



# Investigation of antiparkinsonian activity of new imidazole-4,5-dicarboxylic acid derivatives on the experimental model of catalepsy

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Academic editor: Oleg Gudyrev ♦ Received 04 November 2022 ♦ Accepted 05 March 2023 ♦ Published 31 March 2023

**Citation:** Dergachev VD, Yakovleva EE, Brusina MA, Bychkov ER, Piotrovskiy LB, Shabanov PD (2023) Investigation of antiparkinsonian activity of new imidazole-4,5-dicarboxylic acid derivatives on the experimental model of catalepsy. *Research Results in Pharmacology* 9(1): 41–47. <https://doi.org/10.18413/rrpharmacology.9.10006>

## Abstract

**Introduction:** To study the antiparkinsonian activity of new ligands of the glutamate NMDA receptor complex – 1,2-substituted imidazole-4,5-dicarboxylic acids – on an experimental model of catalepsy caused by haloperidol intraabdominal injections in rats.

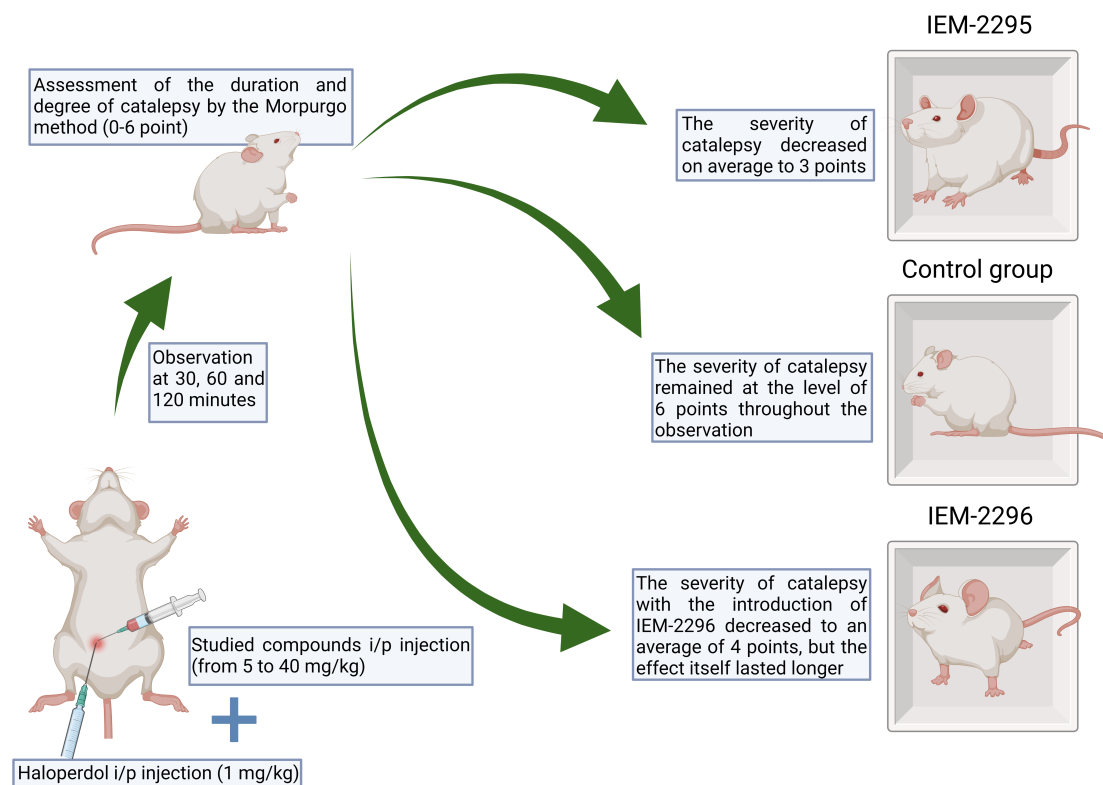
**Materials and methods:** The experiments were performed on Wistar rats weighing 300-350 g, obtained from the Rappolovo nursery of the Russian Academy of Medical Sciences (Leningrad Region). The animals were kept in standard plastic cages in vivarium conditions with free access to water and food at a temperature of 22±2 °C and in the experiment were divided into several groups (6 animals each). All the experiments were carried out in the autumn-winter period. The animals were kept in accordance with the rules of laboratory practice (GLP), regulatory documents "Sanitary Rules for the Device, Equipment and Maintenance of Vivarium" and the Order of the Ministry of Health and Social Development of the Russian Federation dated 23.08.2010 No. 708n "On Approval of the Rules of Laboratory Practice". Imidazole-dicarboxylic acid derivatives (IEM-2295, IEM-2296) were injected intraperitoneally at doses from 5 mg/kg to 40 mg/kg simultaneously with haloperidol at a dose of 1 mg/kg, after which the duration and severity of catalepsy were evaluated after 30, 60, 120 minutes from 0 to 6 points according to the Morpurgo method.

