



# Pharmacological activity of new imidazole-4,5-dicarboxylic acid derivatives in dopaminergic transmission suppression tests in mice and rats

Ekaterina E. Yakovleva<sup>1</sup>, Eugeny R. Bychkov<sup>1</sup>, Maria M. Brusina<sup>1</sup>,  
Levon B. Piotrovsky<sup>1</sup>, Petr D. Shabanov<sup>1</sup>

<sup>1</sup> Institute of Experimental Medicine, St. Petersburg, Russia

Corresponding author: Ekaterina E. Yakovleva ([eeiakovleva@mail.ru](mailto:eeiakovleva@mail.ru))

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## Abstract

**Objective:** To study the antiparkinsonian activity of new 1,2-substituted imidazole-4,5-dicarboxylic acids in dopaminergic transmission suppression tests in mice and rats.

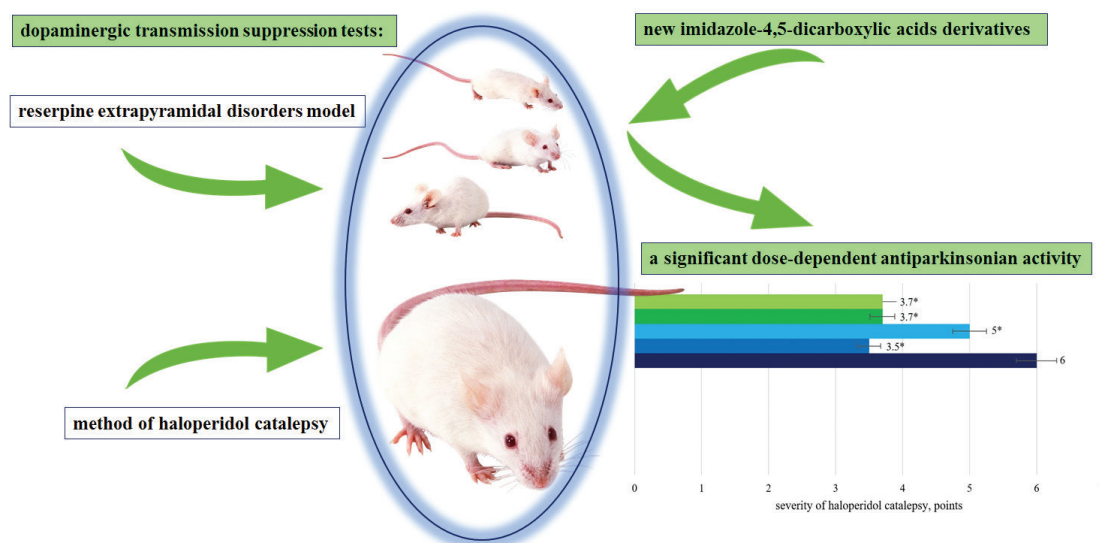
**Materials and methods:** On a model of reserpine extrapyramidal disorders, the derivatives of imidazole-dicarboxylic acids (IEM2258, IEM2248, IEM2247) were injected into the lateral brain ventricles of the mice 30 minutes after injecting **reserpine** at the doses of 0.1–0.5 mmol. Locomotor activity was analyzed in the Open-field test 2 hours later. In the catalepsy model, the studied agents were injected, using a pre-implanted cannula, with a simultaneous intraperitoneal injection of **haloperidol**. The severity of catalepsy was assessed with the Morpurgo method. **Amantadine** was used as a comparator drug in all the tests.

**Results:** It was shown that IEM2258 significantly increased the main indicators of locomotor activity in the Open-field test at all the studied doses. The value of the antiparkinsonian effect of IEM2258 at doses of 0.4–0.5 mmol significantly exceeded that of **amantadine**. The antiparkinsonian effect of IEM2247 was maximally expressed and was significantly different from those in the control and comparator group at doses of 0.2 and 0.3 mmol. For all the experimental groups, a significant decrease in the manifestations of catalepsy in comparison with control indexes was determined.

**Discussion:** The results made it possible to suggest the involvement of imidazole-4,5-dicarboxylic acids derivatives in the process of experimental improvement of dopaminergic neuromodulation and efficiency in animals.

