The Definition of Specific Antiparkinsonian Effects of Rapitalam

Natalia V. Avdeeva[1]*., Mikhail V. Pokrovskiy[2], Zhernakova Nina Ivanovna[3], Elena B. Artyushkova[4]

[1] PhD in medicine, researcher of the Department of Pharmacology and Clinical Pharmacology, Medical Institute, Belgorod State National Research University, 85, Pobedy St., Belgorod, 308015, Russia; E-mail: 7400468@mail.ru
[2] Department of Pharmacology, Medical Institute, Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia
[3] Department of Pharmacology, Medical Institute, Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia
[4] Department of Pharmacology, Medical Institute, Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia

Abstract

Previously, we identified a metabotropic glutamate receptor 4 (mGluR4) as a potential target for drugs and predicted that activation of mGluR4 could provide a palliative advantage in the treatment of Parkinson's disease. Determine the mechanism of action of Rapitalam in vitro on a cell culture with mGluR4 over expression. The HEK293T cell line expressing human mGluR4 was used in the work. Receptor activity was assessed by the level of calcium in the cytoplasm, the release of which is stimulated by the addition of glutamate. It is seen that Rapitalam significantly increases the cellular response induced by an agonist. On cell culture with superexpression of mGluR4 we have shown that Rapitalm is a positive allosteric modulator of mglur4 receptor.

Introduction

Parkinson's disease is a slow-progressive chronic neurodegenerative disease that affects about 1% of people over 55 years of age. At the heart of Parkinson's disease is the progressive destruction and death of neurons that produce the neurotransmitter dopamine, primarily in a black substance [1, 2]. The leading symptoms of Parkinson's disease are: muscle rigidity, hypokinesia, tremor, postural instability [3]. Dopamine-replacement therapy provides a significant improvement in the motor symptoms of Parkinson's disease in the early stages of the disease. However, long-term treatment with these drugs leads to loss of efficacy and the appearance of motor and cognitive side effects [4]. In addition, there is an assumption that l-dopa therapy can accelerate the progression of the disease by increasing oxidative damage [2, 4]. Therefore, the interest of scientists is directed to the development of therapeutic methods of treatment that can bypass the dopamine system. These methods were based on the findings obtained from the results of surgical interventions in the treatment of Parkinson's disease. So pallidotomy or deep stimulation of the brain showed a significant effect in patients with severe manifestations of Parkinson's disease [5, 6]. These methods led to a refinement of the model of basal ganglia dysfunction associated with Parkinson's disease [7]. Basal ganglia are a set of interconnected nuclei that play a key role in the management of movements. The main input nucleus of the basal ganglia is the striatum, which is innervated by the cortex and subcortical structures, such as the thalamus. Corticostrial and thalamocortical pathways are excitatory glutamatergic pathways.

Brain gamma-amino-butyric acid (GABA) is used in the striatopallidarnom and pallidotalamicheskim ways [2, 8]. Thus, this model assumes that there are two ways between the striatum and the output nuclei of the basal ganglia. In Parkinson's disease, the loss of dopaminergic tone of the striatum leads to increased activation of the indirect pathway and a decrease in activity in the direct way, which ultimately leads to an increase and inadequate inhibition of thalamocortical neurons [8]. Surgical interventions circumvent the dopamine system and lead to a reduction in dysfunction in the indirect pathway. Therefore, a pharmacological intervention that mimics these surgical techniques can provide palliative care to a larger number of patients without the need for invasive surgery. In addition, treatment by bypassing the dopamine system should result in fewer side effects and can actually slow the disease process by normalizing the overactive glutamatergic entry into the dopamine-containing neurons of the midbrain [8, 9]. Previously, we identified a metabotropic glutamate receptor 4 (mGluR4) as a potential target for drugs and predicted that activation of mGluR4 could provide a palliative advantage in the treatment of Parkinson's disease [10, 11].

Objectives of the Study

Determine the mechanism of action of Rapitalam in vitro on a cell culture with mGluR4 over expression.

Materials and Methods

Cell line: The HEK293T cell line expressing human mGluR4 was used in the work.

