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ORIGINAL RESEARCH

The Role of Nitric Oxide in Homocysteine Thiolactone-Induced Seizures in Adult Rats

Dragan Hrnčić · Aleksandra Rašić-Marković · Danijela Krstić · Djuro Macut · Dragan Djuric · Olivera Stanojlović

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Abstract The role of NO in epileptogenesis has been studied in different experimental models, and the reported results have been highly contradictory. The current study aimed to determine the role of NO in mechanisms of D,L-homocysteine-thiolactone (H) induced seizures by testing the action of L-arginine (NO precursor) and L-NAME (NOS inhibitor) on behavioral and electroencephalographic (EEG) manifestations of H-induced seizures. The same holds true with the brain Na^+/K^+- and Mg^{2+}-ATPase activity in adult male Wistar rats. We showed that the pretreatment with L-arginine (300, 600 and 800 mg/kg, i.p.) in a dose-dependent manner significantly decreased lethality, seizure incidence and a number of seizure episodes and prolonged latency time to the first seizure elicited by a convulsive dose of H (8 mmol/kg, i.p.). L-Arginine (800 mg/kg) completely reversed the inhibitory effect of H on the Na^+/K^+-ATPase activity in the hippocampus, the cortex and the brain stem and decreased the H-induced spike-and- wave discharges (SWD) formation in EEG. On the other hand, pretreatment with L-NAME (200, 500 and 700 mg/kg, i.p.) potentiated a subconvulsive dose of H (5.5 mmol/kg, i.p) by increasing incidence and severity determined by a descriptive-rating scale (0–4) and shortening the latency time to the first seizure. The L-NAME reversed H-induced alterations in the Na^+/K^+-ATPase activity in the cortex and the brain stem but not in the hippocampus. At last, the potentiated SWD appearance in EEG and an increased number of lethal outcomes occurred. In the present work, the modulation of NO levels, with the NO precursor and NOS inhibitor, was shed more light on its mechanism of action and answered the question whether NO could be included in the list of anticonvulsant agents in the D,L-homocysteine thiolactone experimental model of seizures in adult rats.

Keywords D,L-Homocysteine thiolactone · Nitric oxide · L-Arginine · L-NAME · Seizures · Na^+/K^+- and Mg^+-ATPase · Electroencephalography · Rats

D. Hrnčić · A. Rašić-Marković · D. Macut · D. Djuric · O. Stanojlović (✉)
Laboratory of Neurophysiology, Institute of Medical Physiology "Richard Burian", School of Medicine, University of Belgrade, Višegradska 26/II, 11000 Belgrade, Serbia
e-mail: solja@afrodita.rcub.bg.ac.yu

D. Krstić
Department of Medical Chemistry, School of Medicine, University of Belgrade, 11000 Belgrade, Serbia

Introduction

Homocysteine is an endogenous sulfur-containing amino acid recently recognized as one of the most potent excitatory agents of the central nervous system (Perla-Kajan et al. 2007; Djuric et al. 2008) and the major risk factor for numerous brain disorders (Sachdev 2005) like brain atrophy cognitive decline and dementia, depression, schizophrenia, stroke, Alzheimer's, Parkinson's and Huntington's diseases. Seizures are one of the major symptoms in hyperhomocysteinemia (Van den Berg et al. 1995), but it has been shown that classical antiepileptic drugs like phenytoin, fenobarbiton, carbamazepine and valproate increase plasma homocysteine level, thus showing the complexity of the relationship between homocysteine and epilepsy (Sener et al. 2006). It has been suggested that homocysteine could be particularly harmful to all cells due to its metabolic conversion by methionyl-tRNA synthetases to highly reactive thioester homocysteine thiolactone (H) (Perla-Kajan et al. 2007; Jakubowski 2004). Homocysteine

and its metabolites seem to express direct excitatory effects on *N*-methyl-D-aspartate (NMDA) and group I metabotropic glutamate receptors (mGluRs) (Troen 2005). However, the mechanisms of homocysteine convulsive action are far from being elucidated.

Homocysteine was previously shown to elicit seizures in immature animals (Kubova et al. 1995; Folbergrova 1997). Recently, a model of generalized H-induced seizures in adult rats has been developed (Stanojlović et al. 2009) in which coexistence of convulsive and absence-like sizures, accompanied by characteristic spike-and-wave discharges (SWD) in electroencephalogram, were prooven enabling further investigations of their mechanisms using convulsive (8 mmol/kg) and subconvulsive (5.5 mmol/kg) doses of H. The model has also been shown suitable for testing potential anticonvulsive substances (Rašic-Marković et al. 2009a).

Nitric oxide (NO) is a highly reactive messenger molecule synthesized in a number of tissues, including the brain, with pleiotropic physiological and pathological effects (Guix et al. 2005). It is produced from L-arginine by the action of the family of enzymes known as NO synthases (NOS). Neural (nNOS) and endothelial NOS (eNOS) are Ca^{2+}/calmodulin-dependent enzymes, while inducible NOS (iNOS) shows Ca^{2+}-independent properties. *N*-nitro-L-arginine methyl ester (L-NAME) is a nonselective NOS inhibitor commonly used to decrease NO levels.

NO seems to play the key role in a recently described form of interneuronal communication characterized by the absence of synaptic contacts (Vizi 2000). The main NO-cellular-signaling pathway is the guanylate cyclase activation with the subsequent production of cyclic guanosine-3,5-monophosphate. By modulation of the release of classical neurotransmitters, NO strongly influences the excitability status of neurons, either in basal conditions or during paroxysmal activity (Guix et al. 2005).

