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EXPERIMENTAL STUDY OF N-(γ-AMINOBUTYRYL)-1-AZA-4,7,10,13-TETRAOXACYCLOPENTADECANE HYDROCHLORIDE ANALGESIC ACTIVITY IN PAIN SYNDROMES OF DIFFERENT ETIOLOGY

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The pharmacotherapy of pain syndrome remains a significant and pressing medical issue. Therefore, scientists from various medical, pharmaceutical, and chemical specialties are working towards a solution. The study investigated the analgesic activity of N-(γ-aminobutyril)-1-aza-4,7,10,13-tetraoxacyclopentadecane hydrochloride (gabalgin), a new original compound, on laboratory animals. The severity and duration of the analgesic activity were evaluated using the "hot plate", "tail flick", "acetic writhing test", and the neuropathic pain model, which was reproduced by ligation of the sciatic nerve in rats. The test compound was administered intraperitoneally at a dose of 3 mg/kg. The study found that gabalgin exhibits significant analgesic activity in all pain perception models, with comparable or superior strength to the comparison drugs ketorolac, gabapentin, and diclofenac. Additionally, gabalgin's antinociceptive effect lasts longer than diclofenac.

Key words: analgesic activity, GABA derivatives, crown ether, neuropathic, somatic, thermal pain syndromes, experiment, rats, mice

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ЕКСПЕРИМЕНТАЛЬНЕ ДОСЛІДЖЕННЯ АНАЛЬГЕТИЧНОЇ АКТИВНОСТІ ГІДРОХЛОРИД N-(γ-АМІНОБУТИРИЛ)-1-АЗА-4,7,10,13-ТЕТРАОКСАЦИКЛОПЕНТАДЕКАНУ ПРИ БОЛЬОВИХ СИНДРОМАХ РІЗНОЇ ЕТІОЛОГІЇ

Фармакотерапія больового синдрому наразі залишається важливим та актуальним питанням медицини, тому над її вирішенням працюють науковці різних медичних, фармацевтичних та хімічних спеціальностей. В експериментах на лабораторних тваринах було проведене дослідження виразності та тривалості анальгетичної активність нової оригінальної сполуки гідрохлорид N-(γ-амінобутирил)-1-аза-4,7,10,13-тетраоксациклопентадекану (габальгіну) на моделях «hot plate», «tail flick», «acetic writhing test» та модель нейропатичного болю, який відтворювали шляхом перев'язки сідничного нерва у щурів. Досліджувану сполуку вводили внутрішньочеревно в дозі 3 мг/кг. Результати показали, що габальгін володіє виразною анальгетичною активністю на всіх моделях больової перцепції, за силою якою співставляється та подекуди переважає препарати порівняння кеторолак габапентин та диклофенак, перевершуючи останній також і за тривалістю антиноціцептивного ефекта.

Ключові слова: анальгетична активність, похідні ГАМК, краун-етери, нейропатичний, соматичний, термічний больовий синдром, експеримент, щури, миші

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Pharmacotherapy of pain syndrome is a pressing medical issue, and scientists from various medical, pharmaceutical, and chemical specialities are working to find a solution.

Chronic pain, which persists for more than three months after normal tissue healing, is the most common reason for patients to visit primary care physicians. It is often accompanied by a range of negative impacts on quality of life, including insomnia, immunosuppression, eating disorders, cognitive impairment, maladaptive stress reactions, and severe depression [6]. Chronic pain affects a significant number of individuals worldwide [3]. In Ukraine, chronic pain affects over 60.4 % of the population, with the majority of individuals experiencing pain in multiple locations [15].

Treating pain syndromes is a significant challenge because commonly available medications (opioids, NSAIDs, antidepressants, anticonvulsants, paracetamol) are not always effective, and their long-term use can often result in severe side effects. Opioids, in particular, can lead to addiction [5, 13].

Although researchers have many years of experience in modulating pain syndromes, there is still no definitive understanding of the pathophysiological, psychological, and neurochemical mechanisms of their development. Therefore, their efforts are aimed at creating new drugs for pain treatment that have a separate or complex effect on different levels of pain impulse generation, transmission, and perception [9, 11].