**Results:** The severity of catalepsy with the injection of IEM-2295 decreased on average to 3 points, while in the control group it remained at the level of 6 points throughout the observation. However, the severity of catalepsy with the introduction of IEM-2296 decreased to an average of 4 points, but the effect itself lasted longer than with the introduction of IEM-2295. Thus, it was noted that by the 120th minute of observation, the severity of catalepsy in rats receiving the IEM-2295 compound averaged 5 points, whereas in animals receiving IEM-2296 – 3 points.

**Discussion:** Basing on the results of our work and similar experiments, we can conclude that the studied compounds, which are not channel blockers, have an active effect on dopaminergic neurotransmission, because of which the symptoms of catalepsy that occur when haloperidol is injected to rats were stopped to one degree or another.

**Conclusion:** The studied substances exhibit antiparkinsonian activity on an experimental haloperidol model of catalepsy in rats and are promising for development as potential therapies for neurodegenerative diseases. Further study of these compounds and other ligands from the NMDA-blocker group in a wider sample on the catalepsy model, as well as on other models of Parkinsonism, is required.

## Graphical Abstract



## Keywords

NMDA receptor antagonists, [haloperidol](#), [dopamine](#), parkinsonism.

## Introduction

Parkinson's disease (PD) is one of the urgent problems of world science and medicine. Along with secondary parkinsonism, PD is the most common neurodegenerative disease in the seniors the progression of which leads to disability (Poewe et al. 2017; Chia et al. 2020). Nowadays due to the increase in life expectancy, the tendency to shift middle age towards the seniors population, the number of patients with Parkinsonism is constantly growing, which increases the urgency of finding effective and safe therapy for this neurological pathology (Draoui et al. 2020).

More than 50 years have passed since the first use of [levodopa](#), but it remains the most effective option for drug therapy. All this time, levodopa-induced complications have been the object of careful study and attempts to find a solution to this problem. The arsenal of medical treatment methods aimed at optimizing the motor control of progressive PD is constantly expanding (Aradi and Hauser 2020). The basis for effective lifelong therapy of PD is an individual approach to the treatment of patients, taking into account the peculiarities of the clinical picture, concomitant diseases and other factors (Fox et al. 2018; Jankovic and Tan 2020; Tarakad 2020). Therapeutic strategies for PD are aimed at a number of symptoms, including impaired

motor functions - bradykinesia, rigidity and tremor, as well as relief of complications of long-term use of [levodopa](#) (Kulisevsky et al. 2018).

Pharmacological groups, such as central dopaminomimetics (dopamine receptor agonists, NMDA receptor antagonists), central holinoblockers and drugs that restore [dopamine](#) levels (COMT inhibitors, MAO inhibitors, including [levodopa](#) drugs), are used as medicines aimed at correcting extrapyramidal disorders in PD (Chou et al. 2018; Aradi and Hauser 2020). Unfortunately, modern research shows that long-term combination therapy, helping to cope with the main manifestations of PD, inevitably leads to side effects, such as drowsiness, hallucinations, dyskinesia, nausea, dizziness, constipation and many others (Li et al. 2017). In this regard, the search for effective antiparkinsonian drugs devoid of the above adverse reactions remains an urgent task of modern neuropharmacology.

NMDA receptors are ion channels containing tetrameric groupings of GluN1 and GluN2 receptor subunits that mediate excitatory neurotransmission in the central nervous system. NMDA receptors containing the GluN2D subunit are most relevant as a target for antiparkinsonian therapy due to their expression in some nuclei of the basal ganglia, which demonstrate abnormal

excitation patterns in parkinsonism, in particular in the subthalamic nucleus (Standaert et al. 1994, Bhattacharya et al. 2018). It is proved that glutamate NMDA receptors play a significant role in the development of Parkinsonism, which makes it possible to consider the development of NMDA ligands as one of the priority areas of PD therapy (Haas et al. 2018; Vanle et al. 2018; Groc and Choquet 2020; Vieira et al. 2020).