Among others, the role of NO in epileptogenesis has been studied in different experimental models, and the reported results have been highly contradictory. Currently, the proconvulsant activity of NO has been demonstrated in several studies. On the other hand, the results of other studies indicate that NO may play a role of an endogenous anticonvulsant substance (reviewed in Ferraro and Sardo 2004). Thus, it seems that the activity of NO depends on the experimental seizure model employed, the type and the dose of drugs used in order to modify the cerebral NO levels and the animal strain.

A number of reports have demonstrated a reduction in the Na^+/K^+-ATPase activity in neurodegeneration (Lees 1993), epilepsy (Grisar et al. 1992) and hyperhomocysteinemia (Streck et al. 2003; Matte et al. 2007, 2004) are possibly associated with excitotoxic mechanisms. We have recently reported (Rasić-Marković et al. 2009b) that the Na^+/K^+-ATPase activity was decreased in the rat hippocampus, cortex and brain stem after acute treatment with H. On the other hand, some authors reported the elevated Na^+/K^+-ATPase activity in certain animal models of epilepsy (Bignami et al. 1966). NO-generating compounds have been reported to inhibit the Na/K ATPase activity from the porcine cerebral cortex (Sato et al. 1995), while L-NAME prevented the inhibitory effect of glutamate on the Na^+/K^+-ATPase activity in rat brain synaptosomes (Avrova et al. 1999).

Therefore, the current study aimed to determine the role of NO in the mechanisms of H seizures by testing the action of L-arginine (NO precursor) and L-NAME (NOS inhibitor) on behavioral and electroencephalographic manifestations of H-induced seizures and the brain Na^+/K^+- and Mg^{2+}-ATPase activity in adult rats.

Materials and Methods

Animals

Adult (10-week old at the arrival, 12-week old at allowance to proper experiments) male Wistar albino rats (180–210 g b.w.) were obtained from the Military Medical Academy Breeding Laboratory (Belgrade, Serbia). The animals were housed individually in transparent plastic wire-covered cages (55 × 35 × 15 cm) with free access to food (Purina rat chow) and water. They were kept in a sound-attenuated chamber under controlled ambient conditions (22–23°C, 50–60% relative humidity, 12/12 h light/dark cycle with light switched on at 8 am) and habituated to handling. The acclimatization period lasted for 7 days.

All experimental procedures were in full compliance with The European Council Directive (86/609/EEC) and approved by The Ethical Committee of the University of Belgrade (Permission No 298/5-2).

Experimental Groups

Based on our preliminary experiments and literature data (Stanojlović et al. 2009), the following experimental groups were formed: 1. control (C; 0,9% NaCl, $n = 6$); 2. L-arginine (A_{800}; 800 mg/kg, $n = 8$); 3. L-NAME (N_{700}; 700 mg/kg, $n = 8$); 4. D,L-homocysteine thiolactone 5.5 (subconvulsive dose) and 8 (convulsive dose) mmol/kg ($H_{5.5}$, $n = 11$; and H_8, $n = 9$); 5. L-arginine (300, 600 and 800 mg/kg) 30 min prior to H 8 mmol/kg ($A_{300}H_8$, $n = 6$; $A_{600}H_8$, $n = 6$ and $A_{800}H_8$, $n = 11$); 6. L-NAME (200, 500 and 700 mg/kg) 30 min prior to H 5.5 mmol/kg ($N_{200}H_{5.5}$, $n = 7$; $N_{500}H_{5.5}$, $n = 7$ and $N_{700}H_{5.5}$, $n = 7$).

All drugs were freshly dissolved in saline and after adjusting the pH to 7.0, administered intraperitoneally (i.p.) in a volume of 0.1 ml/100 g rat body weight.

Behavioral Recordings

The rats placed in separate transparent plastic cages (55 × 35 × 15 cm) were observed 90 min for the behavioral manifestations of H-induced seizures in rats. This was assessed by the incidence of motor seizures, a number of seizure episodes per rat and their severity. Seizure severity was determined by a modified descriptive-rating scale reported by Stanojlović et al. (2009) with grades defined as grade 1—head nodding, lower jaw twitching; grade 2—myoclonic body jerks (hot plate reaction), bilateral forelimb clonus with full rearing (Kangaroo position); grade 3—progression to generalized clonic convulsions followed by tonic extension of fore and hind limbs and tail and grade 4—prolonged severe tonic-clonic convulsions lasting over 10 s (status epilepticus) or frequent repeated episodes of clonic convulsions for an extended period of time (over 5 min). In addition, latency to seizure, defined as a time from the H injection to the first seizure response, was also recorded. For the rats without seizures, 90 min latency time was scored. Lethality was recorded 90 min and 24 h after the H administration.

Surgery and EEG Recordings

The rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), placed in a stereotaxic apparatus and three gold-plated recording electrodes were implanted over the frontal (2 mm rostrally to bregma and 2 mm from the median line), parietal (2 mm rostrally to lambda and 2 mm laterally to median line) and occipital (2 mm caudally to lambda) cortices for chronic EEG recordings. The electrodes were fixed to the skull with dental acrylic cement. One week recovery period was allowed prior to further experiments, and the animals had a 24-h-habituation to the recording situation.