Maintenance of the cell line:

Medium: DMEM (Hyclone, USA) with 5% FBS (Hyclone, USA), 1% essential amino acids, 2mM L-Glutamine, 1mM sodium pyruvate, 1% penicillin / streptomycin (Invitrogen), 0.4 mg / ml G418 (Sigma, USA).

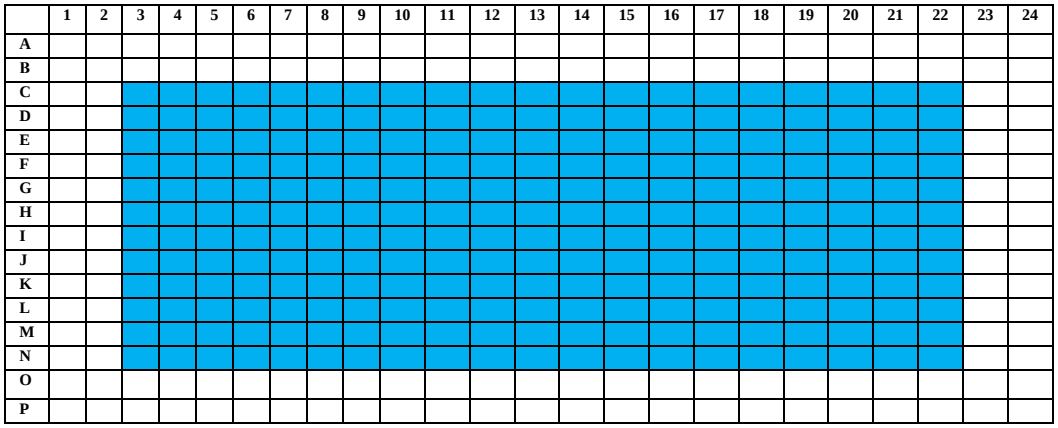
Cells are grown in culture flasks of 175 cm2 to 90% confluency. After the culture medium is removed by a vacuum pump, the cell layer is gently moistened with 0.53 mM EDTA solution to remove traces of the medium and incubated 5-10 minutes before the cells are removed from the substrate. 8 ml of culture medium is added to prepare the cell suspension by neat mixing. The cell suspension is centrifuged for 2 minutes at 120 g. The EDTA-containing medium is removed and replaced with 10 ml of fresh medium. The cells are placed in vials with a dilution of the original culture 4-10 times.

The cell concentration is calculated by a hemocytometer and the required volume of the cell suspension is adjusted with a culture medium without FBS to a concentration of 1000 cells / µl (giving a cell density of 25,000 cells per well) with the addition of aprotinin to a concentration of 10.5 mg / l. The cell suspension is transferred to 25 µl each well of the required number of culture plates with a poly-D-lysine coating using a Biomek NXp384 workstation. The plates are centrifuged at 50g for 30 "and transferred to a CO2 incubator (37 ° C in a humidified atmosphere with 5% CO2) for 12-16 hours.

On the day of the experiment, cells are "loaded" with Ca2 + -sensitive paint in accordance with the protocol of the kit manufacturer. Briefly, 20 ml of Essenia Buffer (HBSS) is added to dissolve the lyophilized Ca2 + -sensitive paint in the vial and the resulting solution is used as a 25X drain as recommended by the manufacturer. A 1x paint working solution is prepared by diluting a 25x stock solution in an Essenia buffer containing 0.2% pluronic acid F-127 and 5mM probenicide.The prepared 1x working solution is poured into the reservoir and transferred with a Biomek NXp384 workstation to 25 µl per each well of the culture plate with the cells placed in it on the previous day. Further, the plates with cells are transferred to storage in a CO2 incubator (37 ° C in a humidified atmosphere with 5% CO2) for 2 hours, after which the plates must be used directly for carrying out the essay on the FLIPR.

