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Analgesic activity of new ligands of the NMDA receptor complex

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ABSTRACT

BACKGROUND: The activation of spinal cord NMDA receptors is a key factor in the pathogenesis of acute and chronic pain. Therefore, the use of existing NMDA antagonists in analgesic schemes and the development of new compounds targeting the NMDA receptor complex are gaining attention. New ligands of the glutamate NMDA receptor complex are derivatives of imidazole-4,5-dicarboxylic acid. The conformational rigidity of the molecules of imidazole-4,5-dicarboxylic acid derivatives allows for increased selectivity of interaction and reduced side effects.

AIM: This study aimed to investigate the analgesic effect of new ligands of the glutamate NMDA receptor complex , which are derivatives of imidazole-4,5-dicarboxylic acid, in rats using the tail-flick test and the formalin test.

MATERIALS AND METHODS: The analgesic activity of the compounds was examined in a model of acute somatic (thermal) pain in the tail-flick test and model of somatic pain induced by algogens in the formalin test. The tested compounds (IEM-303 and IEM-2044) were administered intraperitoneally at doses of 5, 10, 15, and 20 mg/kg. Metamizole was used as the comparison drug.

RESULTS: The experiments demonstrated a significant dose-dependent analgesic effect of the tested compounds on the experimental models of acute pain at doses of 5–20 mg/kg. The tail-flick latency increased by 1.4–1.7 times in the IEM-2044 and IEM-303 groups compared with the value in the control group.

CONCLUSIONS: The analgesic activity of the tested compounds at 10–20 mg/kg doses was comparable to that of metamizole, indicating the prospect of developing these agents and further searching for effective and safe analgesics in this pharmacological class.

Keywords: glutamate; NMDA receptor antagonists; imidazole-4,5-dicarboxylic acid derivatives; analgesic effect; mice.

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Исследование анальгетической активности новых лигандов NMDA-рецепторного комплекса

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АННОТАЦИЯ

Актуальность. Известно, что активация NMDA-рецепторов спинного мозга является ключевым фактором в патогенезе острой и хронической боли. В связи с этим особый интерес представляет использование в схемах обезболивания существующих NMDA-антагонистов, а также разработка новых соединений, действие которых направлено на NMDA-рецепторный комплекс. Новые лиганды глутаматного NMDA-рецепторного комплекса представляют собой производные имидазол-4,5-дикарбоновой кислоты. Конформационная жесткость молекул производных имидазол-4,5-дикарбоновой кислоты позволяет повысить селективность взаимодействия и снизить количество побочных эффектов.

Цель — изучение анальгетического действия новых лигандов глутаматного NMDA-рецепторного комплекса — производных имидазол-4,5-дикарбоновых кислот на крысах с помощью теста tail-flick и теста с введением формалина.

Материалы и методы. Анальгетическую активность соединений изучали в модели острой соматической (термической) боли в тесте отдергивания хвоста (tail-flick) животного и в модели соматической боли, вызванной альгогенами, в формалиновом тесте. Тестируемые соединения (ИЭМ-303 и ИЭМ-2044) вводили внутрибрюшинно крысам в дозах 5, 10, 15, 20 мг/кг. В качестве препарата сравнения использовали метамизол натрия.

Результаты. В ходе проведенных экспериментов установлен достоверный дозозависимый анальгетический эффект исследуемых соединений на экспериментальных моделях острой боли в дозах 5–20 мг/кг. В группах, получавших ИЭМ-2044 и ИЭМ-303, латентный период отдергивания хвоста в тесте tail-flick увеличивался в 1,4–1,7 раза, по сравнению с контрольной группой.

Заключение. Анальгетическая активность тестируемых соединений в дозах 10–20 мг/кг сопоставима с анальгетической активностью метамизола натрия, что свидетельствует о перспективности разработки данных агентов и дальнейшего поиска эффективных и безопасных обезболивающих средств среди соединений данного класса.

Ключевые слова: глутамат; антагонисты NMDA-рецепторов; производные имидазол-4,5-дикарбоновых кислот; анальгетическое действие; мыши.