An 8-channel EEG apparatus (RIZ, Zagreb, Croatia) was used. The signals were digitized using a SCB-68 data acquisition card (National Instruments Co, Austin, TX, USA). A sampling frequency of 512 Hz/channel and 16-bit A/D conversion were used for the EEG signals. The cutoff frequencies for the EEG recordings were set at 0.3 and 100 Hz for the high-pass and low-pass filters, respectively. Ambient noise was eliminated using a 50 Hz notch filter. Data acquisition and signal processing were performed with the LabVIEW platform software developed in the Laboratory (NeuroSciLaBG).

All EEG recordings in the freely moving rats were visually monitored and screened for seizure activity and stored on a disk for the subsequent off-line analysis. The power spectra density (obtained by the Fast Fourier transformation method) of the characteristic 12 s epochs was plotted, and the integrated energy signals expressed as μV^2/Hz. The rats were removed from the recording chamber and returned to their home cage upon completion of the 90 min recording sessions.

The inclusion criteria for the analyzed SWD data were as follows: (1) spontaneous and generalized, rhythmic 5–7 Hz discharges; (2) with typical spike–wave complex lasting >1 s and (3) amplitude of at least twice the background EEG activity (Stanojlović et al. 2009). The number and duration of SWD were calculated during a 90-min period after the H injection. All SWDs were detected visually.

Biochemical Analyses

In a separate series of experiments, the activity of Na^+/K^+-ATPase and Mg^{2+}-ATPase in the cortex, the hippocampus and the brain stem in the following groups C, A, N, $H_{5.5}$ and H_8, $A_{800}H_8$, $N_{700}H_{5.5}$ ($n = 14$ per group) were recorded.

Synaptic Plasma Membrane Preparation

The animals were killed 30 min after the last drug injection without anesthesia by decapitation, and the brains were rapidly excised. The cortex, the hippocampus and the brain stem were dissected out and pooled (4/pool) for immediate preparation of synaptic plasma membranes (SPM). The SPM from the cortex, the hippocampus and the brain stem were isolated as described by Cohen et al. (1977) with the modification described by Towle and Sze (1983). Mitochondrial contamination and protein content were determined according to the standard procedure (Horvat et al. 1995).

ATPase Assays

SPM ATPase activities were assayed in the standard medium consisting of 50 mM Tris–HCl, pH 7.4, 100 mM NaCl, 20 mM KCl and 5.0 mM $MgCl_2$ and supplemented by 25 μg of SPM proteins in a final volume of 200 μl, and the reaction was terminated after 10 min. The inorganic orthophosphate (Pi) released by ATP hydrolysis was measured using a modified spectrophotometric procedure (Vasić et al. 1999) by reading the absorbance at 690 nm. The activity obtained in the absence of NaCl and KCl was attributed to Mg^{2+}-ATPase. The Na^+/K^+-ATPase activity was calculated as the difference between the total ATPase (obtained in the presence of Na^+, K^+ and Mg^{2+} ions) and Mg^{2+}-ATPase activity.

The determination of the SPM ATPase activities was examined in pooled brain tissues (the cortex, the hippocampus and the brain stem). The results are expressed as the mean of the specific enzyme activity ± SD from at least four independent experiments done in triplicates. The

specific enzymatic activities are expressed as μM of inorganic phosphate released per mg protein per hour.

Drugs

All drugs were of analytical purity and purchased from Sigma–Aldrich Chemical Co., USA.

Data Analyses

The significance of the differences in the incidence of seizures and lethality was evaluated by Fisher's exact probability test. Because of the normal distribution of the data on seizure latency, a number and intensity of seizure episodes, as well as a number and duration of SWD has not been estimated by Kolmogorov–Smirnov test, the non-parametric analyses (Kruskal–Wallis ANOVA and Mann–Whitney *U*-test) were used to determine the statistical significance of the differences between the groups (* $P < 0.05$, ** $P < 0.01$). The results were expressed as medians with 25th and 75th percentiles.

The significance of the differences in the activity of Na^+/K^+-ATPase and Mg^{2+}-ATPase between the groups was estimated by Student's *t*-test. The results are expressed as the means ± SD.

Results

Behavioral Findings

All control rats (C), as well as the rats from L-arginine (A_{800}) and L-NAME (N_{700}) groups, expressed the normal gross behavioral activity without any sign of seizures, and no lethality was recorded.

Convulsions were observed in all rats that received D,L-homocysteine thiolactone (H) in the dose of 8 mmol/kg (H_8, incidence 100%). On the other hand, convulsions were observed in 33.33% of rats treated with H in the dose of 5.5 mmol/kg ($H_{5.5}$ group) (Figs. 1, 3).

Effects of L-arginine Pretreatment

L-arginine administered 30 min prior to H 8 mmol/kg decreased seizure incidence in the $A_{600}H_8$ (83.33%; $P > 0.05$) and $A_{800}H_8$ (30%; $P < 0.05$) groups compared to the H_8 group (Fig. 1).

Rats in the groups receiving L-arginine 600 mg/kg ($A_{600}H_8$) and 800 mg/kg ($A_{800}H_8$) displayed a significantly prolonged median latency time to the first seizure episode in comparison with rats from the H_8 group ($P < 0.05$; Fig. 2a).

