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NEUROTROPIC EFFECTS OF GLYCINE MENTHYL ESTER

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The objective of this study was to evaluate toxicity and some pharmacological effects of glycine menthyl ester hydrochloride (2-isopropyl-5-methylcyclohexyl 2-aminoacetate) in animal models.Acute toxicity (LD_{50} value) in mice was determined for different routes of administration – orally and intravenously. Anticonvulsant activity was assessed by using pentylenetetrazole and strychnine induced seizures.The sedative and muscle relaxant actions were investigated with open field and rota-rod tests, accordingly. Present findings indicate that menthyl ester of glycine hydrochloride possesses anticonvulsant activity, prolonged sedative action but does not influences the muscle relaxation.

Key words: *menthyl ester of glycine hydrochloride, acute toxicity, anticonvulsant activity, sedative action.*

Introduction

Menthol is well known for cooling effect due to its ability to chemically activate the cold-sensitive transient receptor potential cation channel (TRPM8) [1]. In addition to this peripheral action, a number of studies have demonstrated that menthol has actions within the CNS. In animal behavioral tests, systemic or direct central administration of menthol produces a variety of effects, including sedative, anticonvulsant, analgesic and nootropic actions [2]. Along with TRPM8 there are other potential cellular targets for menthol such as glycine receptors [3]. Glycine is well known to be the most important inhibitory neurotransmitter in the spinal cord. This amino acid is of particular interest to the neuropharmacology since many commonly studied drugs work by selectively affecting tothis neurotransmitter system [4]. Taking into consideration aforementioned facts, it was of interest to investigate pharmacological properties of compound including both menthol and glycine fragments. So the present study was designed to evaluate the anticonvulsant, sedative and muscle relaxant activity of glycine menthyl ester using animal models.

Materials and methods

Menthyl ester of glycine hydrochloride (2-isopropyl-5-methylcyclohexyl 2-aminoacetate) was synthesized using DCC/DMAP coupling method [5] (fig. 1).

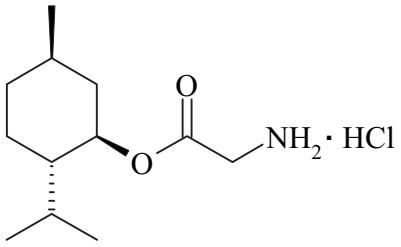

Fig. 1. Chemical structures of glycine menthyl ester hydrochloride.

Pharmacological activity of glycine menthyl ester was studied using outbred male white mice (18-22 g) as experimental animals. They were kept under 12-hour light regime and in a standard animal facility with free access to water and food, in compliance with the European Convention for the Protection of Verte-

brate Animals Used for Experimental and Other Specific Purposes [6] (Strasbourg, 1986) and the principles of the National Ukrainian Bioethics Congress (Kiev, 2003) [7]. All the animals were purchased from Odessa National Medical University, Ukraine. The animal ethics committee of the Odessa National University (Ukraine) approved the study.

Acute toxicity (LD_{50} value) in mice was determined for different routes of administration – orally and intravenously. Mice were divided into groups of six each and menthyl ester of glycine was administered in doses from 1300-1500 mg/kg for orally and in doses from 25-75 mg/kg for intravenousdetermination. The animals were observed for toxic symptoms within 24 hours after the compound administration.

The anticonvulsant activity of the compound was evaluated in model of acute generalized seizures induced by intravenous infusion of 1 % pentylenetetrazole (PTZ) or 0,06 % strychnine solution with the determination of PTZ/strychnine minimum effective doses (MED) inducing clonic-tonic convulsions (CTC) and tonic extension (TE) in test animals. PTZ/strychninedoses for inducing clonic-tonic convulsions (DCTC) and tonic extension (DTE) were calculated relative to control.

The sedative effect was investigated using open field test (OFT).The open field apparatus consisted of a plastic field of half square meter with a series of squares. It had a wall of 50 cm height and was placed in a dimly lit room. Mice were treated with menthyl ester of glycine hydrochloride (87-700 mg/kg), menthol, glycine (equimolar amounts) and were placed in the middle of the open field. Thenlocomotor (lateral, vertical) and research (hole visits) activities were registered for 3 minutes at 3 hours after administration.

