UDC 612.8.01

Mecamylamine modulates epileptiform discharges in low-Mg^{2+} model of epilepsy

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Mecamylamine is a nonselective antagonist of nicotinic acetylcholine receptors that was developed as an antihypertensive medication and is now being studied for its beneficial effects in several pathological conditions, such as substance abuse, depression, anxiety and epilepsy. In this work, we investigate the effect of mecamylamine on the manifestations of seizure-like activity evoked by perfusion of hippocampal slices with low-Mg^{2+} solution of artificial cerebrospinal fluid. Reducing Mg^{2+} concentration in extracellular solution induced two distinct types of epileptiform activity: recurring seizure-like activity and continuous discharges. Application of mecamylamine significantly increased internal frequency of recurring seizure-like activity and significantly decreased inter-event intervals between continuous discharges. We also show that mecamylamine significantly decreased internal frequency of continuous epileptiform discharges. The results of our work show that mecamylamine exerts modulatory effect on the low-Mg^{2+} epileptiform activity induced in hippocampal acute rat brain slices. Additionally, obtained results indicate the role of nicotinic acetylcholine receptors in the modulation of hippocampal network activity, which might explain some of the therapeutic effects of mecamylamine in CNS.

Key words: mecamylamine; rat brain slices; seizure-like activity; CA3 hippocampus.

INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are ionotropic receptors that respond to the neurotransmitter acetylcholine and to drugs such as nicotine. nAChRs are abundant in the central and peripheral nervous system, muscle, and many other tissues. Nicotinic acetylcholine receptors are well-studied ionotropic receptors and their role in memory, learning, LTP are supported by numerous studies [1-3]. However, the role of nAChRs in generation of epileptic discharges is less understood [4, 5]. Injections of nicotine to mice and rats evoked seizures, which were abolished by a subtype non-selective nAChRs antagonist, mecamylamine, while methyllycaconitine, a selective α7 nAChR antagonist, significantly reduced seizures, and an α4β2 nAChR antagonist, dihydro-β-erythroidine, has a weak effect [6]. In another research, MEC was effective against the maximal electroshock seizure test [7]. This was not found in kindled rats, in which nicotinic acetylcholine receptor antagonists exerted less robust effects.

Mecamylamine (3-methylaminoisocamphane hydrochloride, MEC) is a nicotinic ganglionic blocker, originally used to treat hypertension. Because mecamylamine produces several adverse effects at therapeutic concentrations, now MEC's use as antihypertensive agent is limited to severe cases. However, MEC easily crosses the blood-brain barrier to reach brain structures and inhibit all known nAChRs subtypes at safe doses, 3-fold lower than those used to treat hypertension, which diminishes the probability of peripheral side-effects and enables use of MEC for CNS disorders. Since nAChRs play a pivotal role in many physiological and pathological processes, MEC has been recently proposed for its potential therapeutic effects in a variety of pathological states of the CNS [8, 9].

The low-Mg^{2+} model of epilepsy was developed several decades ago, and since that time has been established as a model of chronic

epilepsy [10-13]. Low-Mg^{2+} model of epilepsy has clinical relevance as Mg^{2+} deficits is known to cause seizures in humans [14]. Intravenous injections of Mg^{2+} have an anticonvulsant effect in animal models of epilepsy [15] and are used to treat seizures in women with eclampsia [16].

The aim of present study was to investigate effect of mecamylamine on seizure manifestations evoked by removing of Mg^{2+} from extracellular space in CA3 zone of rat hippocampal slices.

METHODS

Animals. Wistar rat pups of postnatal day 12 (P12) were used throughout the study and treated in accordance with the guidelines set by the Animal Care Committee of Bogomoletz Institute of Physiology of NAS of Ukraine.

Slice preparation. The method used in this study for preparation of acute rat brain slices was described previously [17]. Briefly, animals were deeply anesthetized with sevoflurane and decapitated. Brain was rapidly removed and placed in ice-cold artificial cerebro-spinal fluid (aCSF). Cerebellum and frontal lobe of the brain were removed and transverse brain slices (500 μm) were cut using a vibroslicer. The resulting slices were then transferred to the incubation chamber and left to recover at room temperature for at least one hour before the experiment. All manipulations were performed in constantly oxygenated (95% O_2 – 5% CO_2) aCSF of the following composition (mmol/l): NaCl 1– 25, KCl – 3.5, $MgCl_2$ – 1.3, $CaCl_2$ – 2, NaH_2PO_4 – 1.25, $NaHCO_3$ – 24, glucose – 11, pH 7.35. All drugs were obtained from "Sigma" (USA).