Recently, there has been a lot of focus on developing multitargeted drugs that can impact various aspects of the pathological process and have a more appealing pharmacodynamic profile.

Among a number of polyfunctional macroheterocycles modified with various amino acids, based on the results of studies of the dependence between the chemical structure of crown ether derivatives and the manifestations of psychotropic activity, Bogatsky Institute of Physics and Chemistry of the National Academy of Sciences of Ukraine identified the most active compound N-(γ-aminobutyryl)-1-aza-4,7,10,13-tetraoxacyclopentadecane hydrochloride (gabalgin), in which GABA is combined with a macrocycle, which has anti-amnesic, antihypoxic, anticonvulsant, anxiolytic and sedative effects, and is capable of improving the processes of information fixation [1]. This compound is a derivative of monoaza-15-crown-5 and GABA. The study found that the membrane-active complex of crown ether improves the passage of the pharmacophore group through the blood-brain barrier. Covalent binding of GABA to monoaza-15-crown-5 not only leads to quantitative changes in the activity of the new biologically active compound compared to GABA, but also to a qualitative redistribution of effects in the spectrum of psychotropic activity, resulting in changes in the dominant and secondary manifestations of action. Preliminary studies indicate that this compound exhibits analgesic properties.

The purpose of this study was to investigate the duration and severity of analgesic activity of the new original compound hydrochloride N-(γ-aminobutyryl)-1-aza-4,7,10,13-tetraoxacyclopentadecane (gabalgin) on various pain perception models in experiments.

Materials and methods. The experiments were conducted on male Wistar rats and white nonlinear mice obtained from the vivarium of SI "Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine". All biological experiments were carried out in full accord with the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and the Ukrainian Law No. 3447-IV "On protection of animals from severe treatment" (project ID 3410U14, approved 15 October 2015).

Experimental animals were under quarantine for 10 days. During the experiments, the animals were kept in standard conditions of the 12-hour light-and-dark regimen with access to water and food ad libitum.

The Bioethics Commission of the Pirogov National Medical University (Protocol No. 4 of 13 May 2021) found no violations of moral and ethical standards during the research. The research took into account the seasonal and circadian rhythms of the animals. The experimental animals were randomly divided into groups (10 mice and 7 rats in each group).

The test compound was administered intraperitoneally at an empirical dose of 3 mg/kg, which corresponds to 1/200 of the LD_{50}, previously established. The effectiveness of the action was compared with that of reference drugs diclofenac, ketorolac, and gabapentin, taken at their average effective doses.

Screening studies of analgesic activity were assessed using the "hot plate test" [14]. Rats were placed on a thermostatically controlled surface heated to 49–52°C (Ugo Basil, Hot Plate). The latency period (LP) of the pain response during the 1st phase (time before paw licking) and 2nd phase (time before the first attempt to lift all four limbs off the surface), as well as the total latency time, were recorded. The analgesic effect was determined by a statistically significant prolongation of the latency period of the reaction compared to the control group of animals.

The efficacy of gabalgin in a model of visceral pain was evaluated using the "acetic writhing test" in mice [14]. Control animals received only 0.6 % acetic acid solution, while the test group received intraperitoneal injection of 0.6 % acetic acid solution (0.1 ml/10 g) 60 minutes after gabalgin administration. The number of writhes was counted from 1 to 20 minutes, and the analgesic effect was calculated as the percentage of inhibition of the number of writhes relative to the control animals.

Thermal irritation ("tail-flick test") was modeled by directing a focused light beam to the upper third of the tail using an analgesiometer (Ugo Basile, Italy). The latency time of tail twitching away from the beam source, expressed in seconds, was evaluated to assess the severity of the antinociceptive effect. The latency period's initial values and changes 1, 2, 3, and 4 hours after administering the test compound and reference drugs were compared as a percentage relative to the background value, which was taken as 100 % [14].

