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Short communication

Evaluation of anticonvulsant activity of novel pyrrolidin-2-one derivatives

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ABSTRACT

Background: The aim of this study was to examine the anticonvulsant activity of some novel pyrrolidin-2-one derivatives with considerable affinity to serotonin 5-HT_{1A} and α_1-adrenergic receptors.
Methods: The maximal electroshock-induced seizure (MES) and pentetrazole (PTZ)-induced seizure models in mice were performed.
Results: As a results of the conducted studies, three compounds showing anticonvulsant activity were selected. The **EP-40** molecule significantly reduced incidence of seizures in the maximal electroshock test. The **EP-42** and **EP-46** compounds demonstrated activity in the pentetrazole-induced seizures.
Conclusion: The results may indicate that the decrease in the susceptibility to seizures induced by the new pyrrolidin-2-one derivatives is related to the significant affinity to serotonergic or α_1-adrenergic receptors. Also putative mechanism of action of the test compounds can be linked with their GABA-ergic activity, because these novel derivatives are GABA analogs.

Introduction

Epilepsy is one of the most common neurological disorders. It is characterized by spontaneous recurrent seizures arising from excessive electrical activity in some regions of the brain. Uncontrolled electrical activity in the central nervous system may occur either via a reduction in inhibitory neurotransmission or an increase in excitatory transmission [1]. Changes in ionic conductance through neuronal membranes may underlie the above-mentioned abnormalities. Additionally, there has been a growing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures and its disturbances are involved in the enhanced seizure susceptibility observed in some genetically prone rats [1]. It is well known that serotonin exerts its effects via at least 14 different receptor subtypes but the role of only a few of them (5-HT_{1A}, 5-HT_{2C}, 5-HT_7) has been studied in relation to the control of seizures [2].

Furthermore, the experimental data obtained in animals show that 5-HT_{1A} receptors are predominantly located in the limbic areas and they suggest that serotonin mediates the anticonvulsant effect via these receptors [1,3]. Autoradiographic analysis of 5-HT receptors in fully kindled rat brain showed a selective increase in 5-HT_{1A} binding in the dentate gyrus. These findings suggest that 5-HT_{1A} receptors may have an inhibitory role in the generation of hippocampal seizures. On the other hand, according to the findings of Stean [4], the mixed 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} receptor agonists SFK 99101 and RU 24969 produced marked increases in the seizure threshold. It has also been found that the anticonvulsant action of trifluoromethyl-phenylpiperazine (5-HT_{2A}/5-HT_{2C}) and m-chlorophenylpiperazine (5-HT_{2A}/5-HT_{2C}) in the animal maximal electroshock test [1,5] depended on 5-HT_2 receptor stimulation.

Over 40 antiepileptic drugs are in clinical use worldwide, however, these drugs are effective only in about one third of patients [6]. Many of the clinically used drugs have not been definitively linked with a specific target in the brain or characterized in terms of the exact mechanisms of action. Most antiepileptic drugs possess more than one mechanism of action which may account for their efficacy in different seizure types and patterns,

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and in individual patients. Many of antiepileptic drugs produce undesirable side-effects, such as drowsiness, mental dullness, ataxia, hepatotoxicity, megaloblastic anemia [6]. Therefore, there is a growing demand for new antiepileptic drugs with novel therapeutic targets, enhanced efficacy and minimal side-effects [7].

In the development of new potential anticonvulsant agents and 5-HT_{1A}/5-HT_{2A} receptor ligands, we focused our attention on a group of pyrrolidin-2 one derivatives with a phenylpiperazine fragment. Our earlier studies evidenced the affinity of these compounds for serotonin 5-HT_{1A} and HT_{2A} receptors [8]. In addition, these compounds showed affinity for α_1-adrenergic receptors. The structure of these derivatives was another reason why we decided to test their anticonvulsant activity because a gamma-lactam structure present in the compounds under study is a product of GABA cyclization. Thus, the tested pyrrolidin-2-one derivatives are N-substituted GABA analogs [8].

Materials and methods

Chemicals

The tested compounds **EP-40**, **EP-41**, **EP-42**, **EP-43**, **EP-45**, **EP-46**, **EP-48**, **EP-49**, **EP-50** and **MG-1**, were synthesized at the Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University in Cracow. The synthesis of the tested compounds was described previously [8].