Extracellular recordings. Spontaneous field potential recordings were obtained from CA3 pyramidal cell layers of rat hippocampus using glass micropipettes (2-5 MΩ) filled with aCSF. For induction and recording of epileptiform activity, the slices were transferred to a submersion-type chamber and perfused with low-Mg^{2+} solution containing (mmol/l): NaCl –115, KCl – 5, $CaCl_2$ –1, NaH_2PO_4 – 1.25, $NaHCO_3$ – 24, glucose – 10, pH 7.4, (oxygenated with carbogen). Extracellular field potential recordings were amplified with a differential amplifier, digitized at 10 kHz using an analog-to-digital converter and stored using WinWCP program. All records were made at room temperature (24-25˚C). In this work data were obtained from 10 hippocampal slices, 6 rats. $MgCl_2$ was omitted from the perfusion solution during induction of epileptiform activity.

Data analysis. Analysis was performed using Clampfit 10.2 (Axon Istruments) and Origin 8.5 (OriginLab Northampton, MA). For statistical analysis Shapiro-Wilk test and Kolmogorov-Smirnov test were used. All data are represented as mean ± standard deviation.

RESULTS AND DISCUSSION

Perfusion of hippocampal slices with low-Mg^{2+} aCSF resulted in the appearance of spontaneous epileptiform discharges in CA3 hippocampal zone (Fig. 1). Epileptiform activity appeared in two forms of rhythmic activity. Initially, epileptic activity started with recurring seizures (Fig. 1A) and then either only continuous epileptic discharges appeared (2/10 slices) or both – recurring seizures and continuous discharges were recorded (8/10 slices, Fig. 1A). Recurring seizure-like activity (SLA), which is characterized by long train of epileptic discharge, was evoked in all recorded slices. Recurring seizure-like activity had the mean duration of 60.61 ± 31.98 s and the mean inter-burst frequency was 3.39 ±1.40 Hz. Continuous epileptic discharges are distinct short electrical events that had the mean duration of 1.37 ± 0.42 s, the mean internal frequency of 8.2 ± 1.34 Hz and the mean inter-event interval of 6.73 ± 1.74 s.

Application of MEC did not change the duration of recurring seizure-like activity (70.24 ± 46.65 s), but significantly increased internal frequency of discharges – 3.97 ± 0.99 Hz, ($P = 0.02$, Fig. 1B). After MEC washout in perfusion solution on 6/10 hippocampal slices, the mean internal frequency of discharges decreased to 2.58 ± 0.73 Hz ($P < 0.01$, Fig. 1C).

Application of MEC did not change the duration of continuous discharges (1.11 ± 0.28 s), but significantly decreased the inter-event intervals between these discharges 4.31 ± 0.54 s, (P = 0.001). However, blockade of nicotinic receptors lead to decrease in internal frequency of continuous epileptic discharges (3.18 ± 1.26 Hz, P < 0.001). Washout of MEC from hippo-