Excessive activity of NMDA receptors causes a state of excitotoxicity and promotes cell death, which underlies the potential mechanism of neurodegeneration. Activation of synaptic NMDA receptors initiates plasticity and stimulates cell survival. On the contrary, activation of non-synaptic NMDA receptors promotes cell death and thereby contributes to the development of neurodegeneration, which can be stopped by the action of NMDA receptor antagonists selectively blocking their function (Wang and Reddy 2017).

One of such drugs registered in Russia for the treatment of PD is the non-competitive NMDA blocker amantadine, while various NMDA receptor antagonists are undergoing preclinical and clinical trials (Müller et al. 2019). The first results of the study of the antiparkinsonian activity of aminoadamantane (amantadine) in PD were published in 1969. After 50 years, the drug is still available worldwide and is indicated for the treatment of PD, both in the early and late stages. Amantadine has a unique dual (dopaminergic and non-dopaminergic) pharmacodynamic profile, which makes this drug the only representative of the original class of antiparkinsonian drugs. Initially, an assumption was made about the effect of amantadine on the release and/or reuptake of dopamine, subsequently its uncompetitive antagonism to glutamate NMDA receptors was revealed (Schwab 1969; Perez-Lloret and Rascol 2018; Rascol et al. 2020).

In addition, it is believed that another mechanism of antiparkinsonian action of NMDA receptor antagonists is a decrease in dopamine-dependent acetylcholine release in the striatum. An imbalance of inhibitory and excitatory effects on GABAergic neurons under the tonic influence of dopaminergic projections of the substantia nigra, glutamatergic projections of the cortex and cholinergic projections of the striatum leads to increased dopamine-dependent release of acetylcholine in the striatum. Thus, NMDA blockers have an indirect cholinergic effect, preventing synaptic release of acetylcholine and thus exhibiting antiparkinsonian activity (Iakovleva et al. 2020). This mechanism was studied and described earlier, using a model based on the activation of the cholinergic system (arecoline-induced tremor model) (Dergachev et al. 2021).

It should be remembered at the same time that in pathology, hyperactivation of ionotropic glutamate receptors leads to a sharp increase in transmembrane calcium current into the cell, followed by the release of

Ca<sup>2+</sup> from intracellular depots, depolarization of the mitochondrial membrane and, as a consequence, a prolonged increase in the amount of Ca<sup>2+</sup> in the cytoplasm. The high content of Ca<sup>2+</sup> in neurons triggers neurotoxic processes with activation of proteolytic enzymes and destruction of cellular structures, which eventually leads to increased synthesis of nitric oxide, activation of lipid peroxidation and, consequently, to oxidative stress, impaired synthesis of neurotrophic factors and apoptosis (Szydłowska and Tymianski 2010). It follows from this that glutamate excitotoxicity can not only trigger, but also aggravate the neurodegenerative process in PD (Mironova et al. 2018). In this regard, the safety 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 of the development of compounds from the group of NMDA ligands is the search for means to influence the glutamatergic system by soft, controlled, and safe modulation.

## Materials and Methods

### Animals

The experiments were performed on Wistar rats weighing 300-350 g, obtained from the Rappolovo nursery of the Russian Academy of Medical Sciences (Leningrad Region). The animals were kept in standard plastic cages in vivarium conditions with free access to water and food at a temperature of 22±2 °C and in the experiment were divided into several groups (6 animals each). All the experiments were carried out in the autumn-winter period. The animals were kept in accordance with the rules of laboratory practice (GLP), regulatory documents "Sanitary Rules for the Device, Equipment and Maintenance of Vivarium" and the Order of the Ministry of Health and Social Development of the Russian Federation dated 23.08.2010 No. 708n "On Approval of the Rules of Laboratory Practice".

**The studied compounds:** IEM-2295, IEM-2296 – are the derivatives of imidazole-4,5-dicarboxylic acid.

### Experimental model

A model of catalepsy caused by haloperidol was chosen as a model for studying antiparkinsonian activity, which consists in stimulating dopaminergic transmission of the studied compounds (Mironov et al. 2012). This model is a proven method of studying extrapyramidal disorders, which is used everywhere (Cieślak et al. 2019; Ramírez-Jarquín et al. 2020; Cárcel and De la Casa 2021).

Parkinsonism caused by the use of neuroleptics is the second most common pathogenetic variant in the world after PD. Haloperidol is a first-generation neuroleptic commonly used for the relief of acute and chronic psychoses, the treatment of behavior disorders, personality changes and other psychopathologies. The mechanism of action of haloperidol is associated with

the blockade of dopamine receptors, central alpha-adrenoblocking action and disruption of the process of reverse neuronal capture and deposition of adrenaline. Unfortunately, the use of **haloperidol** is limited by the side effects of the drug, in particular extrapyramidal disorders (Kabra et al. 2020).