All compounds were dissolved in 0.9% sodium chloride and administered intraperitoneally at a dose of 100 mg/kg body weight, at a volume 10 ml/kg, at the time dependent on the test type, i.e. 60 min before the experiment in the maximal electroshock test or 30 min before administration of the convulsant in the pentetrazole test.

Animals

The behavioral experiments were carried out at the Department of Pharmacological Screening, Chair of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University in Krakow. Adult male albino Swiss (CD-1) mice weighing 18–25 g were used in the experiments. The animals were kept in groups of 15 mice in cages at a room temperature of 22 ± 2 °C, under 12/12 h light/dark cycle and had free access to food and water. The ambient temperature in the room and the humidity were kept constant throughout all the tests. Animals for the experiments were selected in a random way. They were killed by cervical dislocation immediately after the assay. Experimental groups consisted of 7–8 animals/dose and all the animals were used only once. The number of animals was kept at a minimum to obtain definite results with the test utilized. Prior to the test, the mice were allowed to acclimatize to the holding cages for a minimum of 2 h. The experiments were performed between 8 a.m. and 3 p.m. All the procedures were approved by the Local Ethics Committee of the Jagiellonian University in Krakow.

Experimental procedures

The maximal electroshock-induced seizure (MES)

Experimental electroconvulsions were induced by a current of 50 mA intensity and 0.2 s stimulus duration delivered *via* standard auricular electrodes attached to an electroshock generator (rodent shocker, type GE; COTM, Białystok, Poland). The percentage of mice that developed a seizure attack ending with tonic hind limb extension (referred to as seizure incidence) of animals in a particular group were recorded. Mortality was assessed just after the maximal electroshock test.

Pentetrazole-induced seizure model

Clonic convulsions were induced in mice by administration of pentetrazole. A dose of 80 mg/kg of pentetrazole which produces seizures in more than 95% of mice, was administered as a 0.5% solution subcutaneously in the posterior midline. Animals were observed for 30 min. Failure to observe even a threshold seizure (a single episode of clonic spasms) was defined as protection and the results were expressed as the number of animals protected/number of animals tested. The clonic seizure activity was defined as the clonus of whole body lasting over 3 s, with an accompanying loss of righting reflex.

Neurotoxicity

The rotorod test was used to evaluate neurotoxicity in mice. Animals were placed on a 1-in. diameter knurled plastic rod rotating at 24 rpm. Non-toxic (normal) mice can remain on a rod rotating at this speed almost indefinitely. Neurological toxicity is defined as the failure of the animal to remain on the rod for 1 min and is expressed as the number of animals exhibiting toxicity/number of animals tested. Animals are considered toxic if they fail this test on three successive attempts.

Statistical analysis

All statistical calculations were carried out with the GraphPad Prism 5 program. The statistical significance was calculated using ANOVA and Dunnet and Fischer test. Differences were considered statistically significant at $p \leq 0.05$.

Results

The maximal electroshock-induced seizure (MES)

All compounds were administered intraperitoneally at a dose of 100 mg/kg. Only the compound **EP-40** exhibited anticonvulsant activity at this dose, inhibiting the seizure incidence by about 71% vs. control. It also decreased mortality by about 38%. The compound **EP-43** did not influence the number electroshock-induced seizure but reduced completely mortality. The compounds **EP-42**, **EP-45**, **EP-48** and **EP-49** at the analogical dose demonstrated a weak statistically non-significant anticonvulsant effect, inhibiting the seizure incidence by about 15% compared to control. The remaining tested compounds were not active in this model of seizures.

Pentetrazole-induced seizure model

In the pentetrazole-induced seizure model, the compounds were tested also at the analogical dose as above, i.e. 100 mg/kg. Anticonvulsant activity was assessed based on the number of seizure attacks during a 30-min observation period, prolongation of the latency time and mortality.

The compound **EP-42** revealed the strongest, statistically significant action inhibiting both the seizure incidence and the number of seizure attacks. It also significantly prolonged the latency time compared to control (1.2 min in the control vs. 22.8 min in **EP-42** treated group). The compound **EP-46** significantly reduced the seizure incidence by 50% and non-significantly prolonged the latency time (1.2 min in the control vs. 16.4 min after **EP-46**). The remaining tested compounds did not show a significant action in this seizure model.

Discussion

Although many new (third-generation) antiepileptic drugs (AEDs) have been introduced in the last decade, there is still a clear