**Conclusion:** The data showed a significant dose-dependent antiparkinsonian activity of new imidazole-4,5-dicarboxylic acid derivatives, which makes it promising to develop these agents and to further search for effective and safe antiparkinsonian drugs in this pharmacological class.

## Graphical abstract



## Keywords

Parkinson's disease, glutamate, NMDA-receptor antagonists, imidazole-dicarboxylic acid derivatives, antiparkinsonian activity.

## Introduction

It is known that the key mechanism for the development of Parkinson's disease (PD) is the degeneration of dopaminergic neurons of the substantia nigra; however, today researchers pay more attention to the involvement of other mediators in the pathogenesis of this disease, including glutamate (Freitas and Fox 2016; Mironova et al. 2018). It is assumed that the clinical efficiency of glutamate NMDA receptor antagonists is based on several complementary mechanisms: the close intertwining of dopamine and glutamatergic projections in the brain regions responsible for movement initiation and motor activity; the mutual regulation of presynaptic dopamine release by glutamate receptors and vice versa; and the undeniable evidence of the interaction of these mediator systems at the postsynaptic and systemic levels (Mingote et al. 2015; Bimpisidis and Wallén-Mackenzie 2019). However, despite the high efficiency of NMDA antagonists, only one non-competitive NMDA antagonist, **amantadine**, has been registered for PD therapy in Russia (Müller et al. 2019; Gonzalez-Latapi et al. 2020). This fact is due to the low safety of the developed pharmacological substances involved in the process of glutamatergic transmission. Glutamate excitotoxicity can not only trigger, but also aggravate the neurodegenerative process in PD (Mironova et al. 2018). In this regard, the problems associated with glutamatergic modulation are still relevant and consist in the extremely important role of glutamate and its receptors in the key functions of the central nervous system. That is why the priority direction for the development of

NMDA ligands remains the search for agents with soft, controlled and safe mechanism of glutamatergic modulation (Perfilova and Tjurenkov 2016). Recent studies have shown that mono- and di-substituted derivatives of imidazole 4,5-dicarboxylic acids (IDA) are of great interest in glutamatergic transmission. A distinctive feature of IDA derivatives is that these agents interact with the NMDA receptor recognition site and are not rigid channel blockers (Piotrovsky et al. 1999). The objective of the investigation was to study the antiparkinsonian activity of new 1,2-substituted imidazole-4,5-dicarboxylic acids in dopaminergic transmission suppression tests in mice and rats.

## Materials and methods

### Animals

The experiments were performed on white male mice weighing 18–25 g and Wistar male rats weighing 180–200 g, obtained from the Rappolovo Laboratory Animal Nursery (Leningrad region, Russia). The animals were kept in standard plastic cages in a vivarium with free access to water and food at a temperature of  $22 \pm 2$  °C and in the experiment were divided into several groups (6 animals in each group). All the experiments were conducted in the autumn-winter period. The animals were kept in accordance with the Good Laboratory Practice (GLP) standards. The experiments were performed in accordance with the principles of humanity (EU Directive No. 86/609 of the EU) and approved by the IEM Ethics Committee (No 12-19 of the Local Ethic Committee meeting of 10.10.18).

### Preparation and administration of the investigated agents

At the screening stage, the psychotropic activity of the studied substances was shown with systemic (intraperitoneal) administration; however, in order to establish and clearly demonstrate the central pharmacological activity, excluding the effect of the blood-brain barrier passing, in this study IDA derivatives: IEM2258, IEM2248 and IEM2247 – were dissolved in distilled water, adjusted with 0.5 n NaOH to pH = 7.0, and injected into the lateral ventricles (LV) of the brain of waking mice at doses of 0.1–0.5 mmol in 5 µl, using a stereotactic device (Lapin 1978).

In rats, IDA derivatives were injected into the LV of the brain using pre-implanted cannulas. Cannula implantation was performed under general anesthesia (xylazine 8 mg/kg + zoletil 40 mg/kg intraperitoneally), unilaterally using a stereotactic device (Medicor, Hungary), at the following coordinates: A = 0.8 mm back from the bregma, L = 1.5 mm laterally from the sagittal suture, H = 3.8 mm from the skull surface (Krasnova et al. 2000). The implanted cannulas were fixed on the skull with self-hardening plastic. After each experiment, all the animals were verified for the penetration of the tested substances into the LV of the brain (Efremov et al. 2005).

