM cyclothiazide, respectively [8].

We also tested whether the ACR, caused by the activation of voltage-gated calcium channels (VGCC), changes in response to dopamine. It was shown that the addition of dopamine does not influence the ACR that occurs under the action of 35 mM potassium chloride, which activates VGCC (Figs. 5a, 5b). Ca^{2+} response to the second KCl application was $82 \pm 11\%$ relative to the control; and this value was $80 \pm 10\%$ in the presence of dopamine.

Thus, it was found that the main target of dopamine is NMDA receptor.

Involvement of dopamine receptors. It is known that dopamine directly suppresses the activity of NMDA receptor; its action can also be mediated by activation of specific dopamine receptors [4, 12].

We tested whether D1- and D2-like receptor antagonists are able to prevent the effect of dopamine on NMDA-induced calcium signal in neurons. Figure 6 shows that the effect of dopamine is not prevented in the presence of the D1-receptors blocker SKF-83566

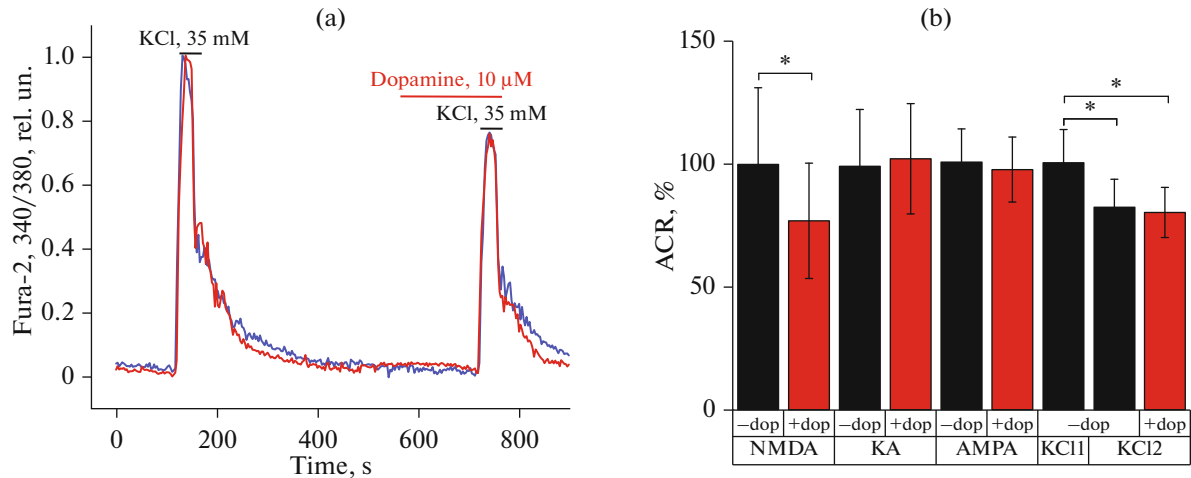


Fig. 5. Dopamine effect on the ACR evoked by glutamate receptor agonists and KCl. (a) Calcium signal in response to 35 mM KCl application in the absence (blue curve) and in the presence (red curve) of 50 μM dopamine; normalized relative to the first application of KCl. (b) ACR evoked by NMDA, KA, AMPA, and KCl applications in the absence (black columns, -dop) and in the presence (red columns) of 50 μM dopamine (+dop) relative to the first application of the agonist. KCl1 and KCl2, the first KCl and the second KCl application, respectively; * $p < 0.05$.