The efficacy of gabalgin in treating neuropathic pain syndrome was evaluated using the Rat Model for Sciatic Nerve Injury (SNI). Rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) The common sciatic nerve was exposed and dissected from surrounding connective tissue near the trochanter, just distal to the branching point of the posterior biceps semitendinosus nerve. Four ligatures (4.0 chromic gut) were tied loosely around the nerve with a 1–1.5 mm interval between ligatures. Sham-operated rats underwent the same surgery, the left sciatic nerve was exposed but no ligation was made. The development of the pathological process lasted 14 days. Mechanical hyperalgesia was evaluated using the Semmes Weinstein kit (Stoelting, Dale Wood, IL, USA) and von Frey filaments. A positive response was recorded when the limb was pulled away from the filament. Each animal made five attempts to determine their pain

sensitivity threshold (PST). The PST was then compared to the threshold before surgery, on day 14 after surgery, and one hour after administration of gabalgin and the reference drug gabapentin, which was administered at the effective analgesic dose (5 mg/kg) used in experimental studies.

The results of the study are expressed as mean±S.E.M and statistical significance between control and treated groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. P<0.05 was considered statistically significant.

Results of the study and their discussion. It was found that the studied compound had a potent analgesic effect, as evidenced by an increase in the latency period of the first and second phases of pain perception by 2.49 and 2.26 times, respectively (p<0.05), in comparison with rats of the control group. The total latency period under its influence was statistically significantly prolonged by 2.35 times. Under these conditions, the analgesic effect of ketorolac was less pronounced: the duration of the latency period of the first and second phases and the duration of the total latency period after its administration to rats increased by 1.96, 1.86, and 1.92 times, respectively (p<0.05), compared with animals receiving equivalent amounts of solvent. The results of the screening studies of gabalgin efficacy on the "hot plate" model are shown in Table 1.

Table 1

Effect of gabalgin on the duration of the latency period of the pain response in the "hot plate test" (M±m, n=7)

Experimental groups	Latency period of pain response, sec		
	phase I	phase II	Total duration of latency
Control	4.97±0.43	5.94±0.67	10.91±1.01
Gabalgin	12.33±0.95*	13.44±1.24*	25.68±1.33*
Ketorolac	9.87±0.36*/**	11.06±0.42*	20.94±0.55*/**

Notes: * – statistically significant differences (p<0.05) compared to control; ** – statistically significant differences (p<0.05) compared to ketorolac.

The next stage of work was the study of analgesic activity of gabalgin on the model of visceral pain syndrome provoked by intraperitoneal injection of acetic acid solution in mice, which showed that in the control group of animals during the observation period the average number of writhes was 54.0±6.02.

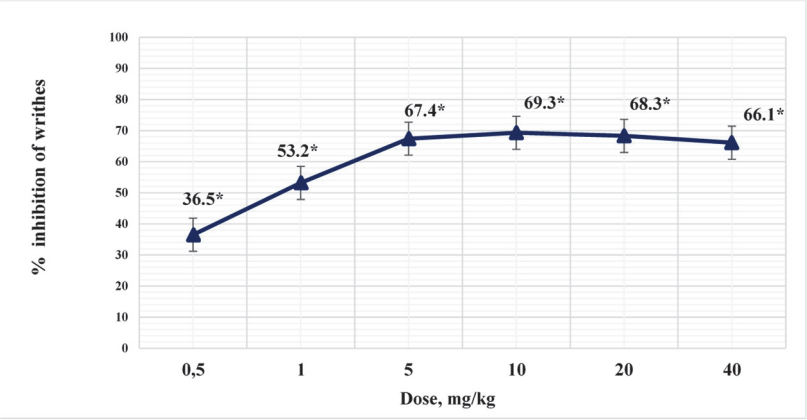

Fig. 1. Analgesic activity of gabalgin on the "acetic writhing test" in mice. Control rats received equal volumes of vehicles, n=10. * – p<0,05 versus control.

Pretreatment with a gamma-aminobutyric acid derivative modified with monoaza-15-crown-5 produced a dose-dependent analgesic effect, statistically significantly reducing the number of writhes (Fig. 1). It is noteworthy that the imaginary dose-response curve of the studied compound has a parabolic configuration: at doses up to 5 mg/kg, the analgesic activity increases, and at higher doses, on the contrary, it begins to decrease.