The median number of seizure episodes per rat was significantly lower in the $A_{800}H_8$ compared with the H_8

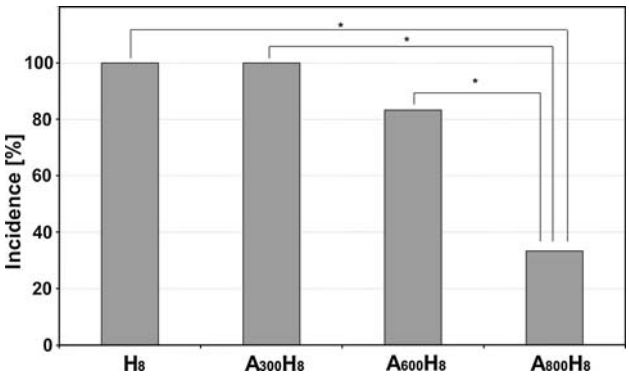


Fig. 1 The influence of L-arginine on seizure incidence (percentage of convulsing rats). Adult Wistar rats were i.p. treated with D,L-homocysteine thiolactone 8 mmol/kg (H_8, $n = 7$) 30 min after L-arginine 300, 600 and 800 mg/kg treatments ($A_{300}H_8$, $n = 6$; $A_{600}H_8$, $n = 6$ and $A_{800}H_8$, $n = 9$). The significance of the differences between the groups was estimated by Fisher's exact probability test (* $P < 0.05$)

($P < 0.05$) group (Fig. 2b). Differences in seizure episode severity among the H_8 and the groups pretreated with L-arginine ($A_{300}H_8$, $A_{600}H_8$, $A_{800}H_8$) were not statistically significant (Fig. 2c). The majority of seizure manifestations in $A_{800}H_8$ were scored as grade 2 (71.43%). In the H_8 group, 20% of grade 4 and 24% of grade 3 seizures were recorded (Table 1).

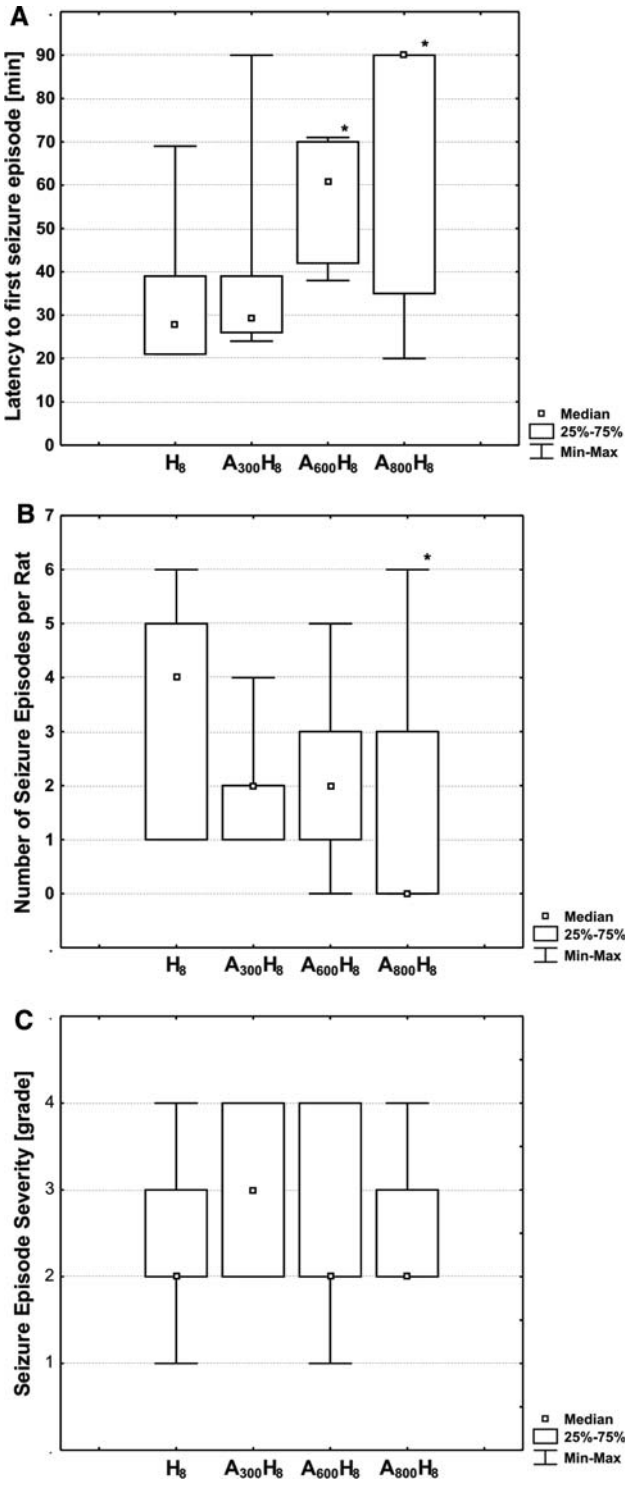
Pretreatment with L-arginine reduced lethality in all experimental groups compared to the H_8 group ($P > 0.05$). Lethality observed 24 h after the H injection was significantly decreased in the $A_{800}H_8$ compared to the H_8 group ($P < 0.05$) (Table 2).

Effects of L-NAME Pretreatment

The seizure incidence in the group receiving L-NAME 700 mg/kg 30 min prior to H 5.5 mmol/kg ($N_{700}H_{5.5}$ group, 85.71%) was significantly higher than in the $H_{5.5}$ group, $N_{200}H_{5.5}$ and $N_{500}H_{5.5}$ group ($P < 0.05$; Fig. 3).