A non-competitive NMDA receptor antagonist **amantadine**, registered in Russia for the treatment of PD and parkinsonism syndrome (solution for infusions, administered to animals intraperitoneally), was used as a comparator drug in all the tests.

### Model of extrapyramidal disorders caused by reserpine

**Reserpine** was administered to mice intraperitoneal at a dose of 2.5 mg/kg in distilled water with Twin-80 to improve solubility 30 minutes before the administration of

the tested agents. Locomotor activity was analyzed in the Open field test and the severity of ptosis and hypothermia (indicators of autonomic (vegetative) nervous system dysfunction) were evaluated 2 hours after **reserpine** administration – a time period of a maximum decrease in the level of monoamines (Mironov et al. 2012).

### Method of haloperidol-induced catalepsy

Catalepsy in rats was induced by intraperitoneal administration of **haloperidol** at a dose of 1 mg/kg simultaneously with the introduction of IDA derivatives and a comparator drug. The severity of catalepsy was studied 120 minutes after **haloperidol** injection in points using the Morpurgo method, which consists in estimating the duration of a rat freezing in an unusual posture on the steps of various heights (Mironov et al. 2012).

### Statistical analysis

Statistical significance was established using one-way ANOVA measurements, followed by the Tukey-Kramer post hoc multiple comparison test. The data were expressed as the mean ± standard error of the mean (SEM). All the statistical analyses were conducted using Graph Pad Prism version 6.0 (Graph Pad Software, San Diego, California, USA). Statistical significance was assumed at p < 0.05.

## Results

On the reserpine model, 1,2-substituted imidazole-4,5-dicarboxylic acids showed different pharmacological activities. The results of the study are presented in Table 1.

Table 1 shows that intraperitoneal administration of **reserpine** led to experimental depletion of catecholamine

**Table 1.** Effect of Imidazole-4,5-dicarboxylic Acid Derivatives (IEM2258, IEM2248, IEM2247) and Amantadine on Locomotor Activity of Mice in the Open-field Test After Reserpine Administration