Как цитировать

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BACKGROUND

According to the Russian Society for the Study of Pain, 90% of the patients seeking medical help experience pain, of which 70% suffer from chronic pain [1, 2]. Recognizably, the traditional methods of analgesia have limited possibilities, and the use of opioids is associated with an increase in the number of complications, the development of drug tolerance and dependence, and the cost of hospital treatment. Thus, the main directions for improving the quality of pain relief are the expedient combination of analgesics, each with a different mechanism of action as well as the development of new, effective, and safe analgesics. Activation of NMDA receptors in the spinal cord is a key factor in the pathogenesis of acute and chronic pain. Thus, the use of existing NMDA antagonists in pain management schemes is of particular interest, in addition to the development of novel compounds whose activity is directed at the NMDA-receptor complex. Novel ligands of the glutamate–NMDA receptor complex are the derivatives of imidazole-4,5-dicarboxylic acid (IDC). Their conformational rigidity enables an increase in the selectivity of the interaction and a reduction in the number of side effects.

Glutamate receptors are present in all central nervous system (CNS) structures and are responsible for pain stimulation response. In recent years, extensive experimental data has indicated that glutamate is one of the main mediators of pain sensitivity at the spinal and supraspinal levels [3]. Glutamate receptors mediate the response to thermal, mechanical, and ischemic pain [3, 4]. Thus, intrathecal administration of most glutamate receptor agonists (e.g., NMDA) to rats and mice reduces pain thresholds with "self-biting," a characteristic behavior that correlates with vocalization [5]. Blockade of NMDA receptors *in vivo* [6] and *in vitro* [7] inhibits the transmission of nociceptive information. These results indicate the analgesic activity of NMDA-receptor antagonists when applied in acute visceral pain models [8].

NMDA-receptor antagonists realize the antinociceptive effect in several ways: directly by blocking potential-dependent Ca^{2+} channels, reducing the Ca^{2+} flow into the cell, suppressing the release of glutamate and substance P; or indirectly through enhancing the production of kynurenic acid, inhibiting receptors, or inhibiting the associative zone and subcortical formations of the thalamus [9].

The target for glutamate action at the suprasegmental level is the central circulatory substance, from where descending serotonergic pathways into the spinal cord pass through the large suture core as a part of the posterolateral cord. Electrophysiology has revealed that the local application of glutamate to the central near-conducting substance enhances the activity of neurons of the large suture nucleus [10]. Injectingexcitatory amino acids into the central circulatory substance leads to pronounced analgesia. This effect is mediated by specific receptors, as it is blocked by glutamate antagonists [8]. A data-based scheme for the interaction of glutamate and opiatergic systems in the central near-conducting substance has been proposed (Fig. 1).

In combination with opiate analgesics, NMDA-receptor channel blockers reduced the analgesic activity of morphine in acute pain models [8]. Intravenous anesthetic — NMDA-receptor inhibitor ketamine — is used for anesthesia induction, self-anesthesia in short-term surgeries, and the treatment of burn surfaces. Ketamine binds to phencyclidine receptors on the inner surface of NMDA receptors, preventing the formation of excitability of spinal neurons, suppressing a progressive increase in the number of nociceptive neuronal responses (wind-up effect), reducing potentiation and summation of pain, and enhancing the analgesic effect of opioids (a decrease in hyperalgesia is demonstrated against the background of its preliminary administration before fentanyl, morphine, and naloxone). In addition, ketamine elevates the activity of antinociceptive processes by acting on monoaminergic mechanisms. Thus, the NMDA-receptor complex remains one of the key targets in the search for new, effective, and safe analgesics, with promise for the treatment of pain syndromes, both in monotherapy and as part of combination regimens to reduce the required dosages of opioid analgesics and prevent the formation of such phenomena as tolerance and dependence on the background of therapy with analgesics from the opioid group.