To evaluate the efficacy of the antinociceptive action of hydrochloride N-(γ-aminobutyryl)-1-aza-4,7,10,13-tetraoxacyclopentadecane and to determine the duration of its action in central pain syndrome, a thermal irritation model ("tail-flick test") based on the spinal flexor reflex in response to a progressive increase in thermal exposure of the tail skin was used. This test involves the sequential activation of thermoreceptors, Ad and C polymodal nociceptors, high threshold mechanoreceptors. The results presented in Figure 2 show that gabalgin significantly prolonged the duration of LP of pain responce almost twice as early as 1 h. It was comparable to ketorolac and significantly superior to diclofenac. The antinociceptive effect increased over the next 4 hours of observation and then decreased, although even in the later stages of the study, the duration of LP exceeded baseline by more than 60 %. The analgesic effect of ketorolac was comparable in strength during the first 2 hours of observation, whereas diclofenac was statistically inferior in analgesic effect at all study times.

Another important aspect of the analgesic effect of biologically active compounds is their efficacy in a model of neuropathic pain. Our results showed that on the 14th day after nerve ligation, the animals showed changes in the fingernails on the injured limbs (signs of autotomy) and also developed a chronic

pain syndrome, which was manifested in the behavioral responses of the rat and expressive hyperalgesia, as evidenced by a decrease in PST on the injured limb by an average of 83–85 % ($p<0.05$) compared to the preoperative value. In this context, the administration of gabalgin produced an analgesic effect, which was confirmed by a statistically significant increase in PST of 59.3 % compared to the value before the compound was given.

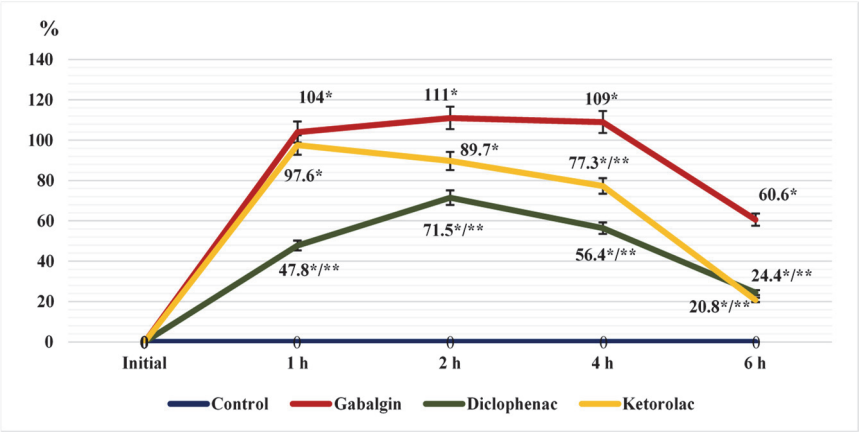

Fig.2 Effect of gabalgin in comparison with diclofenac and ketorolac on withdrawal threshold in "tail-flick test" after parenteral administration. Control rats received equal volumes of vehicles, n=10. * – $p<0,05$ versus initial level; ** – $p<0,05$ versus gabalgin.

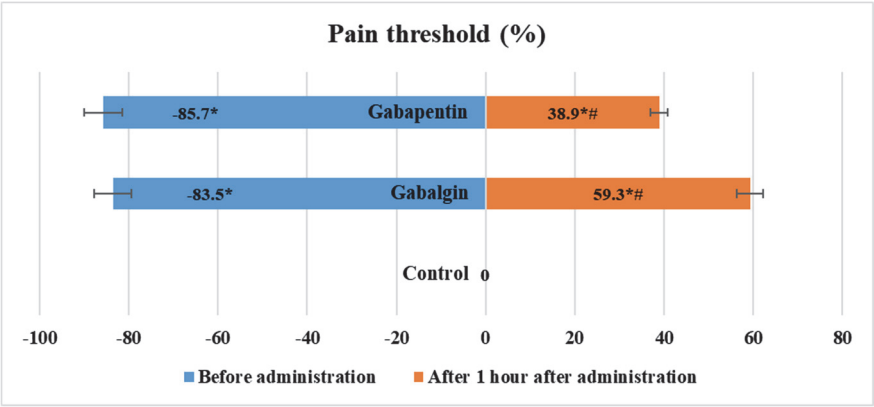

Fig. 3 Analgesic activity of gabalgin and gabapentin in the Rat Model for Sciatic Nerve Injury. * – $p<0.01$ relative to the uninjured limb; # – $p<0.05$ relative to the injured limb before administration of the compounds.