Animal groups	Locomotor activity index						Temperature °C	Ptosis. points
	Number of crossings in the external square	Number of crossings in the internal square	Number of hole sniffing	Number of rearing with support	Number of rearings without support	Grooming		
Intact	96.8 ± 25.7	17.7 ± 11.4	10.5 ± 5.9	36.7 ± 14.5	27.8 ± 19.1	12.2 ± 4.3	37.4 ± 0.18	0.1 ± 0.1
Control (reserpine)	0.17 ± 0.4*	0.2 ± 0.2*	0.1 ± 0.1*	0.2 ± 0.1*	0.2 ± 0.1*	0.67 ± 0.8*	32.4 ± 0.5*	3.0*
Amantadin	5.7 ± 4.4* <sup>a</sup>	1.8 ± 1.3* <sup>a</sup>	0.5 ± 0.4*	2.3 ± 1.9* <sup>a</sup>	0.2 ± 0.1*	0.7 ± 0.5*	33.3 ± 1.2*	2.5 ± 0.5* <sup>a</sup>
IEM2258 0.1 mmol)	2.8 ± 0.75* <sup>a</sup>	2 ± 1.26* <sup>a</sup>	0.67 ± 0.5*	0.2 ± 0.2* <sup>b</sup>	0.2 ± 0.1*	0.83 ± 0.4*	33.7 ± 0.2*	1.5 ± 0.7* <sup>ab</sup>
IEM2258 (0.2 mmol)	4.83 ± 2.5* <sup>a</sup>	2.83 ± 1.2* <sup>a</sup>	1.17 ± 1.17* <sup>a</sup>	0.17 ± 0.1* <sup>b</sup>	0.18 ± 0.1*	0.17 ± 0.1*	33.7 ± 0.2*	1.3 ± 0.7* <sup>ab</sup>
IEM2258 (0.3 mmol)	8.88 ± 1.5* <sup>a</sup>	3.6 ± 1.2* <sup>ab</sup>	2.12 ± 0.1* <sup>ab</sup>	0.16 ± 0.1* <sup>b</sup>	0.18 ± 0.1*	0.4 ± 0.1*	33.9 ± 0.1*	1.1 ± 0.6* <sup>ab</sup>
IEM2258 (0.4 mmol)	15.3 ± 4.7* <sup>ab</sup>	4.33 ± 2.58* <sup>ab</sup>	1.8 ± 1.3* <sup>ab</sup>	1 ± 0.8*	0.2 ± 0.2*	0.8 ± 0.3*	34.4 ± 0.8* <sup>a</sup>	1.1 ± 0.6* <sup>ab</sup>
IEM2258 (0.5 mmol)	19.8 ± 3.4* <sup>ab</sup>	13.7 ± 9.8* <sup>ab</sup>	5.5 ± 1.4* <sup>ab</sup>	3.44 ± 1.8*	2.7 ± 1.2*	1.8 ± 1.3*	35.4 ± 0.7* <sup>a</sup>	0.5 ± 0.5* <sup>ab</sup>
IEM2248 (0.1 mmol)	1.33 ± 1.2* <sup>ab</sup>	0.5 ± 0.5* <sup>b</sup>	0.2 ± 0.1*	0.1 ± 0.1* <sup>b</sup>	0.1 ± 0.1*	0.17 ± 0.1*	32.6 ± 1.1*	1.4 ± 0.2* <sup>ab</sup>
IEM2248 (0.2 mmol)	0.17 ± 0.4* <sup>b</sup>	0.33 ± 0.2* <sup>b</sup>	0.17 ± 0.1*	0.17 ± 0.1* <sup>b</sup>	0.17 ± 0.1*	0.67 ± 0.5*	33.8 ± 0.1*	1.2 ± 0.7* <sup>ab</sup>
IEM2248 (0.3 mmol)	0.29 ± 0.4* <sup>b</sup>	0.2 ± 0.1* <sup>b</sup>	0.37 ± 0.1*	0.2 ± 0.1* <sup>b</sup>	0.17 ± 0.2*	0.82 ± 0.5*	33.6 ± 2.1*	1.2 ± 1.2* <sup>ab</sup>
IEM2248 (0.4 mmol)				↑RR. convulsions. sprawling posture				
IEM2248 (0.5 mmol)				↑RR. convulsions. sprawling posture				
IEM2247 (0.1 mmol)	4.8 ± 2.2* <sup>a</sup>	0.83 ± 0.4* <sup>a</sup>	1.33 ± 1.1* <sup>a</sup>	0.67 ± 0.5*	0.33 ± 0.2*	0.33 ± 0.1*	33.8 ± 0.4*	2.1 ± 0.5* <sup>a</sup>
IEM2247 (0.2 mmol)	18.8 ± 6.11* <sup>ab</sup>	10.8 ± 1.5* <sup>ab</sup>	7.67 ± 3.4* <sup>ab</sup>	0.5 ± 0.36* <sup>ab</sup>	0.2 ± 0.1*	0.67 ± 0.4*	34.3 ± 1.6* <sup>a</sup>	1.6 ± 0.7* <sup>ab</sup>
IEM2247 (0.3 mmol)	11.8 ± 4.1* <sup>ab</sup>	3.8 ± 1.5* <sup>ab</sup>	2.63 ± 0.4* <sup>ab</sup>	0.5 ± 0.1* <sup>b</sup>	0.2 ± 0.1*	0.62 ± 0.5*	34.4 ± 0.6* <sup>a</sup>	1.7 ± 0.2* <sup>ab</sup>
IEM2247 (0.4 mmol)	5.5 ± 3.6* <sup>a</sup>	1.8 ± 0.9* <sup>a</sup>	1.8 ± 1.3* <sup>ab</sup>	1 ± 0.8*	0.2 ± 0.2*	0.8 ± 0.3*	34.3 ± 0.8* <sup>a</sup>	1.33 ± 0.5* <sup>ab</sup>
IEM2247 (0.5 mmol)	5.4 ± 2.2* <sup>a</sup>	1.1 ± 0.4* <sup>a</sup>	0.8 ± 0.7* <sup>ab</sup>	1.6 ± 0.4*	0.17 ± 0.2*	0.7 ± 0.3*	34.5 ± 1.5* <sup>a</sup>	1.2 ± 0.3* <sup>ab</sup>