Recently, mono- and di-substituted derivatives of IDC have garnered great interest concerning glutamatergic transmission. New IDC derivatives can be considered quinolinic acid analogs, in which the six-membered heterocycle is replaced by a five-membered one [4, 12]. The tested compounds belong to a fundamentally different class of NMDA-receptor antagonists: they interact with the receptor recognition site. The selectivity of the action of alkyl-substituted IDC derivatives on the NMDA-receptor complex was demonstrated in isolated rat hippocampal pyramidal neurons by the patch-clamp method

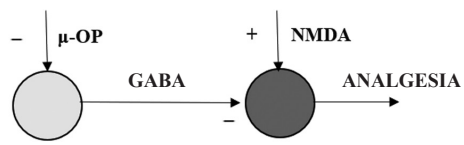

Fig. 1. Scheme of the interaction between the opiate and glutamatergic systems in the central periductal substance [11]. The diagram shows postsynaptic receptors (NMDA, GABA, and μ-OR-opiate). "–" and "+" indicate inhibitory and excitatory influences

Рис. 1. Схема взаимодействия опиатной и глутаматергической систем в центральном околоводопроводном веществе [11]. На схеме указаны постсинаптические рецепторы (NMDA, ГАМК, μ-ОР-опиатные), «–» и «+» — тормозные и возбуждающие влияния

in the "whole cell" configuration [13]. The fact that the tested compounds do not belong to channel blockers has been confirmed by the radioligand method. The conformational rigidity of the IDC derivatives makes it possible to increase the selectivity of the interaction and reduce the number of side effects of potential analgesics.

This study aimed to determine the analgesic effect of novel ligands of the glutamate–NMDA receptor complex — derivatives of IDC in rats applying the tail-flick and formalin injection tests.

MATERIALS AND METHODS

Selection of animals

The work was carried out on male Wistar rats. The animals were kept in a vivarium at 22–24 °C and with free access to water and food. Rats were placed in plastic cages measuring 61 × 44 × 22 cm with a lattice metal lid of six animals each. All experiments were carried out per the Good Laboratory Practice (GLP), GOST 33647-2015, and the provisions of the International Convention on "Rules for Working with Experimental Animals" (European Communities Council Directives, November 24, 1986, 86/609/EEC).

Study of the analgesic activity on a model of acute somatic (thermal) pain in the tail-flick test

The study included six groups, each of six male rats with an average weight of 300 g. The compounds studied, IEM-303 and IEM-2044 were administered intraperitoneally at doses of 10, 15, 20, and 25 mg/kg. The control group of animals received 0.9% NaCl, and the comparison group received 20 and 50 mg/kg sodium metamizole — a reference drug with pronounced analgesic activity. After 15 min of injection, a tail-flick test, a standard test for evaluating the analgesic activity of a drug (assessment of thermal somatic pain) was performed using an analgesimeter for each group of rats. Analgesic activity was assessed by analyzing the duration of the latent period of the tail-flicking reaction when exposed to thermal radiation (in this case, thermoreceptors, C-fibers and Ad-fibers of polymodal receptors, nociceptors, and mechanoreceptors are sequentially activated). The criterion for the analgesic effect was a significant increase in the duration of the latent reaction period after administration of the test substance compared with the control group [11].

Study of the analgesic activity in a somatic pain model caused by algogens using a formalin test

An acute inflammatory reaction was caused by the sub-plantar injection of 50 μl of 2% formalin to a rat 20 min after the intraperitoneal administration of IEM-303 and IEM-2044 at 10, 15, 20, and 25 mg/kg. The number of pain reaction patterns, such as tapping the paw on the floor, shaking, and licking and/or gnawing of the paw was recorded in the first 5–10 min — phase I, and from the 30th to the 50th min — phase II [11]. The criterion considered for the analgesic effect was a marked reduction in the number of pain reactions in the groups of rats receiving IDC derivatives or sodium metamizole relative to the control group. The test simulates the reactions that occur during surgical skin incisions. One of the mechanisms of the nocigenic action of formalin is the activation of TRPA1 channels, which normally react to cold and stimulate inflammation [14]. The first phase characterizes the acute pain that occurs in response to the injection of a chemical stimulus and is mainly associated with the direct activation of thin non-myelin C-fibers. The second phase is the result of the development of an inflammatory process in the peripheral tissues and changes in the function of the posterior horn neurons of the spinal cord gray matter, where neurons of the pain ascending pathways are located. Local anesthetics inhibit only the first phase, while nonsteroidal anti-inflammatory drugs, the second phase.