Preparation of dies with a solution of test compounds in DMSO:

The ratals were dissolved in DMSO to give a 30 mM solution. Further serial dilutions were made in 384-well plates with a dilution step of 3.16, 10 concentration points and two repetitions at each concentration point, respectively, in the following scheme (see Figure 1):



Columns 2.23 and rows B, O - DMSO

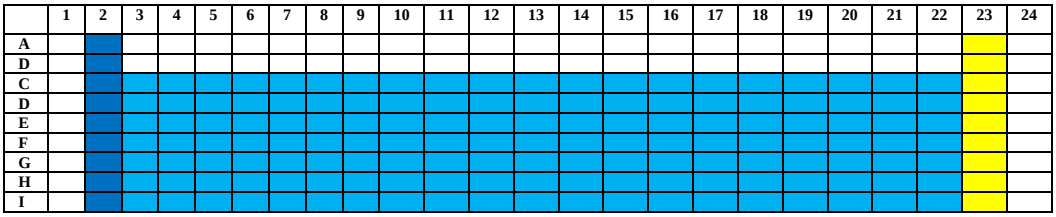
Columns 1.24 and rows A, P are not used in view of the presence of an edge effect.

Figure 1. Diagram of a die with compounds dissolved in DMSO

3-22 in rows C-N: serial dilutions of the compounds from 30 mM, 40 µl per well

Preparation of intermediate dissolution plates:

Using the Biomek FXp384 workstation, transfer 199 µl of Essenia buffer containing 0.1% pluronic acid F-127 to the required number of Axygen Scientific dies (VWR, Cat. No. 47743-466) from the reservoir. Using a Biomek FXp384 workstation, 1 µl from each well of the plate with substances dissolved in DMSO at a concentration of 30 mM is transferred to the corresponding wells of the plate containing 199 µl of Essenia buffer and mixed 6 times to obtain a 5x working solution of the substances. The control wells are filled according to the scheme (see Fig. 2):



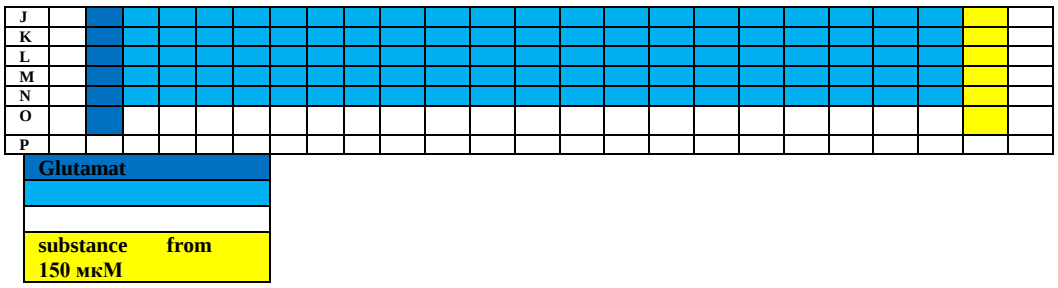


Figure 2. Diagram of the intermediate dissolution plate of the compounds.

Preparation of a plate with agonist:

Glutamate is dissolved in Essenia buffer containing 0.1% Pluronic acid F-127 to produce a 6x working solution (E

C20) and transferred from the reservoir using the Biomek FXp384 workstation to the Axygen Scientific plate. The scheme of filling holes in a plate with an agonist is given below (see Fig. 3):