The latency time to the first seizure episode was significantly shorter in the $N_{700}H_{5.5}$ group [33 (30–53) min] compared with the $H_{5.5}$ group [90 (46–90) min] and $N_{200}H_{5.5}$ [90 (52–90) min] ($P < 0.05$; Fig. 4a). No statistically significant differences in the number of seizure episodes between the $H_{5.5}$ and the groups pretreated with L-NAME ($N_{200}H_{5.5}$, $N_{500}H_{5.5}$, and N700H5.5) were observed (Fig. 4b).

The rats in the $N_{500}H_{5.5}$ and $N_{700}H_{5.5}$ groups developed seizure episodes of higher severity compared with the rats from the $H_{5.5}$ group ($P < 0.01$), as well as from the $N_{200}H_{5.5}$ group ($P < 0.05$; Fig. 2c). The majority of seizure episodes in the $H_{5.5}$ group were scored as grade 1 (62.50%); in the $N_{700}H_{5.5}$ group, grade 2 (53.85%, $P < 0.05$) was dominant, and grade 4 (66.04%) was dominant in the $N_{500}H_{5.5}$ group



◀**Fig. 2** The influence of L-arginine on median latency to the first seizure episode (**a**), a number of seizure episodes per rat (**b**) and their severity (**c**). Seizure severity was assessed by descriptive-rating scale with the following grades: 1—head nodding, lower jaw twitching; 2—myoclonic body jerks (hot plate reaction), bilateral forelimb clonus with full rearing (Kangaroo position); 3—generalized clonic convulsions followed by tonic extension of fore and hind limbs and tail and 4—prolonged severe tonic-clonic convulsions lasting over 10 s (status epilepticus) or frequent repeated episodes of clonic convulsions for an extended period of time (over 5 min). The significance of the differences between the groups was estimated by Kruskal–Wallis ANOVA and Mann–Whitney *U*-test (* $P < 0.05$ comparing to H_8). For the details see the caption of Fig. 1

(Table 3). The appearance of grade 1 was only 7.70% in $N_{700}H_{5.5}$ (vs. $H_{5.5}$, $P < 0.05$).

No lethal outcomes either 90 min or 24 h upon the H administration were recorded in the $H_{5.5}$ and $N_{200}H_{5.5}$ group of animals. On the other hand, in more than half of the $N_{700}H_{5.5}$ animals (57.14%), lethality was observed 90 min after the H injection, and the difference was significant in comparison with the $H_{5.5}$ group ($P < 0.05$). The same holds true for lethality after 24 h upon the H injection (Table 4).

EEG Activity

Bioelectrical activity recorded from the frontal, parietal and occipital cortex in the groups of rats treated only with L-arginine (A_{800}, 800 mg/kg) or L-NAME (N_{700}, 700 mg/kg) was similar to the one in control (C), revealing no epileptiform graphoelements, while spectral power density was dominant in the alpha frequency range (8 Hz). During

Table 1 Effects of L-arginine on seizure episode severity grade distribution

Grade (%)	Experimental groups			
	H_8	$A_{300}H_8$	$A_{600}H_8$	$A_{800}H_8$
1	16	0	7.69	0.00
2	40	50$^{\#}$	46.15$^{\#}$	71.43$^{\#}$
3	24	0	15.39	7.14
4	20	50$^{\#}$	30.77	21.43

Severity of seizure episode was assessed by descriptive-rating scale with defined grades 1–4. For details see the captions of Figs. 1 and 2. Statistical significance of the differences was estimated by Fisher's exact probability test ($^{\#}$ $P < 0.05$ vs. grade 1)

Table 2 The effects of L-arginine on lethality recorded 90 min and 24 h after D,L-homocysteine-thiolactone administration

Lethality (%)	Experimental groups			
	H_8	$A_{300}H_8$	$A_{600}H_8$	$A_{800}H_8$
After 90 min	48.85	0.00	16.67	22.22
After 24 h	85.71	66.67	33.33	22.22*

Lethality—number of exited rats out of total number of rats in group expressed in percentage

For details see the caption of Fig. 1. Significance of the differences between the groups was estimated by Fisher's exact probability test (* $P < 0.05$ vs. H_8)

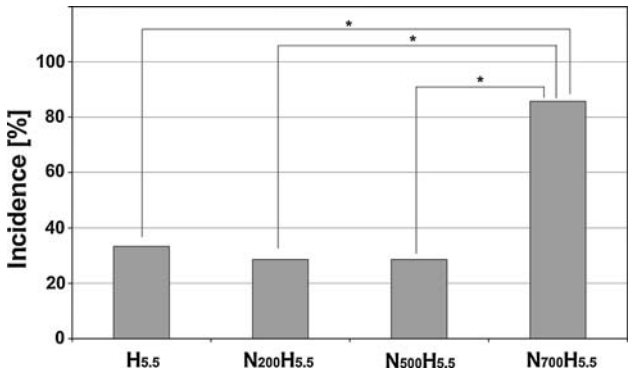


Fig. 3 The influence of L-NAME on seizure incidence (percentage of convulsing rats). Adult Wistar rats were i.p. treated with L-NAME (200, 500 and 700 mg/kg) 30 min prior to D,L-homocysteine thiolactone 5.5 mmol/kg ($N_{200}H_{5.5}$, $n = 7$; $N_{500}H_{5.5}$, $n = 7$ and $N_{700}H_{5.5}$, $n = 7$). The significance of the differences between the groups was estimated by Fisher's exact probability test (* $P < 0.05$)

recordings, the rats were quiet but awake (Fig. 5c, A_{800} and N_{700}).