Statistical processing of the data

The results were statistically processed by employing MS Excel 2010 and BioStat 2009 softwares. The data distribution normality was determined by the Shapiro–Wilk criterion. The reliability of the differences in values between the groups was ascertained using nonparametric Kruskal–Wallis and Fisher's exact criterion.

RESULTS OF THE STUDY

The study results of the analgesic activity of new NMDA ligands in the acute pain model using the tail-flick test are shown in Figs. 2 and 3.

In the rats of the passive control group that were intraperitoneally injected with 0.9% NaCl, the latent period of tail flicking during painful (thermal) irritation was 17.9 ± 0.9 s. In rats of the active control groups treated intraperitoneally with sodium metamizole, a classic analgesic widely used in clinical practice, at 20 and 50 mg/kg, the latent period was 12.2 ± 4.1 and 23 ± 5.8 s, respectively. Thus, 50 mg/kg of sodium metamizole remarkably prolonged the latent period duration of tail flicking by 5 s, compared with that of the passive control group that did not receive an analgesic.

In the experimental groups receiving IEM-303 at 10, 15, 20, and 25 mg/kg, the latent period duration was 5.6 ± 2.2, 13.6 ± 0.8, and 15.9 ± 1.2 s, respectively, indicating no analgesic effect at these concentrations. Only on the background of the introduction of an IDC derivative at 25 mg/kg, the latent period was 23.9 ± 4.4 s, which was 5.9 s more than the passive control group. In comparison with the active control group, this indicator increased by 0.9 s, indicating an analgesic activity of the high doses of the new IDC derivative comparable with that of sodium metamizole.

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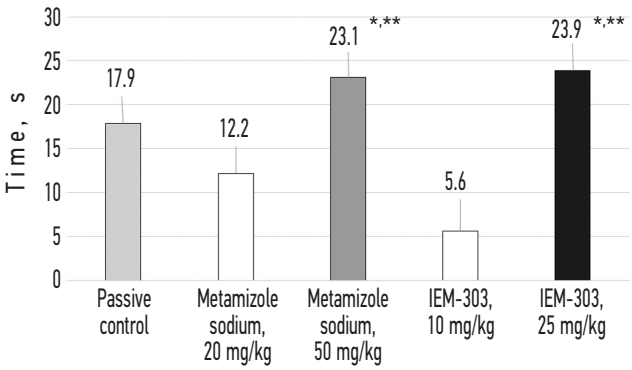

Fig. 2. Duration of the latent period of tail withdrawal in the tail-flick test in the IEM-303-treated rats. *The differences are significant compared with the passive control group (physical solution) at $p < 0.01$. **The differences are significant compared with the group receiving metamizole sodium at a dose of 20 mg/kg at $p < 0.01$

Рис. 2. Продолжительность латентного периода отдергивания хвоста в тесте tail-flick у крыс, получавших соединение ИЭМ-303. *Различия достоверны по сравнению с группой пассивного контроля (0,9 % раствор натрия хлорида) при $p < 0,01$; **различия достоверны по сравнению с группой, получавшей метамизол натрия в дозе 20 мг/кг при $p < 0,01$

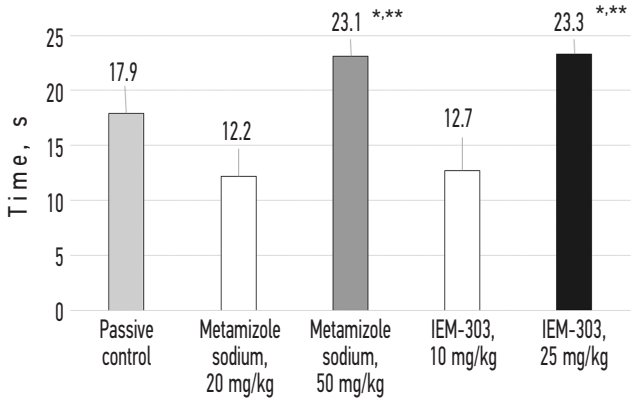

Fig. 3. Duration of the latency of tail withdrawal in the tail-flick test in the IEM-2044-treated rats. *The differences are significant compared with the passive control group (physical solution) at $p < 0.01$. **Differences are significant compared with the group receiving metamizole sodium at a dose of 20 mg/kg at $p < 0.01$