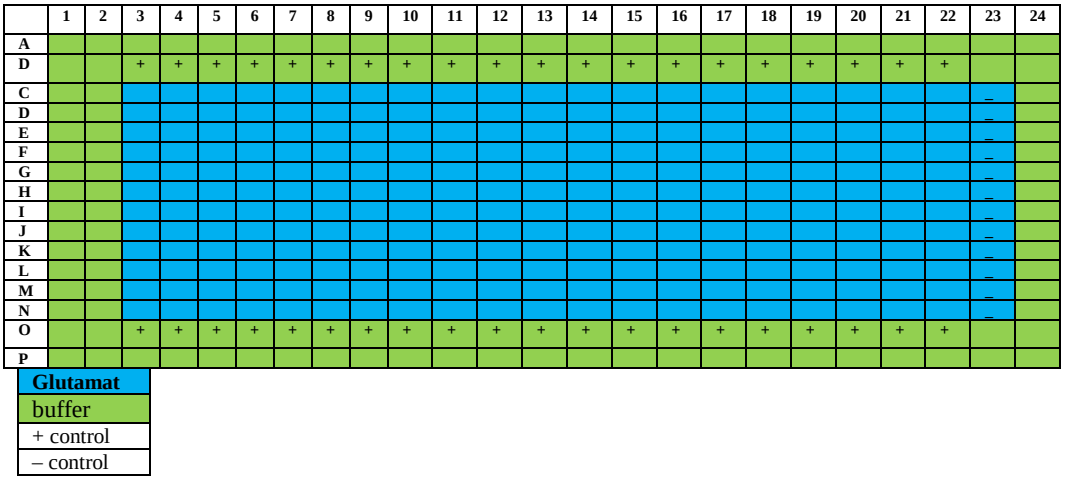


Figure 3. Diagram of a plate with an agonist. Reading plates on FLIPRtetra

For 12.5 µl of a 5 × solution from each well of a plate with intermediate dissolution of the substances is transferred by the FLIRPtetra device (Molecular Devices, CA) to the corresponding wells of the plate with the cells preloaded with Ca2 + -sensitive paint as described above and read for 0.1 sec every 1 second for 5 minutes and once every 6 seconds for 10 minutes to determine whether there is agonism or the cytotoxic effect of the analytes (total incubation time with the substances before adding the agonist 15 minutes). Further, 12.5 µl of 6 × Glutamate solution is transferred to the plate and the signal is read out for 0.1 second every 1 second for 5 minutes.

Parameters of control wells: Positive control of agonistic activity (EC100 Glutamate = 100% mGluR4 activation) - wells B2-O2. Negative control of agonist activity (0% mGluR4 activation - buffer): wells B23-O23. Total reaction volume: 75 µl. The final concentration of DMSO: 0.01%

Results

Receptor activity was evaluated on the culture of cells superexpressing the human mGluR4 receptor, according to the level of calcium in the cytoplasm, the release of which is stimulated by the addition of an agonist (glutamate). It can be seen that in itself, the Ratalam does not activate the release of calcium (Figure 4, black triangles). In this case, Rapitalam significantly increases the cellular response (Figure 4, blue) induced by the agonist (glutamate, Figure 4, red).

Thus, the obtained data prove the mechanism of the action of Rapitalam as a positive allosteric modulator of human mGluR4 receptor.

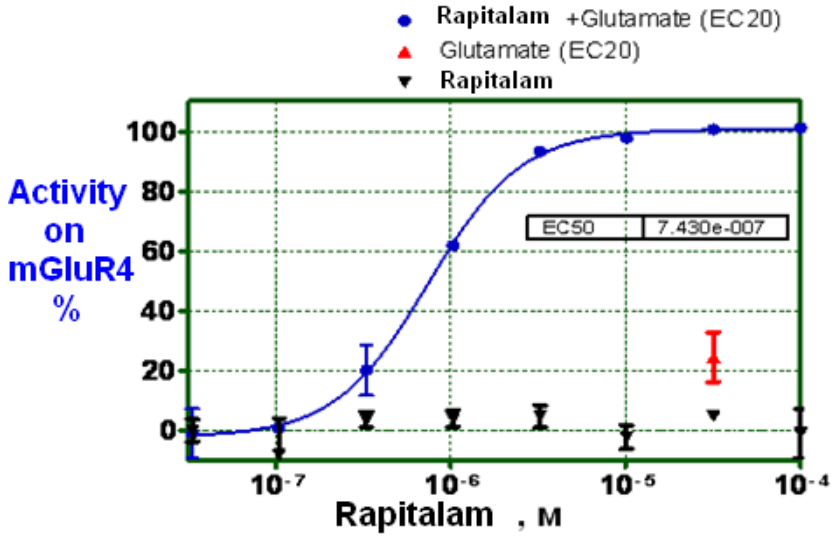


Figure 4. Rapitalam enhances the activity of glutamate. The experiment was performed on HEK293T - mGluR4 cells. Red cells in the presence of glutamate, black cells in the presence of Rapitalam, blue cells in the presence of Rapitalam and glutamate.