The dissociation between the EEG pattern and the motor phenomena, as well as low electro-clinical correlations, was found in all recordings after the H_8 injection. The same holds true for the recordings after $N_{700} + H_{5.5}$. The convulsions of grade 4 were usually accompanied by bursts of polyspikes in EEG (Fig. 5 $N_{700}H_{5.5}$). Generalized high-voltage synchronous, spindle-like electrical oscillations in EEG, defined as SWD, were associated with the absence-like behavior (a sudden motor immobility with minor clinical signs like the loss of responsiveness (Fig. 5 last panels). The appearance of SWD in EEG was analyzed in the experimental groups. The median number of SWD per rat was lower in the $A_{800}H_8$ compared to H_8 group, while this number was significantly higher in the $N_{700}H_{5.5}$ group compared to the $H_{5.5}$ group ($P < 0.05$, Fig. 6a). No statistically significant differences were found in duration of SWD between the $A_{800}H_8$ and the H_8, as well as between the $N_{700}H_{5.5}$ and the $H_{5.5}$ group (Fig. 6b).

Na^+/K^+-ATPase and Mg^{2+}-ATPase Activities

The activity of Na^+/K^+-ATPase was significantly reduced in the H_8 group in all the examined brain structures compared to the C group (Fig. 7a), while the activity of Mg^{2+}-ATPase was not affected (Fig. 7c). The subconvulsive H dose (5.5 mmol/kg) did not affect the activities of either Na^+/K^+-ATPase or Mg^{2+}-ATPase, compared to controls (Fig. 7b, d).

L-arginine in the A_{800} group significantly increased the activity of Na^+/K^+-ATPase in the cortex (265.94%, $P < 0.01$), the hippocampus (93.59%, $P < 0.01$) and the brain stem (262.17%, $P < 0.01$) compared to the C group (Fig. 7a). The same effect was observed in the $A_{800}H_8$ group where the activities of ATPase were increased in all the examined brain structures compared to the C and H_8 group.

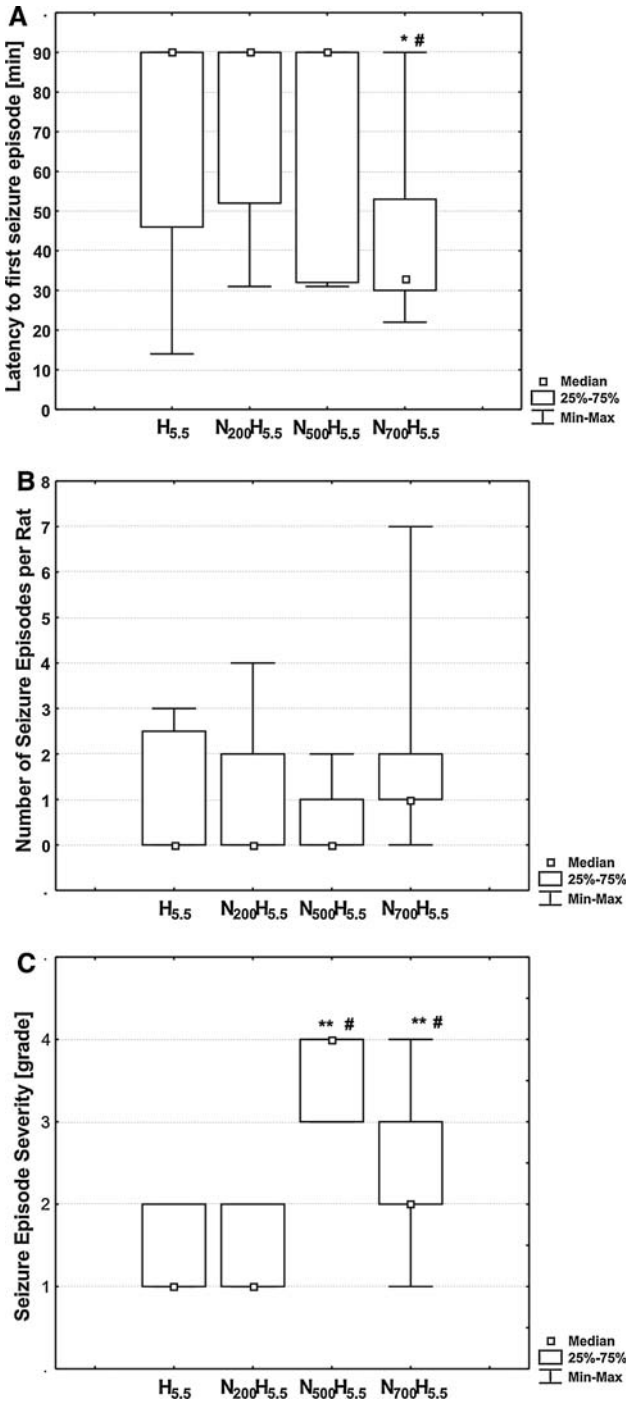


Fig. 4 The influence of L-NAME on latency to the first seizure episode (**a**), a number of seizure episodes per rat (**b**) and their severity (**c**). The significance of differences between the groups was estimated by Kruskal–Wallis ANOVA and Mann–Whitney *U*-test (* $P < 0.05$, ** $P < 0.01$ comparing to $H_{5.5}$ and # $P < 0.05$ compared to $N_{200}H_{5.5}$). For the details see the caption of Figs. 2 and 3