Imidazole-dicarboxylic acid derivatives (IEM-2295, IEM-2296) were injected intraperitoneally at doses from 5 mg/kg to 40 mg/kg simultaneously with **haloperidol** at a dose of 1 mg/kg, after which the duration and severity of catalepsy were evaluated after 30, 60, 120 minutes from 0 to 6 points according to the Morpurgo method (Mironov et al. 2012).

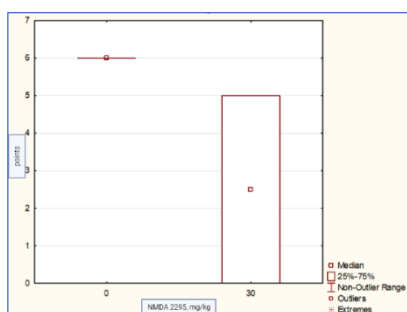
### Statistical analysis

Statistical processing of the results was carried out using

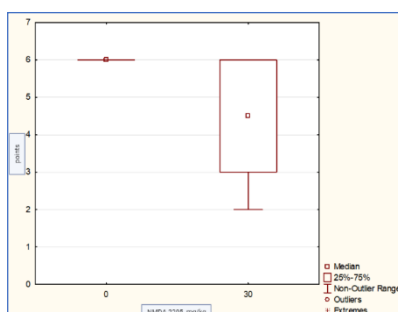
MS Excel 2010 and BioStat 2009. The normality of the data distribution was determined by the Shapiro-Wilk criterion. The reliability of the differences in values between the groups was defined using nonparametric criteria: Kruskal-Wallis and Fisher's exact criterion.

## Results

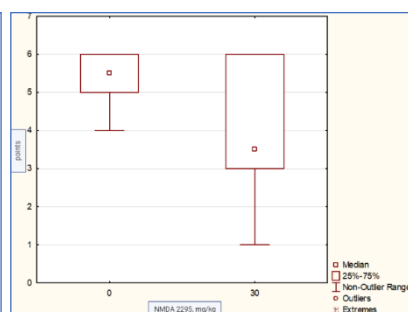
It is necessary to accentuate a concentration of 30 mg/kg with the injection of the test substance IEM-2295. Against the background of the injection of the test agent at this dose, there was a significant difference from the control group at 30<sup>th</sup> min ( $p=0.002671$ ), 60<sup>th</sup> min ( $p=0.028441$ ) and 120<sup>th</sup> min ( $p=0.027851$ ) of observation (Figs 1, 2, 3).



**Figure 1.** Differences in the severity of catalepsy between the control group and the group receiving IEM-2295 at a concentration of 30 mg/kg at the 30<sup>th</sup> minute of the experiment. ( $p=0.002671$ ).



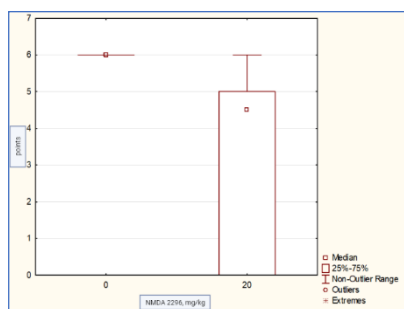
**Figure 2.** Differences in the severity of catalepsy between the control group and the group receiving IEM-2295 at a concentration of 30 mg/kg, at the 60<sup>th</sup> minute of the experiment. ( $p=0.028441$ ).



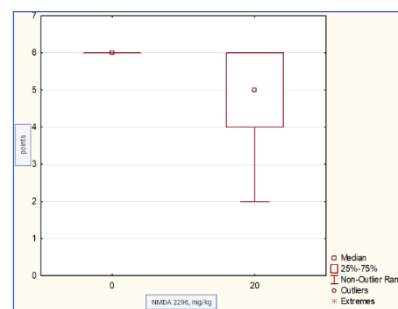
**Figure 3.** Differences in the severity of catalepsy between the control group and the group receiving IEM-2295 at a concentration of 30 mg/kg at the 120<sup>th</sup> minute of the experiment. ( $p=0.027851$ ).