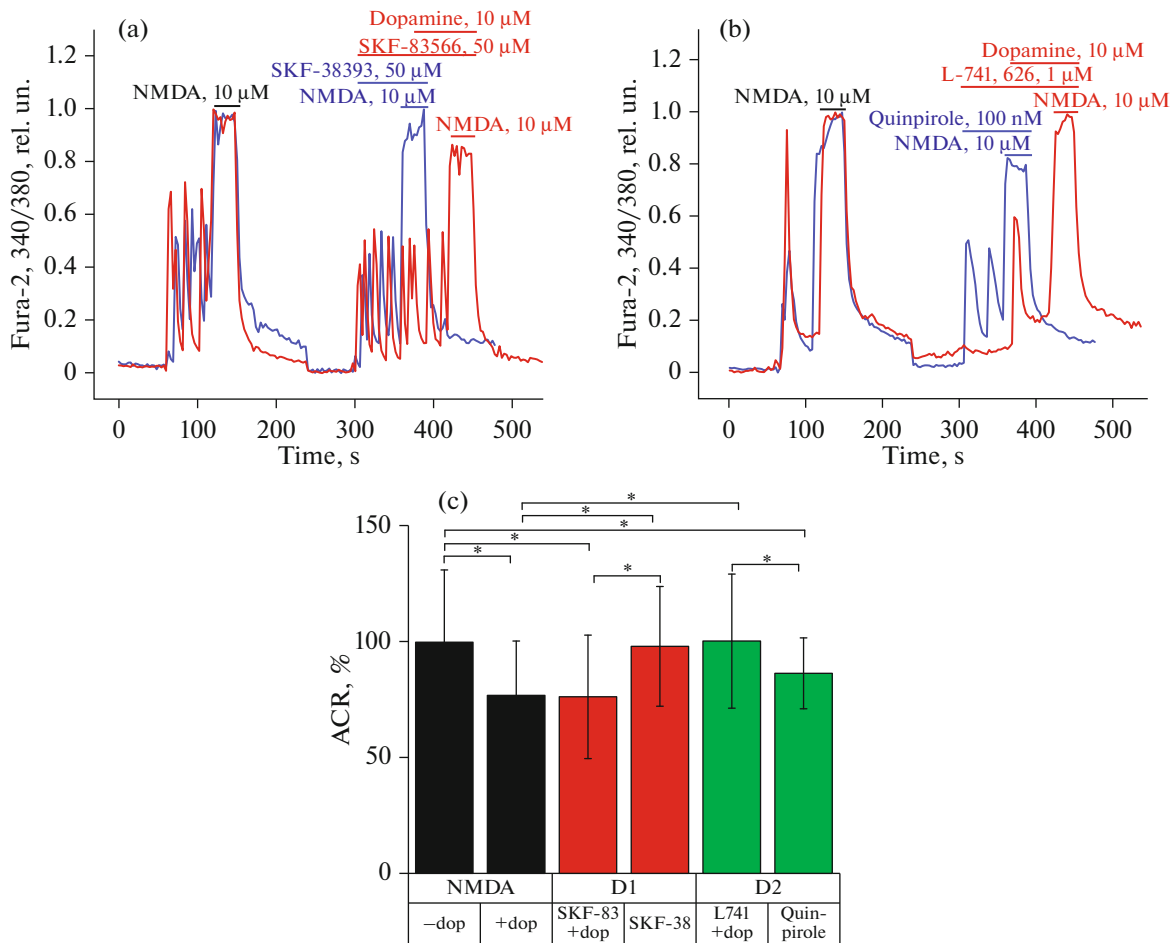


Fig. 6. Effects of D1- and D2-like receptor agonists and antagonists on the ACR induced by NMDA. (a) Calcium signal in response to 10 μM NMDA application in the presence of 50 μM D1-receptor agonist SKF-38393 (blue curve) and 50 μM D1-receptor antagonist SKF-83588 together with dopamine (red curve); normalized relative to the first NMDA application. (b) Calcium signal in response to 10 μM NMDA application in the presence of 100 nM D2-receptor agonist Quinpirole (red curve) and 1 μM D2-receptor antagonists L-741,626 together with dopamine (blue curve); normalized relative to the first NMDA application. (c) ACR induced by NMDA in the presence of D1- and D2-receptor activators and their blockers together with dopamine (+dop) relative to the first NMDA application (100%), * $p < 0.05$.