Muscle relaxant activity was examined by rota-rod test that was performed using horizontal rotation rod set at a rate of 5 revolutions per minute. Three hours after menthyl ester administration (87-700 mg/kg) each mouse was placed on the rod for 2 minutes. Motor coordination was then assessed by recording the falling time from the rotating rod for each mouse.

Results and discussion

Acute toxicity study of glycine menthyl ester revealed the following LD_{50} values: 1350 mg/kg by oral route administration and 50 mg/kg intravenously.

Glycine menthyl ester wasnot shown to demonstrate significant anticonvulsant activity at the doses of 87-350 mg/kg via oral routeat 3 hour after administration. However, at the dose of 700 mg/kg it produces anticonvulsant effect as indicated by increasing of DCTC and DTE values that amounts to 2.0 and 1.7 timeswith comparison of control, respectively.

Strychnine has been demonstrated to have a well-defined mechanism of convulsant action reported to be by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing spinal reflexes [8]. Taking into account this fact, we have evaluated ester anticonvulsant effect in strychnine induced seizure model. Menthyl ester at the dose of 700 mg/kg was found to modify strychnine actionslightly with DCTC and DTE values 1.5 and 1.4 times with comparison of control, accordingly; this might be assigned to menthol predominant contribution into anticonvulsant activity of synthesized ester.

Investigation of sedative effect was performed by recording spontaneous locomotor activity of mice in open field test. In this test any agent with sedative properties produces a decrease in the number of movements, interpreted as a decrease in curiosity of the new environment. As far as menthol and glycine were reported to possess sedative activity [9], they also have been investigated under experiment conditions. Our results demonstrate that the oral administration of menthyl ester at doses of 350-700 mg/kg

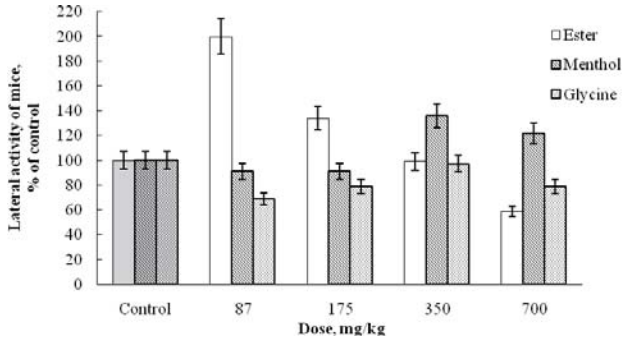

Fig. 2. Comparable lateral activity of mice in 3 h after oral administration of glycine menthyl ester, menthol and glycine (dose-response relationship).

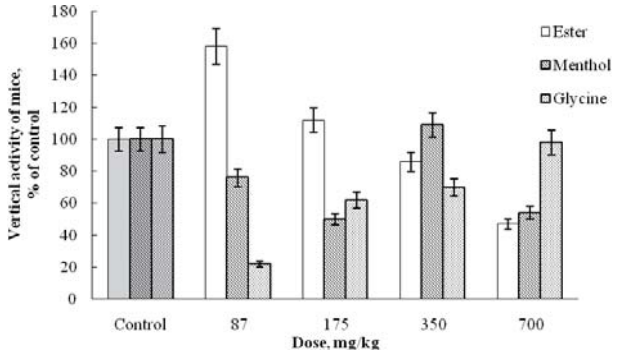

Fig. 3. Comparable vertical activity of mice in 3 h after oral administration of glycine menthyl ester, menthol and glycine (dose-response relationship).

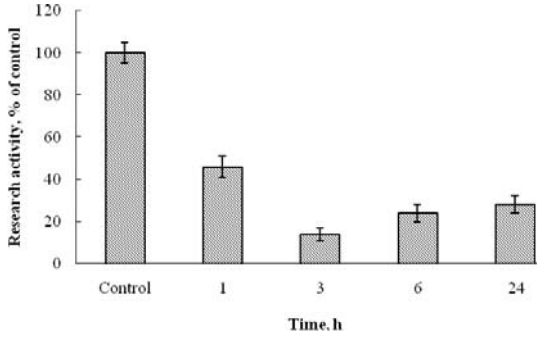

Fig. 4. Research activity of mice after oral administration of glycine menthyl ester at dose175 mg/kg (time-response relationship).