Note: n = 6, \* – p < 0.05 compared with the intact group of animals; <sup>a</sup> – p < 0.05 compared with the control group of animals; <sup>b</sup> – p < 0.05 compared with the group of animals treated with amantadine; RR – respiration rate.

reserves and simulated a dopaminergic deficit with reduced motor activity in the animals and the development of autonomic disorders. The number of crossings in the external and internal squares of the open field decreased by 569 and 88.5 times, respectively, in comparison with the intact animals' indexes. There was also a significantly reduced number of rearings, hole sniffings and grooming acts. The mice body temperature decreased in the reserpine group by 5 °C, while the level of ptosis increased by 2.9 points.

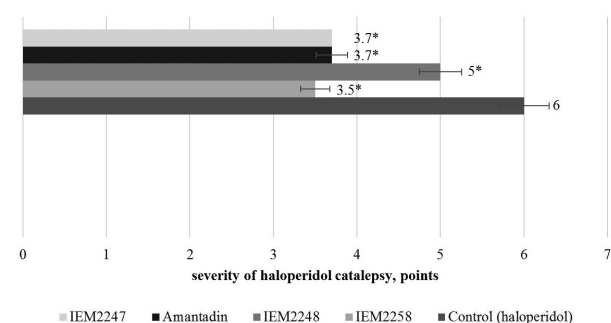
The results of the experiment showed that intraventricular administration of the IEM2258 against the background of reserpine led to a significant increase in the number of crossings in the external and internal squares of the open field in all the studied doses (0.1–0.5 mmol), compared with the control animals which had only received reserpine. Locomotor activity in the group treated with IEM2258 rose proportionally to a dose increase. In the mice receiving IEM2258 at doses of 0.4 and 0.5 mmol, locomotor activity in the external sector exceeded that in the control by 90 and 116 times and that in the amantadine-treated group by 2.7 and 3.5 times, respectively. The same trend was noted during the investigation of the animal motor activity in the central square of the open field: the number of lines crossed by the mice receiving IEM2258 at doses of 0.4 and 0.5 mmol was 21.6 and 68.5 times higher than that in the control animals, and 0.7 and 7.6 times higher than that in the amantadine-treated group, respectively. The number of hole sniffings was also dose-dependent: it was the highest at a dose of 0.5 mmol and exceeded that in the control and comparison group indexes by 55 and 11 times, respectively. The autonomic nervous system indicators also indicated the protective effect of IEM2258: at doses of 0.4 and 0.5 mmol, the mice body temperature increased (by 2 and 3 °C, respectively), and the level of ptosis decreased (by 1.4 and 2.5 scores, respectively), compared with the control values. In addition, the ptosis level upon IEM2258 administration had significant differences with the corresponding indicators in the amantadine-treated group in all the studied doses.

The antiparkinsonian effect in the group receiving IEM2247 was the most expressed in a dose of 0.2 mmol: the number of crossings in the external and internal squares was 110.5 and 54 times higher than that in the control and 3.2 and 6 times higher than that in the amantadine group. Significant differences in comparison with the control group and the comparator group are also shown in relation to the number of hole sniffings and autonomic indicators: temperature and a ptosis level. Substantial preventive action against monoamine depletion (significantly higher than that in the amantadine group) was also identified for IEM2247 at a dose of 0.3 mmol. With a further increase in the dose of IEM2247, the antiparkinsonian effect persisted, but was less pronounced.

The administration of IEM2248 in this test did not lead to manifestation of any significant antiparkinsonian effect: the locomotor activity indexes in the animals recei-

ving this substance differed slightly from the control values. In addition, the administration of IEM2248 into the mice brain lateral ventricles at doses of 0.4 and 0.5 mmol led to the development of subtoxic reactions: increased respiratory rate, tachycardia and convulsions which made it impossible to assess an antiparkinsonian activity.