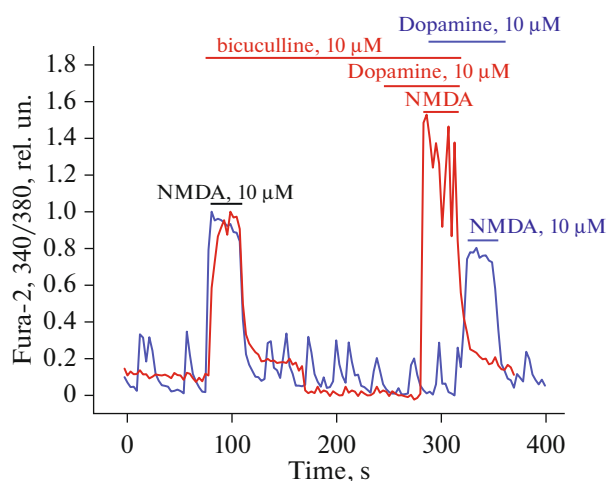


Fig. 7. Effect of GABA(A)-receptor activity on the ACR induced by NMDA. Calcium signal in response to 10 μ M NMDA application in the presence of 10 μ M GABA(A)-receptor inhibitor bicuculline with 10 μ M dopamine (*red curve*) and in the absence of bicuculline but with dopamine (*blue curve*); normalized relative to the first NMDA application.

(50 μ M); this is evidenced by the decrease in the amplitude of the calcium response upon the combined action of NMDA and dopamine in the presence of SKF-83566 ($76 \pm 26\%$, Figs. 6a, 6c). The application of D1-receptor agonist SKF-38393 (50 μ M) did not lead to a significant change in the calcium signal in response to NMDA ($98 \pm 26\%$, Figs. 6a, 6c).

In the case of the D2-receptor inhibitor L-741,626 (1 μ M), dopamine effect was not observed: calcium signal to the second application of NMDA was $100 \pm 28\%$ (Figs. 6b, 6c), and the addition of D2-receptor agonist Quinpirole (100 nM) to the cells reduced the calcium signal caused by NMDA ($86 \pm 15\%$, Figs. 6b, 6c). This suggests that the action of dopamine is mediated by the activation of G_i -coupled D2 receptor.

Thus, it became clear that dopamine exerts its inhibitory effect on the activity of NMDA-receptor through the activation of D2-like receptor.

It was shown previously that the effect of this neurotransmitter can be cancelled by inhibitors of GABA(A)-receptors [13, 14]. In this study, we found that 10 μ M bicuculline (GABA-receptor inhibitor) together with 10 μ M dopamine did not suppress the calcium signal to NMDA application (Fig. 7).

Dopamine protects mitochondria from glutamate-induced depolarization. It is known that the toxic effect of glutamate is based on an increase in the level of cytosolic calcium in neurons, which is characterized by the so-called delayed calcium dysregulation (DCD), occurring due to the suppression of ATP production in mitochondria and the inability of cells to maintain ion homeostasis. In this case, a characteristic feature is the depolarization of mitochondria [2]. We tested whether

dopamine affects glutamate-induced mitochondrial depolarization. It was shown that glutamate induces mitochondrial depolarization by $15 \pm 7\%$ in the presence of dopamine less than in the control (Figs. 8a, 8b). Thus, the amplitude of the change in Rhodamine 123 (Rh123) fluorescence was 0.40 ± 0.12 in response to glutamate, while this value was 0.35 ± 0.11 after pre-treatment with dopamine (the data are normalized relative to the signal in response to 2 μ M FCCP, which causes complete depolarization of mitochondria).

Dopamine inhibits glutamate excitotoxicity. In order to confirm the protective role of dopamine under the action of toxic doses of glutamate, we carried out experiments to study the neuronal survival. It was shown that 100 μ M glutamate causes the death of $30.0 \pm 1.7\%$ of neurons in culture after 24 hours of exposure, that is significantly higher than in the control (non-treated cells, $18.1 \pm 1.6\%$, Fig. 9). If 10 μ M dopamine is present in the bathing solution, the number of damaged neurons decreases by 53% after 24 h. Thus, this value under the combined action of glutamate and dopamine was $14.2 \pm 1.6\%$. Incubation with dopamine did not significantly affect the number of damaged cells relative to the control ($17.1 \pm 1.7\%$).