Table

Locomotor activity of mice after oral administration of glycinementhyl ester at dose175 mg/kg (time-response relationship)

Time after oral administration, h	Locomotor activity	
	lateral	Vertical
Control	100.0 ± 8.1	100.0 ± 7.7
1	130.3 ± 7.4	80.9 ± 7.2
3	64.7 ± 5.2	19.2 ± 5.4
6	48.1 ± 6.0	31.3 ± 6.6
24	78.7 ± 4.9	83.6 ± 9.1

causes a marked reduction both in lateral (fig. 2) and vertical (fig. 3) activities, but does not affect the research activity.

We should point outthat underadministration of lowdose (87-175 mg/kg) of ester, we observed an increase the motor activity of mice in 1.4-2.0 times, as compared with the control. With increasing doses, we observed a decrease inlocomotor activity that can be explained by the appearance of estersedative activity.

Considering the possible prolonged action of obtained ester, sedative effect was estimated over the time range: 1-24 hours. This enables the pharmacokinetics of synthesized compound to be expressed as a function of time after oral administration. Our data reveal that menthyl ester of glycine at 175 mg/kg dose causes a time-dependent reduction of locomotor (table) and research activity (fig. 4). Maximum suppressive effect was found within the time of 3-6 hours and continued up to 24 hours after oral administration, indicating prolonged sedative action.

Results obtained from rota-rod test reveal no mice fallings for all concentrations of glycine menthyl ester (87-700 mg/kg) in 3 h after oral administration. Thus, we can assume that there is no significant muscle relaxant activity of synthesized compound at aforementioned concentrations and time point.

Conclusions

In conclusion,the present findings in our study indicate that menthyl ester of glycine hydrochloride possesses anticonvulsant activity, prolonged sedative action but does not influences the muscle relaxation in animal models. Additionally, investigation of acute toxicity for the compound was carried out both for oral and intravenous routes of administration.

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Резюме

НЕЙРОТРОПНЫЕ ЭФФЕКТЫ МЕНТИЛОВОГО ЭФИРА ГЛИЦИНА

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Целью данного исследования служит определение токсичности и фармакологических свойств ментилового эфира глицина гидрохлорида (2-изопропил-5-метилциклогексил 2-аминоацетат) на экспериментальных моделях с использованием лабораторных животных. Острая токсичность (значение $ЛД_{50}$)соединения определена при пероральном и внутривенном путях введения. Противосудорожную активность оценивали на модели острых генерализованных судорог, индуцируемых пентилентетразолом и стрихнином. Седативный и миорелаксантный эффекты исследовали с помощью тестов открытого поля и вращающегося стержня. Полученные результаты свидетельствуют о наличии противосудорожной и пролонгированной седативной активности у изучаемого соединения при отсутствии миорелаксации.

Ключевые слова: *ментиловый эфир глицина гидрохлорида, острая токсичность, противосудорожная активность, седативное действие.*

Резюме

НЕЙРОТРОПНІ ЕФЕКТИ МЕНТИЛОВОГОЕСТЕРУГЛІЦИНУ

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Метою даного дослідження є вивчення токсичності та фармакологічних властивостей ментилового естеру гліцину гідрохлориду (2-ізопропіл-5-метилциклогексил 2-аміноацетат) на експериментальних моделях із використанням лабораторних тварин. Гостра токсичність (значення $ЛД_{50}$) сполуки визначена для перорального та внутрішньовенного шляхів введення. Протисудомну активність оцінювали на моделі гострих генералізованих судом, індукованих пентилентетразолом та стрихніном. Седативний та міорелаксантний ефекти досліджували за допомогою тестів відкритого поля та обертового стрижня. Отримані результати свідчать про наявність у досліджуваної сполуки протисудомної та пролонгованої седативної дії за відсутності міорелаксації.

Ключові слова: *ментиловий естер гліцину гідрохлориду, гостра токсичність, протисудомна активність, седативна дія.*

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