On the model of catalepsy, the rats that received only haloperidol got 6 points by the Morpurgo catalepsy scale. The average score in IEM2258 group was  $3.5 \pm 0.5$  points; in IEM-2247 and amantadine group –  $3.7 \pm 0.5$  points, which indicates a significantly less severe catalepsy in these experimental groups (Fig. 1). The poorest antiparkinsonian activity in the haloperidol catalepsy test was identified in the group of rats receiving IEM2248, where the average score was  $5 \pm 0.9$  points, which correlates with the test data in mice with reserpine depression.



**Figure 1.** Effect of imidazole-4,5-dicarboxylic acid derivatives (IEM2258, IEM2248, IEM2247) and amantadine on the severity of haloperidol catalepsy in rats. **Note:** n = 6; the dose of IEM2258 – 0.4 mmol; the dose of IEM2248 – 0.2 mmol; the dose of IEM2247 – 0.2 mmol; \* – p < 0.05 compared with the group of animals receiving haloperidol.

Thus, the data indicate that new imidazole-4,5-dicarboxylic acid derivatives exhibit a substantial antiparkinsonian activity, preventing excessive dopaminergic transmission blockade, which confirms the literature data about the considerable involvement of the glutamatergic system and, in particular, NMDA-receptor transmission in the processes of dopaminergic regulation and pathophysiological mechanisms of Parkinson's disease.

## Discussion

Today along with the theory of impaired dopaminergic neurotransmission, an important role of NMDA-specified excitotoxicity in the development of PD is discussed (Ambrosi et al. 2014; Himmelberg et al. 2018; Mironova et al. 2018).

A considerable difference between NMDA-glutamate receptors and other ionotropic receptors is that NMDA-receptor channel transmits not only Na<sup>+</sup> and K<sup>+</sup> ions, but also Ca<sup>2+</sup> and works as a secondary intermediary that can modulate the cell response depending on an external signal (Zhang et al. 2019). The highest density of

NMDA receptors is found in the hippocampus, cerebral cortex, amygdala, and striatum (Furuyama et al. 1993). This is probably why the activation of NMDA receptors in physiological conditions is associated with the plasticity of structures in central nervous system, learning and memory processes (Hansen et al. 2018). However, in pathology, the same receptors can be activated by smaller, micromolar concentrations of glutamate (Perrella and Bhavnani 2005). Hyperactivation of ionotropic glutamate receptors leads to a sharp increase in transmembrane calcium current inside the cell, followed by the release of  $Ca^{2+}$  from intracellular depots, depolarization of the mitochondrial membrane, and, as a result, a long-term increase in the amount of  $Ca^{2+}$  in the cytoplasm. High  $Ca^{2+}$  concentration in neurons triggers neurotoxic processes with activation of proteolytic enzymes and cellular structures destruction, which eventually leads to increased synthesis of nitric oxide, activation of lipid peroxidation and, as a result, to oxidative stress, impaired synthesis of neurotrophic factors, and apoptosis (Ureshino et al. 2019). Thus, the effect on the process of glutamate excitotoxicity, which causes and intensifies the neurodegenerative process in PD, is one of the most important directions of pharmacological correction of this pathology.

PD is traditionally considered as a disease that affects mainly the motor sphere (hypokinesia, rigidity, resting tremor – classic motor symptoms of PD), but in the clinical practice, there are also non-motor manifestations of PD. Moreover, some non-motor symptoms (vegetative, dissomnic, sensory, etc.) may dominate in the clinical picture, having a negative impact on the quality of life of patients (Levin 2002; Karaban 2011; Pfeiffer 2016). Low-affinity channel NMDA blockers, including amantadine, were first NMDA receptor antagonists, which were successfully used for treating Parkinson’s disease (Kulisevsky et al. 2018). These drugs stimulate the release of dopamine from neuronal depots, increase the sensi-

tivity of receptors to dopamine, and inhibit the process of mediator reuptake in the neurons (Inzelberg et al. 2006). It was shown that amantadine significantly weakens the excitatory glutamate corticostriate effects on cholinergic neurons, due to which glutamate receptor antagonists are classified also as indirect anticholinergic agents and neuroprotectors (Fig. 2). Amantadine is prescribed in monotherapy in the treatment of the initial stages of PD and is used in a complex therapy to reduce the daily dose of levodopa and the severity of side effects, such as dyskinesia (Oertel and Schulz 2016).