Under similar experimental conditions, gabapentin also produced an analgesic effect, as it increased the PST of the animals by 38.9 % ($p<0.05$). In other words, the investigated compound was able to eliminate hyperalgesia in a model of neuropathic pain syndrome, with a higher efficacy than the reference drug gabapentin.

It is known from numerous data in the literature that modern strategies for finding new potential analgesics are associated with changes in the sensitivity of nociceptors (the effect on the family of transient receptor potential (TRP) channels, ryanodine receptors (RyR), which are intracellular calcium channels activated by hyperpolarization of cyclic nucleotide-gated (HCN) channels, which belong to the superfamily of voltage-dependent K^+ channels), normalization of nociceptors disturbed by inflammation, reduction of pathological excitability of neurons involved in nociceptive signaling (blockers of tetrodotoxin-insensitive Na^+ channels, blockers of Ca^{2+} channels). New compounds with analgesic and antihyperalgesic activity can be developed among COX-3 inhibitors, ASIC3 proton-sensitive channel blockers, P2 X2 and P2 X3 purinergic receptor channels, bradykinin receptor blockers, calcitonin gene-related peptide (CGRP), cannabinoids, etc. [4, 7, 12].

The results obtained in this study demonstrate the feasibility of using the GABA receptor as an important and promising target for the search and development of new drugs with antinociceptive activity. GABA is known to be involved in the mechanisms of pain and hyperalgesia in inflammatory and neurological peripheral lesions. The GABA receptor is the target of a well-known class of drugs with numerous neurotrophic effects – benzodiazepines. Among the derivatives of this class, drugs with anxiolytic, anticonvulsant, hypnotic, and analgesic properties have been developed [2, 8]. GABA is a universal inhibitory mediator involved in neuronal regulation of almost all physiological functions. Many drugs have been developed and used based on GABA, but they all have one major drawback: low bioavailability and thus poor penetration across the blood-brain barrier. To overcome this problem, the drugs gabapentin and pregabalin, which are synthetic analogs of the aminoacid, were developed. The mechanisms of action of these drugs are fundamentally similar: they bind to the α2–δ subunit of voltage-dependent calcium channels and reduce the flow of calcium ions, which play an important role in the onset of neuropathic pain. These compounds also reduce glutamate-dependent neuronal death, increase endogenous GABA synthesis, and suppress the release of monoamine neurotransmitters [10]. Their widespread use is limited by serious side effects, including drug dependence. Therefore, the search for

other analgesic drugs in this area with an improved pharmacological profile continues. A promising step in this regard is the search for biologically active substances among macroheterocyclic compounds. The discovery of membrane-active complexes allowed us to obtain their synthetic analogues: crown ethers, cryptans, etc., among which gabalgin occupies one of the leading positions. Most of these compounds are ionophores, transporting cations across biological membranes along an electrochemical potential gradient, so they can be used to improve the permeability of ions and molecules across biological barriers.

Conclusions

The studies have shown that gabalgin (hydrochloride N-(γ-aminobutyryl)-1-aza-4,7,10,13-tetraoxacyclopentadecane) has a marked analgesic activity in models of somatic, visceral and neuropathic pain syndromes, which is comparable to and sometimes superior to the reference drugs ketorolac, gabapentin and diclofenac, surpassing the latter also in the duration of the antinociceptive effect.

A detailed study of the strength and mechanisms of analgesic effect of gabalgin will provide an experimental basis and help determine the vector for further preclinical and clinical studies of this compound as a potential drug in the treatment of diseases accompanied by pain.

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