Conclusion

Studies of specific antiparkinsonian activity and the mechanism of action of the pharmaceutical substance, in vivo, have been carried out. On cell culture with mGluR4 overexpression, we have been shown that Rapitalam is a positive allosteric modulator of the mGluR4 receptor. The Rapitalam have a concentration-dependent increase in the cellular response induced by glutamate.

The search for new safe compounds that affect synaptic transmission is an important task of modern pharmacology [12]. In addition, the choice of adequate in vitro models can optimize and accelerate preclinical studies [13].

References

[1] Avdeeva, N.V., Nikitina, V.A., Kochkarova, I.S., Litvinova, A.S., 2016. The possibility of administration of glutamate receptors antagonists in the treatment of parkinson's disease. Research result: pharmacology and clinical pharmacology., 2(3): 86–94.

[2] Marino, M.J, Williams, D.L., Jr, O'Brien, J.A., et al., 2003. Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to Parkinson's disease treatment. Proc. Natl Acad. Sci. USA., 100(23):13668–13673.

[3] Chaudhuri, K.R., Healy, D.G., Schapira, A.H.V., 2006. Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol., 5(3):P. 235–245.

[4] Hopkins, C.R., Lindsley, C.W., Niswender, C.M., 2009. mGluR4-positive allosteric modulation as potential treatment for Parkinson's disease. Future Med Chem., 1(3):501–513.

[5] Schapira, A.H.V., 2009. Neurobiology and treatment of Parkinson's disease. Trends Pharmacol. Sci., 30(1): 41–47.

[6] Voronkov, A.V., Pozdnyakov, D.I. 2018. Endothelotropic activity of 4-hydroxy-3,5-di-tret-butylcinnamic acid in the conditions of experimental cerebral ischemia. Research Results in Pharmacology, 4(2):1-10.

[7] Breit, S., Schulz, J.B., Benabid, A.L., 2004. Deep brain stimulation. Cell Tissue Res., 318(1):275–288.

[8] Johnson, K. A., Conn, P. J., and Niswender, C. M., 2009. Glutamate receptors astherapeutic targets for Parkinson's disease. CNS and Neurological Disorders., 8(6):475–491.

[9] Marino, M.J, Conn, P.J., 2006. Glutamate-based therapeutic approaches: allosteric modulators of metabotropic glutamate receptors. Curr. Opin. Pharmacol., 6(1):98–102.

[10] Avdeeva, N.V., Kulikov, A.L., Pokrovskii, M.V., Avtina, T.V., 2016. Pharmacokinetic studies of new antiparkinsonian drug Rapitalam. Research result: pharmacology and clinical pharmacology., 2(4): 3–8.

[11] Marino, M.J., Hess, J.F., Liverton, N., 2005.Targeting the metabotropic glutamate receptor mGluR4 for the treatment of diseases of the central nervous system. Curr. Top. Med. Chem., 5(9):885–895.

[12] Bogus, S., Galenko-Yaroshevsky, P., Suzdalev, K., Sukoyan., G, Abushkevich, V., Soldatov V., 2018. 2-phenyl-1-(3-pyrrolidin-1-il-propyl)-1H-indole hydrochloride (SS-68): Antiarrhythmic and Cardioprotective Activity and Its Molecular Mechanisms of Action (Part II). Research Results in Pharmacology 4(3): 73-86. https://doi.org/10.3897/rrpharmacology.4.30329

[13] Dzhimak, S.S., Basov, A.A., Volchenko, N.N., Samkov, A.A., Fedulova L.V., Baryshev, M.G.,

2017. Changes in the functional activity of mitochondria isolated from the liver of rat that passed the preadaptation to ultra-low deuterium concentration. Doklady Biochemistry and Biophysics 476: 323–325. DOI: 10.1134/S1607672917050088