MODULATION OF EPILEPTIC ACTIVITY IN RATS: FOCUS ON SLEEP, PHYSICAL EXERCISE AND NITRIC OXIDE-MEDIATED NEUROTRANSMISSION IN A MODEL OF HOMOCYSTEINE THIOLACTONE-INDUCED SEIZURES

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MODULACIJA EPILEPTIČNE AKTIVNOSTI KOD PACOVA: SPAVANJE, FIZIČKA AKTIVNOST I NEUROTRANSMISIJA POSREDOVANA AZOT MONOKSIDOM U MODELU EPILEPSIJE IZAZVANE HOMOCISTEIN TIOLAKTONOM

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ABSTRACT

Epilepsy is a chronic neurological disorder characterised by recurrent epileptic seizures. Understanding the mechanisms by which it initiates and develops, as well as its modulating factors, are of great scientific interest. Experimental models of epilepsy are useful for understanding these mechanisms.

Homocysteine, an amino acid endogenously generated in the body, together with its reactive metabolite, homocysteine thiolactone (HCT), is recognised as a risk factor for a variety of diseases. HCT-induced seizures are a model of generalised epilepsy in which the coexistence of two types of epileptic activity has been documented. The complex interplay between sleep and epilepsy is still only poorly understood. Additionally, the relationship between physical exercise and epilepsy is quite intriguing, especially the mechanism underlying this relationship. The role of nitric oxide (NO)-mediated neurotransmission in the development of epileptic activity is highly debated in the existing scientific literature .

In this review article, we described the modulation of epileptic activity in rats and focused on sleep, physical activity and NO-mediated signalling. First, we explain the characteristics of the experimental models of epileptic activity and the unique features of HCT-induced seizures. Second, the modulating effects of sleep and regular physical exercise training on epileptic activity, along with works from the authors, are discussed. Finally, the anticonvulsive effects of NO that is produced via nNOS and iNOS in HCT-induced seizures are reviewed.

Keywords: *homocysteine, seizures, sleep, physical activity, nitric oxide*

SAŽETAK

Epilepsija je hronično neurološko oboljenje koje karakteriše rekurentna pojava epileptičnih napada. Razumevanje menahizama nastanka i širenja epileptične aktivnosti, kao i foktora modulacije ovih procesa, od izuzetnog je naučno-stručnog značaja. Eksperimentalni modeli epilepsije su značajni za razumevanje upravo ovih mehanizama.

Homocistein, aminokiselina koja se endogeno sintetiše u organizmu, zajedno sa svojim reaktivnim metabolitom homocistein tiolaktonom (HCT), je prepoznat kao faktor rizika za nastanak različitih bolesti. Epilepsije izazvane HCT-om predstavljaju model generalizovane epileptične aktivnosti u kome je pokazana koegzistencija dva tipa napada. Složeni uzajamni odnos između spavanja i epilepsije još uvek nije dovoljno razjašnjen. Takođe, međuodnos fizičke aktivnosti i epilepsije je krajnje interesantan, naročito mehanizmi ovih odnosa. Uloga neurotransmisije posredovane azot monoksidom (NO) u nastanku epileptične aktivnosti je vrlo kontradiktorna u postojećoj literaturi.

Predmet ovog rada bila je modulacija epileptične aktivnosti kod pacova sa posebnim osvrtom na spavanje, fizičku aktivnost i neurotransmisiju posredovanu NO. Najpre su razmotreni koncepti eksperimentalnih modela epileptične aktivnosti sa osobenostima HCT epilepsija. Zatim su diskutovani modulatorni efekti spavanja i regularnog fizičkog veržbanja na epileptičnu aktivnost zajedno sa rezultatima radova autora. Na posletku, dat je osvrt na antikonvulzivnu ulogu NO i doprinos nNOS i iNOS u epileptičnoj aktivnosti izazvanoj HCT-om.