A number of NMDA receptor antagonists with various mechanisms of interaction with glutamate receptors, besides amantadine, have undergone and are undergoing preclinical and clinical trials (Vanle et al. 2018). However, at present, there is no complete understanding of the role of glutamate as a neurotransmitter and neurotoxin in the pathogenesis of PD. At the same time, it is known that all brain cells have glutamate receptors, and many neurons use glutamate as a neurotransmitter, so any change in the activity of glutamate receptors inevitably affects important structural and functional parameters of brain activity. Therefore, the pharmacological influence on glutamate receptors should be mild and safe.

In the study of new derivatives of IDA used for the synthesis of drugs, such as etymizol and cardosal, it was found that these substances had their own pharmacological activity, in particular, the effect on NMDA-ergic transmission. The advantage of these pharmacological agents is their interaction with the NMDA receptor recognition site, in contrast to non-selective NMDA receptor channel antagonists, which have significant side effects. The assumption that IDA derivatives do not belong to channel blockers was confirmed by the radioligand method. To determine potential competitive interactions of the studied substances with the NMDA receptor (IC50), a series of experiments on radioligand binding, using a tritium la-

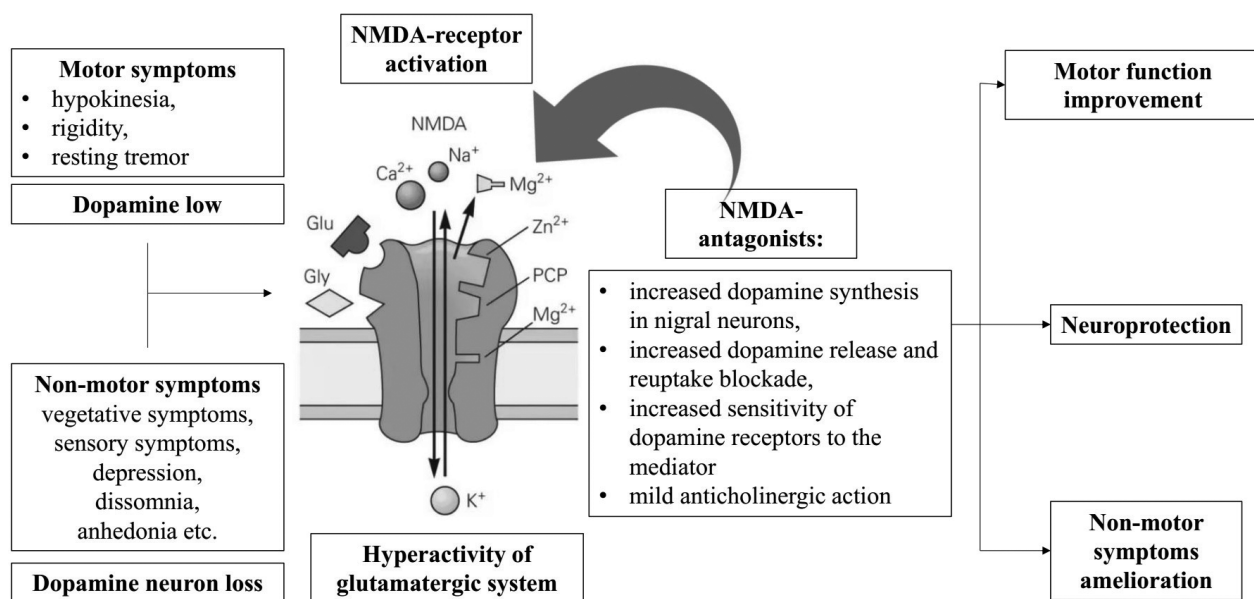


Figure 2. Antiparkinsonian action of NMDA antagonists.

beled MK 801(+), was conducted. It was established that the concentration of half-maximal inhibition of IDA derivatives is  $>100 \mu\text{mol/L}$ , regardless of the length, nature and position of the substituent in the imidazole cycle, in contrast to  $\text{IC}_{50} \text{ MK 801} = 7 \cdot 10^{-3} \mu\text{mol/L}$ .