Table 3 Effects of L-NAME on seizure episode severity grade distribution

Grade (%)	Experimental groups			
	$H_{5.5}$	$N_{200}H_{5.5}$	$N_{500}H_{5.5}$	$N_{700}H_{5.5}$
1	62.50	57.14	0	7.70*
2	37.50	42.86	0	53.85$^{\#}$
3	0$^{\#}$	0$^{\#}$	33.33	15.38
4	0$^{\#}$	0$^{\#}$	66.04*	23.08

Severity of seizure episode was assessed by descriptive-rating scale with defined grades 1–4. For details see the caption of Figs. 2 and 3. Statistical significance of the differences was estimated by Fisher's exact probability test (* $P < 0.05$, vs. $H_{5.5}$; $^{\#}$ $P < 0.05$ vs. grade 1)

Table 4 The effects of L-NAME on lethality recorded 90 min and 24 h after D,L-homocysteine-thiolactone administration

Lethality (%)	Experimental groups			
	$H_{5.5}$	$N_{200}H_{5.5}$	$N_{500}H_{5.5}$	$N_{700}H_{5.5}$
After 90 min	0	0	28.57	57.14*,$^{\#}$
After 24 h	0	0	28.57	57.14*,$^{\#}$

Lethality—number of exited rats out of total number of rats in group expressed in percentage

For details see the caption of Fig. 3. Significance of the differences between the groups was estimated by Fisher's exact probability test (* $P < 0.05$ vs. $H_{5.5}$; $^{\#}$ $P < 0.05$ vs. $N_{200}H_{5.5}$)

In the A_{800} group, the activity of Mg^{2+}-ATPase was increased only in the brain stem (61.48%, $P < 0.05$) in comparison with controls, while in the $A_{800}H_8$ group, the activity of this enzyme was significantly increased in the cortex and the brain stem compared to the C and H_8 group (Fig. 7c).

The activity of Na^+/K^+-ATPase was significantly increased in the N_{700} group, measured in the cortex (211.51%, $P < 0.01$) and the brain stem (156.9%, $P < 0.01$) compared to the controls (Fig. 7b). An increased Na^+/K^+-ATPase activity was found in the cortex and the brain stem in the $N_{700}H_{5.5}$ group, and the statistical significance was found compared with the C and $H_{5.5}$ group ($P < 0.05$).

The same was true with an increased activity of Mg^{2+}-ATPase in the N_{700} group that was found in the cortex (85.28%, $P < 0.05$) in comparison with C, while in the group pretreated with L-NAME ($N_{700}H_{5.5}$), this enzyme activity was increased in the cortex and the hippocampus in comparison with the C and $H_{5.5}$ group (Fig. 7d).

Discussion

The detailed mechanisms of homocysteine convulsive action have not been clear yet, but there is evidence that numerous excitatory homocysteine effects are due to ionotropic and metabotropic glutamate receptor overstimulation, promotion of oxidative stress, DNA damage and apoptosis (Troen 2005; Djuric et al. 2008; Stanojlović et al. 2009).

In the present study, we showed that the systemic administration of increasing doses of L-arginine, NO precursor, in a dose-dependent manner significantly decreased seizure incidence and the number of seizure episodes and the prolonged latency time to the first seizure (Figs. 1, 2) elicited by the convulsive dose of H (8 mmol/kg, i.p.).

It was found that L-arginine in doses similar to ours acted as anticonvulsant in numerous models of epilepsy such as kainate (Przegalinski et al. 1994), lithium-pilocarpine (Noyan and Gulec 2000), the sound-induced convulsions in DBA/2 mice, picrotoxin (Paul and Ekambaram 2005) and the penicillin-induced epileptiform activity in rats (Marangoz and Bagirici 2001; Ayyildiz et al. 2007). Tutka et al. (2007) reported the involvement of NO in nicotine convulsions in mice. But NO has been demonstrated as a proconvulsant agent in several seizure models opposed to our results (reviewed in Ferraro and Sardo 2004).

The results of this study, for the first time, showed the functional involvement of NO in the convulsive activity of the H-induced seizures in adult rats. Pretreatment with L-NAME, in a dose-dependent manner, increased seizure incidence and severity and shortened latency time to the first seizure following the injection with the subconvulsive dose of H (5.5 mmol/kg, i.p.). This H dose was subthreshold and subconvulsive, and it could be used for some additional manipulations like cortical cobalt lesion, constant-current stimulation (Kubova et al. 1995; Walton et al. 1996; Mareš et al. 2002) or L-NAME, like in our experiment, to induce a series of generalized clonic-tonic homocysteine-seizures.

NOS inhibitors have been shown to increase the seizure severity in rats induced by kainate, amygdale kindling (Alabadí et al. 1999), potentiate the seizures induced by quinolinate to rats (Haberny et al. 1992) and facilitate focal seizures induced by aminopyridine in rats (Boda and Szente 1996). NOS inhibitors augmented (Del-Bel et al. 1997), inhibited (Van Leeuwen et al. 1995) or were without effects (Noyan et al. 2007) on the epileptic activity induced by pilocarpine. Similarly, NOS inhibitors were found to either inhibit (Bashkatova et al. 2000; Han et al. 2000) or have no effect on PTZ-induced convulsions (Przegalinski et al. 1996; Urbanska et al. 1996). A recent study revealed that mice lacking the nNOS gene exhibited severe convulsions following the subconvulsive dose of PTZ and lethal outcome after the convulsive dose in all mice (Itoh and Watanabe 2009). In mice model of generalized epilepsy induced by NMDA, a model similar to homocysteine generalized seizures in rats, L-NAME caused an increase in duration and seizure severity (Buisson et al. 1993).