Ključne reči: *homocistein, epilepsije, spavanje, fizička aktivnost, azot monoksid*

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INTRODUCTION

Epilepsy is a chronic neurological disorder with an incidence rate of approximately 50 per 100,000 people per year (1). It is characterised by a variety of cellular and molecular alterations of the brain, especially the cerebral cortex, that result in recurrent epileptic seizures (2). Despite extensive improvements in the pharmacotherapy for epilepsy, it remains poorly controlled in almost 40% of patients (3). Therefore, understanding the mechanisms by which it initiates and develops, as well as its modulating factors, are of great scientific interest. Experimental models of epilepsy are useful in these attempts, but no single model system can be useful for all types of epilepsy (4).

Homocysteine is an amino acid that is endogenously generated in the body during methionine metabolism (5). Homocysteine, together with its reactive metabolite, homocysteine thiolactone (HCT), is recognised as a risk factor for a variety of diseases and is one of the most potent excitatory agents in the central nervous system (CNS) (6-9). The primary mechanism of its epileptic properties has been attributed to the activation of glutamate receptors (10). HCT-induced seizures are a model of generalised epilepsy in which the coexistence of two types of epileptic activity has been documented (11). There is a complex interplay between sleep and epilepsy. This interplay is of special interest for neuroscientists because it is still only poorly understood (12,13). Additionally, the relationship between physical exercise and epilepsy is quite intriguing, especially the mechanism underlying this relationship.

Nitric oxide (NO) belongs to the family of gasotransmitters. The role of NO-mediated neurotransmission in the development of epileptic activity is highly debated in the existing scientific literature (14). The results of studies on the role of NO in epileptogenesis have indicated that it depends on the type of NO production, among other factors (15-17).

With these considerations, we described the modulation of epileptic activity in rats while focusing on sleep, physical activity and NO-mediated signalling in this review article. First, we explained the characteristics of experimental models of epileptic activity and the unique features of HCT-induced seizures.

Second, the modulating effects of sleep and regular physical exercise training on epileptic activity, along with works from the authors, are discussed.

Finally, the anticonvulsive effects of NO that is produced via nNOS and iNOS in HCT-induced seizures are reviewed.

Modelling of epileptic activity in animals: unique features of homocysteine thiolactone seizures

Epileptogenesis is defined as a process of by which a neuronal network transforms into a network of synchronised hyperexcitable neurons. Primarily, it is a consequence of an imbalance between inhibitory and excitatory neurotransmission systems, with overstimulation of the latter (1). The main goal in treatment of epilepsies is the reestablishment of the homeostasis between these systems. Therefore, the only way to develop new antiepileptic drugs and treatments is to further understand the process of epileptogenesis and the mechanisms contributing to it.

A variety of experimental models for epilepsy have been developed, and it is highly likely that no single model system could be useful for all types of epilepsy (4). Modelling the epileptic activity in animals involves two main approaches: administering different chemical compounds and electrical stimulation kindling. In our Laboratory for Neurophysiology, different animal models have been developed, including metaphit audiogenic seizures (18-21) and lindane- (22-24) and homocysteine thiolactone-induced seizures (11). In addition to these models, the other commonly used experimental epilepsy models are those induced by factors such as N-methyl-D-aspartate (NMDA), pentylenetetrazol, pilocarpine, kainic acid, and 4-aminopyridine amygdala kindling (for review see 4). These experimental models of generalised epilepsy each have distinct advantages and disadvantages and are suitable for research on epileptogenic mechanisms, as well as for preclinical evaluation of antiepileptic drugs (25,26).

Of the chemically induced seizures, those induced by HCT are of particular interest because of both the properties of homocysteine and its related compounds and some unique features of this model. In particular, Stanojlovic et al. (11) showed that acute administration of HCT to adult rats significantly alters neuronal circuits, leading to epileptogenic activity in the electroencephalogram (EEG) with characteristic spike-and-wave discharges (SWDs) and convulsive episodes in the animal behaviour. SWDs are ictal phenomena accompanied with absence-like behaviour and characterised in the EEG as follows (11): a) spontaneous and generalised, rhythmic 5-7 Hz discharges, b) with a typical spike-wave complex lasting more than 1 s, and c) an amplitude of at least twice the background EEG activity. The convulsive behaviour elicited by HCT includes motor phenomena ranging from lower jaw twitching to tonic whole body convulsions. Therefore, HCT-induced seizures are widely accepted as a suitable model of generalised epilepsy in which the coexistence of two types of epileptic activity, i.e., convulsive and absence-like seizures, has been documented (27-28).