Рис. 3. Продолжительность латентного периода отдергивания хвоста в тесте tail-flick у крыс, получавших соединение ИЭМ-2044. *Различия достоверны по сравнению с группой пассивного контроля (0,9 % раствор натрия хлорида) при $p < 0,01$; **различия достоверны по сравнению с группой, получавшей метамизол натрия в дозе 20 мг/кг при $p < 0,01$

In the experimental groups receiving the new NMDA-ligand, IEM-2044, a reliable dose-dependent analgesic effect at 10–25 mg/kg was established. The latent period of the tail-flicking reaction in the group of rats receiving the test substance at 10, 15, 20, and 25 mg/kg was 12.7, 12.8, 13.1, and 23.3 s, respectively. Thus, the analgesic activity of IEM-2044 at 25 mg/kg was comparable to that of sodium metamizole at 50 mg/kg.

During a study of the analgesic activity of new IDC derivatives in a test involving the introduction of algogen-2% formalin — it was observed that on the background of preliminary analgesia with sodium metamizole (at 50 mg/kg intraperitoneally), the number of pain patterns significantly decreased in rats, compared with those receiving only formalin sub-plantarly. A marked decline was observed: the number of acts of lifting the damaged paw and "turning off" the support on it — by 1.9-fold; the amount of shaking the paw and tapping it on the floor of the cage — by 3.6-fold; and the extent of licking and biting the diseased limb — by 2.8-fold. On the background of the preliminary intraperitoneal administration of IEM-303, remarkable variations with the control group receiving formalin were obtained in the groups receiving 20 and 25 mg/kg of the new IDC derivative; significant differences were identified in the group receiving IEM-303 at 25 mg/kg with the comparison group (sodium metamizole) (Fig. 4).

The introduction of IEM-2044 did not lead to the registration of analgesic activity in modeling acute somatic pain in the algogen introduction test (Fig. 4). Moreover, 20 min after the intraperitoneal injection of 2% formalin at high doses of 20 and 25 mg/kg, behavioral patterns of aggression toward individuals of the same sex were observed in a vast majority of the animals, when the rats were in the same cage. These included standing on their hind legs, squeaking, attacking, and biting. However, this behavior was not found in rats that were administered with lower doses of 10–15 mg/kg, which indicates the possible development of glutamate transmission hyperactivation syndrome during the use of this NMDA-ligand against the background of an increase in concentration in combination with a decrease in the threshold of pain sensitivity against the background of the introduction of the pain-causing agent — formalin. After 20–30 min of the administration of formalin, the manifestations of aggression decreased until complete disappearance, and the animals, as well as the rats of other test groups, fell asleep.

DISCUSSION OF RESULTS

Analgesics used in clinical practice affect the nociceptive system at varying levels. These include exogenous opioids, α2-adrenergic receptor agonists, serotonin reuptake blockers, capsaicin receptor activity modulators, drugs that affect the GABAergic structures of the CNS, glutamatergic transmission, and other drugs. Modern pharmacological approaches to pain management require improvement, and the most effective class of analgesics — opioid painkillers — can cause pathological addiction, which limits their clinical use.