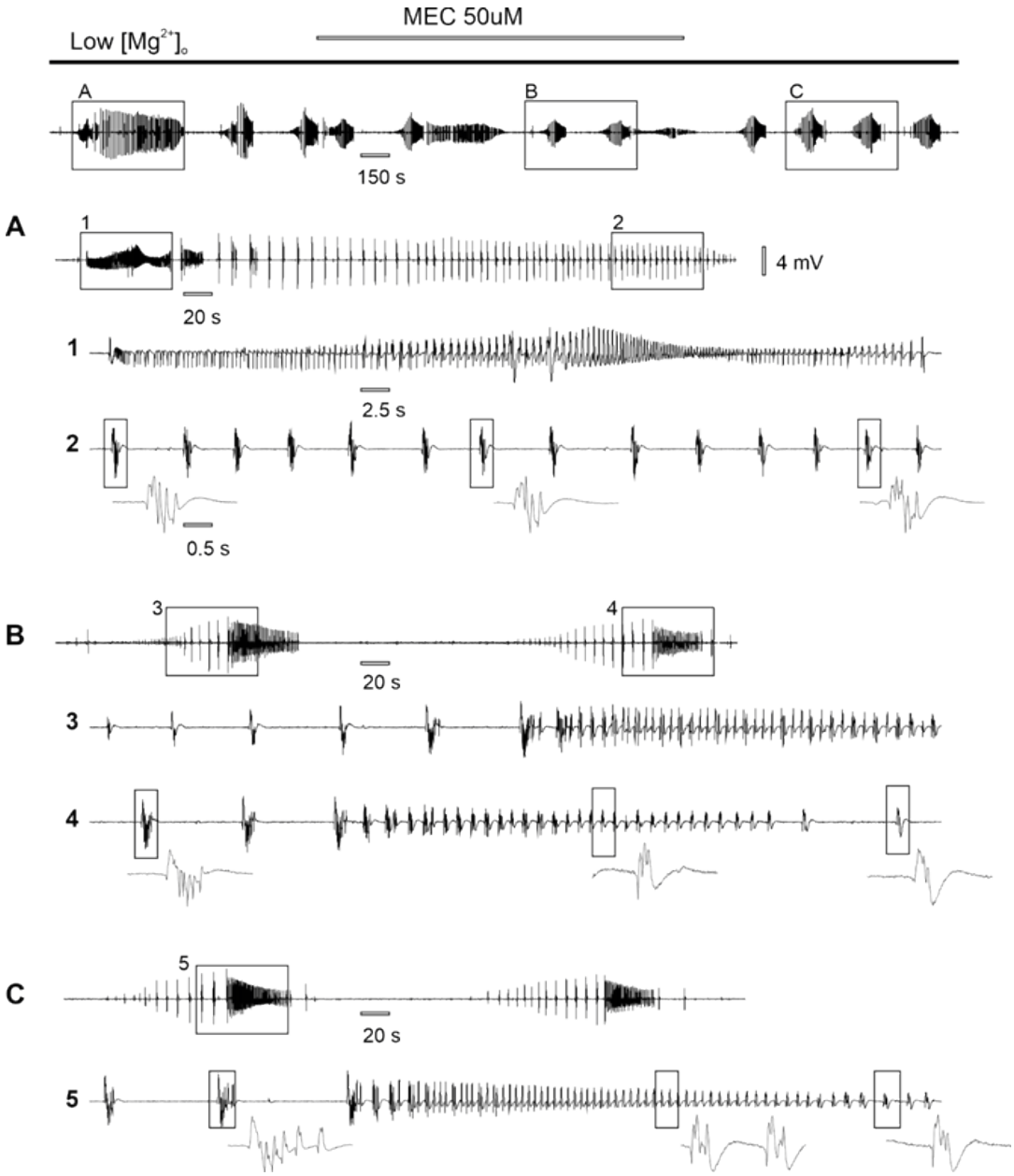

Fig. 1 – Extracellular recording of epileptiform activity during perfusion of hippocampal slices with low-Mg^{2+} aCSF, during and after application of 50 μM mecamylamine. A – Typical electrophysiological response of CA3 neuronal net on Mg^{2+} omitting: (1) recurring seizure-like activity; (2) continuous epileptic discharges. B – Field electrical activity of CA3 neurons in low Mg^{2+} during application of mecamylamine: (3) continuous epileptic discharges; (4) recurring seizure-like activity. C – Field electrical activity of CA3 hippocampus after recovery from mecamylamine application: (5) continuous epileptic discharges and recurring seizure-like activity

campal slices increased internal frequency of continuous epileptic discharges to 7.02 ± 1.24 Hz, ($P < 0.01$).

In this study, we investigated the role of nicotinic receptors in manifestations of epileptic discharges in CA3 hippocampus, evoked by omitting Mg^{2+} from extracellular solution. Application of MEC increased frequency of recurrent seizures and continuous epileptic discharges, however decreased the internal frequency of continuous epileptic discharges. Our results demonstrate that nicotinic receptors have modulatory role, rather than proepileptic or anticonvulsive effect in low Mg^{2+} model of epilepsy.