Sleep and epilepsy: bidirectional relationship

Sleep is a cyclic and vital physiological process. On average, it constitutes one-third of human life (29). Electrophysiological studies have shown the existence of two sleep types: rapid eye movement (REM) and non-REM sleep (30). REM sleep is characterised by intensive brain activity, which is similar to the awake state, while the body is relaxed; thus, REM sleep is termed paradoxical sleep.

Non-REM sleep is characterised by delta activity in the EEG (also known as slow-wave sleep, SWS). (31). Tonic events include the suppression of electromyographic activity to the level of atonia; a desynchronizised, high-frequency, low-voltage (20–30 μV) EEG; a high awakening threshold; and reduced body temperature. Phasic events include rapid eye movements; muscle contraction in the eardrum, lip and tongue; muscle movement in the limbs; and respiratory and cardiac changes (32). Numerous findings revealed similarities between rats and humans, justifying the use of rats in preclinical sleep studies (32-34)

The intimate and bidirectional relationship between sleep and epilepsy has been known since the time of Aristotle and Hippocrates (32, 35). The sleep state is known to influence seizure onset, especially in certain epilepsy syndromes. The converse is also true; epilepsy may disrupt sleep, either directly through convulsive nature of disease or indirectly through the effects of antiepileptic drugs (reviewed in (36)). Neuroscientists have remained particularly interested in the complex interplay between sleep and epilepsy because it is still only poorly understood (32).

Although most studies on this issue are based on clinical trials, experimental studies are of great significance for understanding this relationship (13). Most experimental techniques for selective REM sleep deprivation are based on the single platform method of Jouvet (37,38), which was modified by Susic and Markovic (33). Alterations in behaviour upon REM sleep deprivation are mostly a consequence of imbalance in neurotransmitter systems (39). It has been shown that the generalised down-regulation of muscarinic receptors (40), which are necessary for the initiation and coordination of paradoxical sleep, is included in this effect (41). This change also involves dopamine neuronal circuits, inducing the up-regulation of postsynaptic dopamine receptors (42). Moreover, REM sleep deprivation affects the levels of the excitatory amino acid glutamate and of aspartate, which become elevated in the cortex and hippocampus (43).

Sleep modulation and its effects on homocysteine thiolactone seizures

The effects of sleep modulation on the epileptic activity induced by HCT have been recently investigated (44) using the single platform method to selectivity deprive rats of REM sleep. This study showed that selective REM sleep deprivation increased the incidence and number of seizure episodes per rat that are induced by a subconvulsive dose of HCT. Moreover, the REM-sleep-deprived animals showed a shorter latency time to seizures in behaviour and a significant rate of lethality after HCT administration; in contrast, no significant effects were observed on the seizure severity. EEG analysis in the same study showed a significant increase in the number and duration of SWDs and a decrease in latency of its EEG appearance in the REM-sleep-deprived rats upon treatment with a subconvulsive dose of HCT. In the EEG of rats receiving 0.9% NaCl instead of HCT in the corresponding condition, SWD was not registered. Such behavioural and EEG outputs revealed that selective REM sleep deprivation aggravated the HCT-induced process of epileptogenesis.

Physical activity in epileptic patients: should patients participate in exercise and sport activities?