Thus, dopamine reduces glutamate-induced mitochondrial depolarization and improves cell viability in culture, thereby exerting a neuroprotective effect on cells.

DISCUSSION

In this study using fluorescence microscopy and inhibitory analysis it was shown that dopamine is neuroprotective against glutamate toxicity in hippocampal cell cultures. In particular, dopamine at a concentration of 10 μ M reduces the ACR to glutamate, primarily due to the suppression of the activity of NMDA-receptor, a subtype of ionotropic glutamate receptors. In addition, dopamine prevents the development of glutamate-induced mitochondrial depolarization and increases neuronal survival in the presence of toxic glutamate levels. This effect depends on the activity of D2-like dopamine receptors but does not depend on D1-like receptors. It has also been shown that the effect of dopamine on glutamate-induced changes in the studied cell parameters is mostly determined by the activation of GABAergic system, since the effect of dopamine also depends on the activity of GABA(A) receptor (see also our previous study [14]). It was shown that the discovered effect of dopamine is sensitive to GABA(A)-receptor antagonist bicuculline since the presence of 10 μ M bicuculline in the bathing solution completely suppresses the effect of dopamine on NMDA-induced calcium signal in neurons.

Several possible mechanisms can be proposed to explain the protective effect of dopamine.

First, dopamine can act via the direct interaction with NMDA-receptor channel. Studies have shown

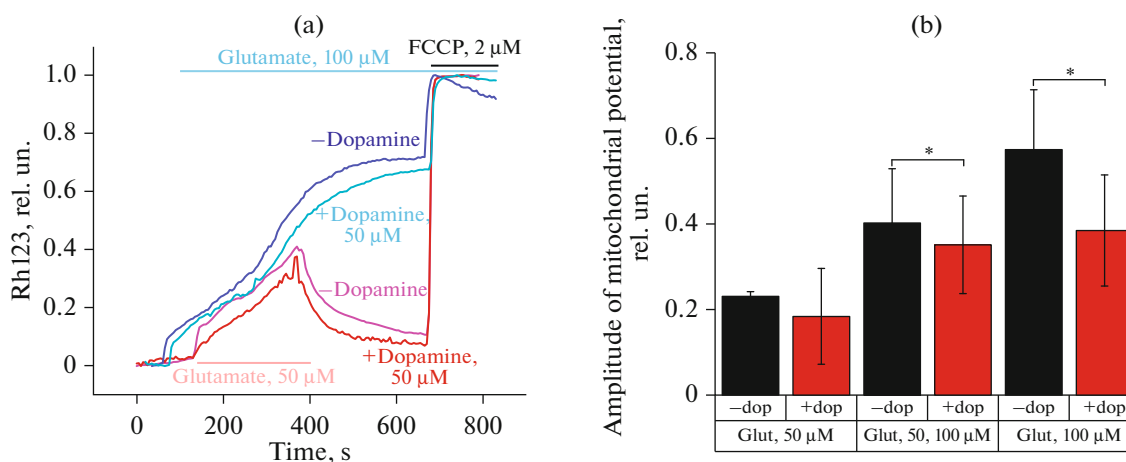


Fig. 8. Dopamine effect on glutamate-induced mitochondrial depolarization in rat hippocampal neurons. (a) Change in the fluorescence of rhodamine 123 (Rh123) induced by 50 and 100 μM glutamate application in the presence (blue and red curves) and in the absence (blue and pink curves) of 50 μM dopamine; normalized to FCCCP. (b) Amplitude of change in mitochondrial potential caused by short-term applications of 50 and 100 μM glutamate (Glut, 50 μM and 100 μM) in the absence (black columns, -dop) and in the presence (red columns) of 50 μM dopamine (+dop); the data presented correspond to the experimental scheme with glutamate washout (panel (a), red and pink curves); * $p < 0.05$.

that dopamine receptor ligands, including dopamine itself, as well as other monoamines, can interact with NMDA receptor, acting as voltage-dependent blockers of the open state of the receptor and having a binding site inside NMDA-receptor channel [15, 16].