According to the literature, many NMDA-receptor antagonists synthesized during the last century did not show any analgesic activity in basic experimental

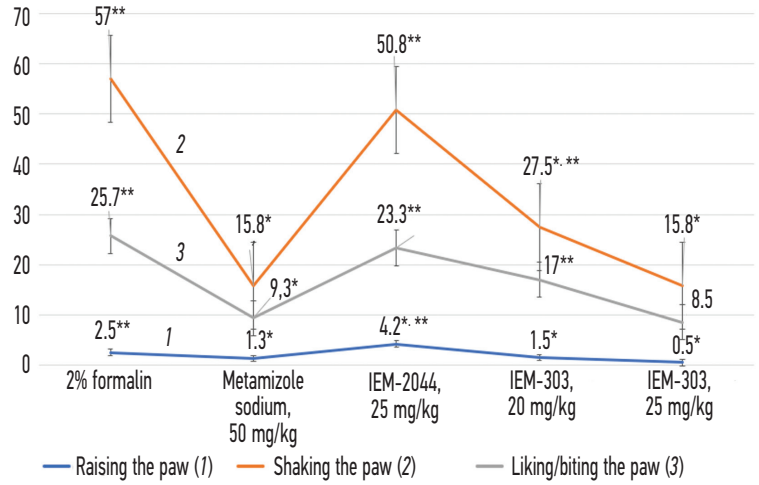

Fig. 4. Number of pain patterns in the formaldehyde test in rats treated with IEM-303 and IEM-2044. *The differences are significant compared with the control group (2% formalin solution) at $p < 0.01$. **The differences are significant compared with the group receiving metamizole sodium at a dose of 50 mg/kg at $p < 0.01$

Рис. 4. Количество паттернов боли в формалиновом тесте у крыс, получавших соединения ИЭМ-303 и ИЭМ-2044. *Различия достоверны по сравнению с группой контроля (2 % раствор формалина) при $p < 0,01$; **различия достоверны по сравнению с группой, получавшей метамизол натрия в дозе 50 мг/кг при $p < 0,01$

models of acute pain, such as hot plate, tail flicking, paw squeeze, and tail clipping tests. Notably a majority of the negative results were obtained concerning channel blockers [13, 15]. When employing NMDA-receptor channel blockers in animals, a decrease in the response to pain stimuli of a thermal and mechanical nature was observed only when these compounds were administered at doses that markedly disrupted motor coordination and/or muscle tone. However, isolated reports from previous years indicate the presence of analgesic activity in NMDA-receptor antagonists, probably not belonging to the class of glutamate channel blockers, when used in acute pain models [11]. In addition, evidence supports the complex nature of the functioning of the NMDA receptor indicating the possibility of the combined use of two or more ligands of this receptor complex [16, 17]. Studies have reported that the new derivatives of IDC at doses of 20–25 mg/kg exhibit analgesic activity in acute somatic pain models. Long-term examinations on the clinical use of NMDA-receptor antagonists suggested that the functions of these receptors should be modulated, but not blocked. The NMDA receptor is an ionotropic receptor that selectively binds NMDA. Crucially, several binding sites in the receptor structure selective to different molecules must be identified. The development of drugs that selectively bind to a single site as opposed to complete blockade of the receptor channel is a promising area of modern pharmacology. In the inactive form, the receptor channel is closed by Mg^{2+}, which is removed during depolarization. Concomitantly, glutamate should enter the synaptic cleft. Such activation promotes the opening of an ion channel that is not selective to cations, altering the potential of the synaptic membrane. Simultaneously, the activation of glutamate receptors can mediate the processes of neurotoxicity but also plays a pivotal role in the implementation of neuroplasticity mechanisms. Therefore, compounds that gently and controllably modulate the function of glutamate receptors may have a more significant effectivity and safety. Thus, the data obtained allow us to consider new derivatives of IDC as promising compounds for the further study of models of visceral and neuropathic pain, as well as for use in combinations with narcotic analgesics to investigate the possible potentiation of the analgesic effect of the latter.

ADDITIONAL INFORMATION

Authors' contributions. All authors made significant contributions to the conception, conduct of the study and preparation of the article, and read and approved the final version before publication. Personal contribution of each author: E.E. Yakovleva, M.T. Kamalova, M.A. Brusina, E.R. Bychkov — conducting experiments, analyzing data, writing the article; L.B. Piotrovsky, P.D. Shabanov — developing the general concept.

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Competing interests. The authors declare that they have no competing interests.

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