In 1968, the American Medical Association (AMA) recommended that contact sports and physical education should be limited in epileptic patients whose seizure activity is uncontrolled or not controlled sufficiently well. Since then, the recommendations have been constantly revised: the AMA revised them in 1974, and other institutions, such as the American Pediatric Association, have also revised the recommendations. For a long time, there were established beliefs that epilepsy patients should avoid physical exercise and involvement in sports due to the paroxysmal nature of seizure attacks and the higher possibility of head injuries. Therefore, an increase in sedentary lifestyles in epileptic patients is observed in population-based studies (45). Namely, studies based on large population studies showed that epileptic patients are significantly more sedentary than the general population (46-48). It should be noted that these populations displayed differences in the form of physical activity. The dominant activity among epileptic patients was walking for physical exercise, possibly because of the restrictions imposed on driver's license for patients suffering from epilepsy (48). However, regular physical activity is known to improve both physical and mental health and to contribute to improved quality of life and better social integration. Moreover, it generally known that a lack of physical exercise is a risk factor for a variety of disorders, including cardiovascular diseases, obesity, diabetes and many others (49). Therefore, the beneficial aspects of physical exercise could be lost for these patients (50). With these considerations, the relationship between physical exercise and epilepsy is quite intriguing, particularly the mechanism underlying this relationship. Further investigations that elucidate the role of physical activity in epilepsy are of particular interest.

Experimental epilepsy models have been extremely useful in understanding the role of exercise in epilepsy. There are some reports showing aggravation of epileptiform EEG activity upon physical exercise (51,52). Moreover, studies using the kainate model of seizures report that physical activity aggravated neuronal damage in rats (53). However, opposite results are also reported. Initially, the Arida group used the kindling model of epilepsy to determine the effect of physical activity on rats (54). Arida et al. (54) have shown that chronic physical exercise increased the seizure threshold in an amygdala kindling model of epilepsy. The same group also reported that a physical exercise program decreased the seizure incidence in a model of temporal lobe epilepsy (55). Souza et al. (56) used swimming training as a paradigm of physical activity

and showed that this type of physical exercise prevented the neuronal hyperexcitability induced by pentylenetetrazol (PTZ); this change manifested as increased seizure latency and attenuated seizure duration. It has also been reported that aerobic physical exercise beneficially affects pilocarpine seizures (57). The possible beneficial effects of physical exercise on neuronal hyperexcitability have also been reported in some clinical trials (58, 59).

Physical activity in a model of homocysteine thiolactone seizures

Hrncic et al. (60) investigated the effects of regular physical activity on the epileptic activity induced by homocysteine thiolactone using an experimental paradigm of aerobic physical activity on a treadmill. Namely, rats were made to run on treadmill apparatus for 30 minutes once a day for 30 consecutive days while the belt speed was set to 20 m/min with a 0° incline. Physical exercise using this protocol could be considered aerobic (61) because it has been reported that rats reached the maximal lactate steady state (MLSS) at this belt speed.

The results of that study (60) showed that the rats subjected to regular physical exercise training on a treadmill for 30 consecutive days had a significantly prolonged latency time to development of the first seizure sign and a significantly lower number of HCT-induced seizure episodes per rat comparing with the rats that were sedentary during the same period of time. EEG analysis in the same study showed congruent results. Namely, statistical analysis of the SWD appearance in this study showed a significantly lower number of SWDs in the rats subjected to the physical exercise protocol vs. their sedentary mates with no differences in the duration of SWD. These results suggested that physical activity decreased the susceptibility of rats to developing HCT-induced seizures. In the same study, Hrncic et al. showed that this physical exercise protocol partially prevented the elevation of lipid peroxidation after HCT administration and prevented an HCT-induced decrease in SOD and CAT activity.

NO-mediated signalling in epileptogenesis

NO is synthesised from L-arginine by the activity of the family of enzymes known as NO synthases (NOS). Three different forms of NOS have been identified: neural (nNOS) and endothelial NOS (eNOS), which are Ca^{2+}/calmodulin-dependent enzymes, and inducible NOS (iNOS), which shows Ca^{2+}-independent activity (62). It should be noted that all NOS isoforms have been identified in the brain (63). nNOS is found to be expressed in the hippocampus, cerebral cortex, corpus striatum and cerebellum, as well as in some cells of the autonomic nervous system (64). iNOS is reported to be expressed in the brains of humans with epilepsy. In some spontaneously epileptic mice, overexpression of iNOS is also found (65,66). Moreover, iNOS has been found to be a major contributor to the initiation/exacerbation of CNS inflammatory/degenerative conditions via the production of excessive NO (67).