Secondly, dopamine through the receptor causes hyperpolarization of neurons. In 1986, it was shown on neurons of the *nucleus accumbens* that dopamine

can induce D1-receptor-dependent hyperpolarization in most neurons; in addition, depolarization mediated by activation of D2 receptors was observed [17]. Our experiments help to expand our understanding of these neurotransmitters interplay mechanisms. So, one of the targets of dopamine is the D2 receptor. However, blocking two types of receptors (D1 and D2 receptors), we did not see the abolition of the dopamine action. Perhaps this is due to the fact that this effect is not only mediated by these types of receptors.

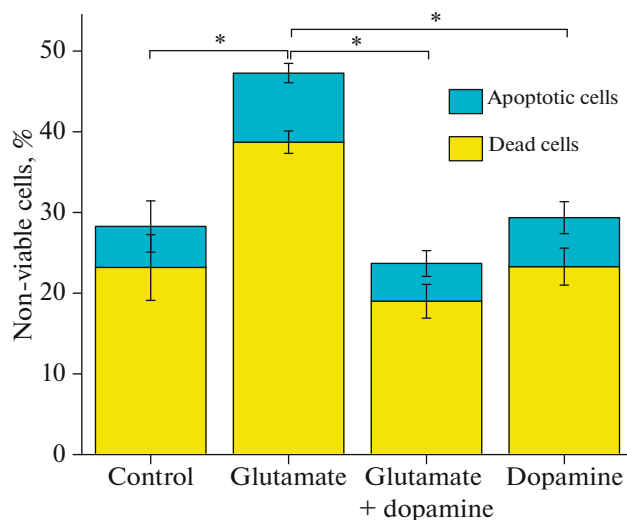


Fig. 9. Dopamine effect on the viability of rat hippocampal cells in the neuroglial culture. The percentage of damaged cells, dead (yellow) and apoptotic (blue), relative to the total number of cells in the field of view (%) after 24-h incubation in the presence of 100 μM glutamate, 10 μM dopamine, or both (100 μM glutamate and 10 μM dopamine); * $p < 0.05$, square brackets show the compared groups of damaged cells (dead + apoptotic).

It is also known that dopamine can affect AMPA receptors, but this occurs at the level of modulation of their externalization. In this case, dopamine activates the incorporation of GluR1 subunits into the plasma membrane of neurons in a D1-receptor-dependent manner [18, 19]. Our data on the effect of dopamine on calcium signals in response to the activation of KA and NMDA receptors are in some conflict with the data of the authors [20]. In this study, it was demonstrated that although dopamine protects cells from kainate-induced neurotoxicity, the mechanisms associated with the modulation of calcium homeostasis are not involved in this effect. Moreover, NMDA-receptor activity was not regulated by dopamine at all. In contrast, we found that the effect of dopamine is associated with NMDA receptor, while the calcium response upon activation of KA receptor was not affected by dopamine.

CONCLUSIONS

We have shown that dopamine has a neuroprotective effect against glutamate neurotoxicity, since the addition of dopamine to the hippocampal cell culture reduces the calcium response and mitochondrial

depolarization caused by the addition of glutamate and increases the number of viable cells after exposure to glutamate. We also found that dopamine, acting through D2-like receptors, is able to activate NMDA receptors and influence the functioning of GABAergic system.

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest.

All procedures were performed in accordance with the European Communities Council Directive (November 24, 1986; 86/609/EEC) and the Declaration on human treatment of animals. The Protocol of experiments was approved by the Guidelines for Working with Laboratory Animals of the ICB RAS No. 57 (December 30, 2011).

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