Our research supports previous data indicating that blockade of nicotinic acetylcholine receptor might be a valuable therapeutic approach to treat generalized epileptic seizure rather than complex partial seizures [18].

CONCLUSIONS

1. Omitting Mg^{2+} from extracellular solution results in the appearance of epileptiform activity in CA3 rat hippocampus.

2. Two types of seizure manifestations are induced by low-Mg^{2+} aCSF: recurring seizures and continuous discharges.

3. Application of mecamylamine significantly increases internal frequency of recurring seizure-like activity and significantly decreases inter-event intervals between continuous discharges.

4. Mecamylamine significantly decreases internal frequency of continuous epileptic discharges.

5. Blockade of nicotinic acetylcholine receptors with mecamylamine has multidirectional effect on epileptiform activity, evoked by omitting Mg^{2+} from extracellular solution.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

О.С. Запухляк, Д.С. Ісаев

МЕКАМИЛАМИН МОДУЛИРУЕТ ЭПИЛЕПТИФОРМНУЮ АКТИВНОСТЬ В МОДЕЛИ ЭПИЛЕПСИИ С НИЗКИМ СОДЕРЖАНИЕМ MG^{2+}

Мекамиламин – неселективный антагонист никотиновых ацетилхолиновых рецепторов, который был разработан как антигипертензивный препарат, а сегодня исследуется его терапевтическое действие при таких патологических состояниях, как депрессия, тревожность и эпилепсия. В этой работе мы исследовали влияние мекамиламина на проявления судорожноподобной активности, вызванной перфузией срезов гиппокампа раствором искусственной спинномозговой жидкости с низким содержанием Mg^{2+}. Следует отметить, что во внеклеточной растворе наблюдали два различных типа эпилептиформной активности: повторяющаяся судорожноподобную активность и длительные разряды. Аппликация мекамиламина увеличила внутреннюю частоту повторяющейся судорожноподобной активности и значительно уменьшила интервалы между длительными эпилептиформными разрядами. Также снижалась внутренняя частота длительных эпилептиформных разрядов. Результаты нашей работы показывают, что влияние мекамиламина на эпилептиформную активность гиппокампа, вызванную в растворе с низким содержанием Mg^{2+}, имеет модулирующий характер. Дополнительно, наши результаты указывают на роль никотиновых холинорецепторов в синхронизации активности гиппокампа и могут частично объяснить терапевтическое действия мекамиламина в ЦНС.

Ключевые слова: мекамиламин; срезы мозга крыс; эпилептиформная активность; СА3-гиппокамп.

О.С. Запухляк, Д.С. Ісаєв

МЕКАМІЛАМІН МОДУЛЮЄ ЕПІЛЕПТИФОРМНУ АКТИВНІСТЬ В МОДЕЛІ ЕПІЛЕПСІЇ З НИЗЬКИМ ВМІСТОМ MG^{2+}

Мекаміламін – неселективний антагоніст нікотинових ацетилхолінових рецепторів, який було розроблено як антигіпертензивний препарат, а нині ведуться дослідження щодо його терапевтичної дії при таких патологічних станах, як депресія, тривожність та епілепсія. У цій роботі ми досліджували вплив мекаміламіну на прояви судомоподібної активності, викликаної перфузією зрізів гіпокампа розчином штучної спинномозкової рідини з низьким вмістом Mg^{2+}. Слід відмітити, що у позаклітинному розчині спостерігали два різних типи епілептиформної активності: повторювану судомоподібну активність та тривалі розряди. Застосування мекаміламіну суттєво збільшило внутрішню частоту повторюваної судомоподібної активності та значно зменшило інтервали

між тривалими розрядами. Також суттєво знизилася внутрішня частота тривалих епілептиформних розрядів. Результати нашої роботи вказують, що вплив мекаміламіну на епілептиформну активність гіпокампа, індуковану в розчині з низьким вмістом Mg^{2+}, має модулюючий характер. Додатково, наші результати вказують на роль нікотинових холінорецепторів у синхронізації активності гіпокампа, які можуть частково пояснити ефект терапевтичної дії мекаміламіну в ЦНС.

Ключові слова: мекаміламін; зрізи мозку щурів; епілептиформна активність; СА3-гіпокамп.

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Received 02.12.2020