Numerous studies have demonstrated the anticonvulsive activity of NO in different experimental models of epileptic activity (68-72). However, NO has been reported to play a proconvulsive role in several epilepsy models (73-77). These issues have been reviewed recently in more detail (14).

Anticonvulsive effects of NO in homocysteine thiolactone seizures: the role of NO that is produced via nNOS and iNOS

Using L-arginine and L-NAME as modulators of NO production, we investigated the role of NO in HCT-induced epileptic activity (78). We showed that the systemic administration of L-arginine significantly decreased the seizure incidence and the number of seizure episodes and prolonged the latency time to the first seizure elicited by a convulsive dose of HCT (79). In contrast, pretreatment with L-NAME increased the seizure incidence and severity and shortened the latency time to the first seizure following the injection with a subconvulsive dose of HCT. Moreover, EEG analysis showed that L-arginine decreased but L-NAME increased the median number of SWDs per rat; the duration of individual SWDs was not altered. These results showed the functional involvement of NO in HCT-induced convulsive activity.

As noted, the role of NO could depend on how it is produced. Therefore, further investigations of the roles of nNOS and iNOS in HCT-induced seizures were undertaken. Pharmacological inhibition of nNOS by 7-nitroindazole has been used to investigate the involvement of nNOS in HCT-induced seizures (79). In this study, the intraperitoneal application of 7-nitroindazole showed a tendency to increase seizure incidence, decrease the latency time to first seizure, increase the number of seizure episodes per rat and increase the severity of HCT-induced seizures.

The involvement of iNOS-derived NO in HCT-induced seizures was demonstrated using aminoguanidine, a selective iNOS inhibitor (80). Treatment with aminoguanidine increased the following behavioural seizure properties: seizure incidence, number of seizure episodes per rat and severity of HCT-induced seizures, as well as the number and duration of SWDs in the EEG. Quantitative analysis of the EEG ictal activity showed similar results. Namely, aminoguanidine increased the number and duration of SWDs induced by HCT in that study.

Numerous attempts were also made to elucidate the effects of various NOS inhibitors on the activity of different antiepileptic drugs to determine the potential therapeutic effects of NO modulation. However, the obtained results were inconsistent to some level. In particular, the results showed that NOS inhibitors could increase (76,

81), decrease (82) or not affect antiepileptic efficacy (81). Recently, it has been shown that co-administration of an iNOS inhibitor significantly decreased the beneficial effects of pioglitazone on PTZ-induced seizures in mice (83). Constitutive forms of NOS, but not iNOS, were involved in the anticonvulsant effect of lithium in PTZ-induced seizures (84). The NO modulation of NMDA receptor activity by the process of S-nitrosylation and the co-localisation of NOS and GABA to some extent suggest that the role of NO-mediated signalling involves excitability balance .

The anticonvulsive properties of NO that is produced via nNOS and iNOS in HCT-induced epileptic activity could result from several mechanisms and the interplay between NO and HCT at various levels, i.e., interaction at NMDA and GABA receptors, including the relationship of NO with the NMDA and GABA receptors, neurodegeneration, cytoprotection and oxidative stress (85-89).

CONCLUSION

The only way to develop new antiepileptic drugs and treatments is to improve the understanding of the process of epileptogenesis and the mechanisms that contribute to it. Experimental epilepsy models have been extremely useful in achieving this goal. HCT-induced seizures are particularly interesting because HCT induces two types of epileptic activity in rats. Selective REM sleep deprivation potentiates HCT-induced seizures, whereas regular physical activity beneficially affects them. The role of NO in the HCT model of epileptic activity is demonstrated to be anticonvulsive, regardless of how it is produced. Translational research on the complex interplay between the modulating factors of epileptogenesis described in this review will result in new strategies to fight epilepsy.

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