It is necessary to accentuate a concentration of 20 mg/kg with the injection of the test substance IEM-2296. Against the background of the injection of the test agent at this dose, there was a significant

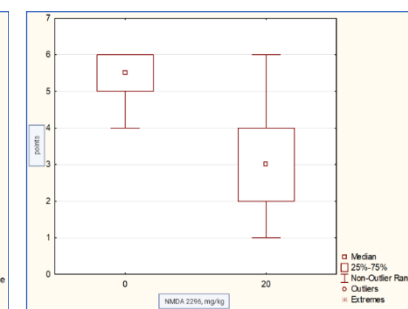
difference from the control group at 30<sup>th</sup> min ( $p=0.020241$ ), 60<sup>th</sup> min ( $p=0.045553$ ) and 120<sup>th</sup> min ( $p=0.045328$ ) of observation, as evidenced by the data presented in Figures 4, 5 and 6.



**Figure 4.** Differences in the severity of catalepsy between the control group and the group receiving IEM-2296 at a concentration of 20 mg/kg, at the 30<sup>th</sup> minute of the experiment. ( $p=0.020241$ ).



**Figure 5.** Differences in the severity of catalepsy between the control group and the group receiving IEM-2296 at a concentration of 20 mg/kg, at the 60<sup>th</sup> minute of the experiment. ( $p=0.045553$ ).



**Figure 6.** Differences in the severity of catalepsy between the control group and the group receiving IEM-2296 at a concentration of 20 mg/kg, at the 120<sup>th</sup> minute of the experiment. ( $p=0.045328$ ).

## Discussion

The first NMDA receptor antagonists for the treatment of Parkinson's disease were successfully used low-affinity channel blockers amantadine and memantine (Danysz et al. 1997). The clinical efficacy of NMDA receptor antagonists is obviously based on several complementary mechanisms: firstly, the close interweaving of dopamine and glutamatergic projections in the brain regions responsible for the initiation of movements and motor activity; secondly, the mutual regulation of presynaptic dopamine release by glutamate receptors and vice versa; and thirdly, there is indisputable evidence of the interaction of these mediator systems at the postsynaptic and systemic levels (Christoffersen and Meltzer 1995).

The deep interrelation of the dopamine and glutamatergic systems is confirmed by numerous studies indicating that a violation of NMDA-receptor transmission contributes to the clinical manifestation of motor and non-motor symptoms of Parkinson's disease and dyskinesia caused by taking L-DOPA drugs (Mellone and Gardoni 2018). It has been shown that, in addition to the main agonist of L-glutamate, such amino acids as D-aspartate and D-serine also play a role in NMDA receptor transmission in neurodegenerative diseases, a decrease in the level of which in the substantia nigra was associated with dopaminergic denervation, and a reduced concentration in spinal fluid – with clinical manifestations of Parkinson's disease in patients without substitution therapy with L-DOPA drugs (Wolosker et al. 2016; Nuzzo et al. 2019).

The severity of catalepsy with the injection of IEM-2295 decreased on average to 3 points, while in the control group it remained at the level of 6 points throughout the observation. However, the severity of catalepsy with the introduction of IEM-2296 decreased to an average of 4 points, but the effect itself lasted longer than with the introduction of IEM-2295. Thus, it was noted that by the 120<sup>th</sup> minute of observation, the severity of catalepsy in rats receiving the IEM-2295

compound averaged 5 points, whereas in animals receiving IEM-2296 – 3 points.

Basing on the results of our work and similar experiments, we can conclude that the studied compounds, which are not channel blockers, have an active effect on dopaminergic neurotransmission, because of which the symptoms of catalepsy that occur when haloperidol is injected to rats were stopped to one degree or another.

Possible steps in future research will be the study of the selectivity of the interaction of the studied compounds, as well as experimental work on other models of Parkinsonism in order to expand the understanding of the therapeutic possibilities of the studied compounds (Ugale et al. 2021; Vecchia et al. 2021). Thus, we will be one step closer to creating safe and cost-effective medicines in the near future.

## Conclusion

It can be concluded based on the obtained results that the studied substances exhibit antiparkinsonian activity on an experimental haloperidol model of catalepsy in rats and are promising for development as potential therapies for neurodegenerative diseases. Considering the proposed mechanism of action of the studied substances, ligands of the glutamate NMDA receptor complex – 1,2-substituted imidazole-4,5-dicarboxylic acids, which is based on non-channel-blocking antagonism of NMDAR, one can hope for the possibility of safer therapy of Parkinsonism compared to the existing medicinal substances. The studied doses of compounds showed an interesting result, especially doses of 30 mg/kg for IEM-2295 and 20 mg/kg for IEM-2296. Further study of these compounds and other ligands from the NMDA-blocker group in a wider sample on the catalepsy model, in higher dosages, as well as on other models of Parkinsonism, is required.

## Conflict of interests

The authors declare no conflict of interests.

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