Accordingly, considering potentially high safety of new IDA derivatives, we have obtained the substantial results, indicating the ability of new imidazole-4,5-dicarboxylic acid derivatives to have a significant controlled dose-dependent antiparkinsonian effect. In the model of experimental monoamine depletion, locomotor activity of the mice receiving IEM2258 and IEM2247 in all the studied doses was significantly higher in comparison to that in the control group, and increased proportionally to a dose increase. The introduction of IEM 2258 at doses of 0.4–0.5 mmol and IEM2247 at doses of 0.2–0.3 mmol led to an increase in the main locomotor activity indexes (number of crossings in the external and internal squares of the open field, number of hole sniffings) to the levels significantly higher than those in the group of the animals treated with comparator drug **amantadine**. The characteristics of vegetative reactions, such as body temperature (at doses of 0.4–0.5 mmol for IEM2258 and 0.2–0.5 mmol for IEM2247) and the level of ptosis (for all the studied doses of IEM2258 and IEM2247) also significantly differed from the characteristics of the control group. In the

model of haloperidol-induced catalepsy, there were also significant differences for IEM2248 and IEM2247 from the control animals in the scores characterizing the severity of catalepsy. Thereby, both models of dopaminergic transmission suppression made it possible to suggest the involvement of new glutamate-sensitive ligands – imidazole-4,5-dicarboxylic acids derivatives – in the process of experimental improvement of dopaminergic neuromodulation and their pharmacological effectiveness in animals.

## Conclusion

The data obtained in the tests based on inhibition of dopaminergic transmission (a model of non-selective extrapyramidal disorders caused by **reserpine**, and a method of haloperidol catalepsy) showed a significant dose-dependent antiparkinsonian activity of new imidazole-4,5-dicarboxylic acids derivatives, which makes it promising to develop these agents and further search for effective and safe antiparkinsonian drugs in this pharmacological class.

## Conflict of interests

The authors declare no conflict of interests.

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## Author contributors

- **Ekaterina E. Yakovleva**, PhD in Medicine, research fellow of The Laboratory of Chemistry and Pharmacology of Drugs, Department of Neuropharmacology, Institute of Experimental Medicine; e-mail: [eeiakovleva@mail.ru](mailto:eeiakovleva@mail.ru), **ORCID ID** <https://orcid.org/0000-0002-0270-0217> (main idea, experimental work, obtaining the results, statistical analysis, writing the article).
- **Eugeny R. Bychkov**, PhD in Medicine Head of The Laboratory of Chemistry and Pharmacology of Drugs, Department of Neuropharmacology, Institute of Experimental Medicine; e-mail: [bychkov@mail.ru](mailto:bychkov@mail.ru), **ORCID ID** <http://orcid.org/0000-0003-1068-4701> (organization of the experimental study, experimental work).
- **Maria A. Brusina**, PhD in Chemistry, junior research fellow of The Laboratory for the Synthesis and Nanotechnology of Drugs, Department of Neuropharmacology, Institute of Experimental Medicine, e-mail: [mashasemen@gmail.com](mailto:mashasemen@gmail.com) (synthesis of the studied substances).
- **Levon B. Piotrovsky**, Doctor Habil. of Biological Sciences, Head of The Laboratory for the Synthesis and Nanotechnology of Drugs, Department of Neuropharmacology, Institute of Experimental Medicine, e-mail: [levon-piotrovsky@yandex.ru](mailto:levon-piotrovsky@yandex.ru) (main chemical idea, organization of the chemical work).
- **Petr D. Shabanov**, Doctor Habil. of Medicine, Professor, Head of Department of Neuropharmacology, Institute of Experimental Medicine; Head of Department of Pharmacology, Kirov Military Medical Academy, e-mail: [pdshabanov@mail.ru](mailto:pdshabanov@mail.ru), **ORCID ID** <https://orcid.org/0000-0003-1464-1127> (general supervision of the experiment, proofreading).