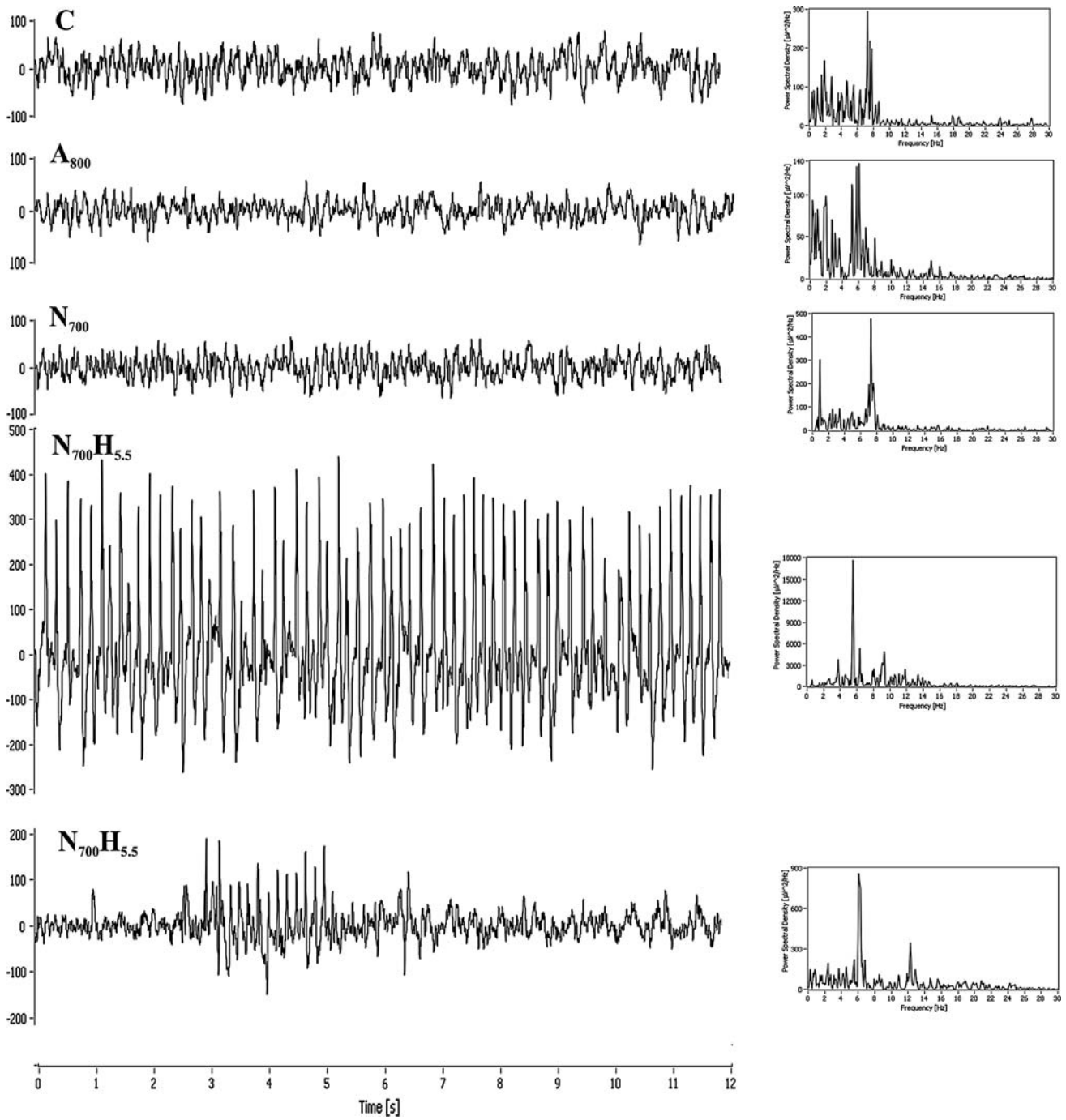


Fig. 5 Representative EEG tracings (the *left panels*) and corresponding power spectra density (the *right panels*) recorded in the control group of rats (*C*), 30 min after L-arginine 800 mg/kg, i.p. (A_{800}); 30 min after L-NAME 700 mg/kg, i.p. (N_{700}). High-voltage polyspikes during grade 4 seizures in the $N_{700}H_{5.5}$ treatment. Characteristic SWD pattern during absence-like seizure in the $N_{700}H_{5.5}$ group (the *last panel*). Lead: the left frontal–right parietal cortex

The anticonvulsive relation of NO and homocysteine could be explained by several mechanisms including the relationship of NO with the NMDA and GABA receptor.

NO could modulate the NMDA receptor activity by interacting with the –SH group of its redox modulatory site via the process of *S*-nitrosylation. It results in the downregulation of this receptor complex (Lipton et al. 1993) and prevents neurotoxic effects of an excessive Ca^{2+} influx during homocysteine induced "overstimulation" of NMDA and mGluRs I receptors. In addition, Kim (1999) demonstrated that NO ameliorated homocysteine adverse effects by *S*-nitrosylation in cultured rat cortical neurons.

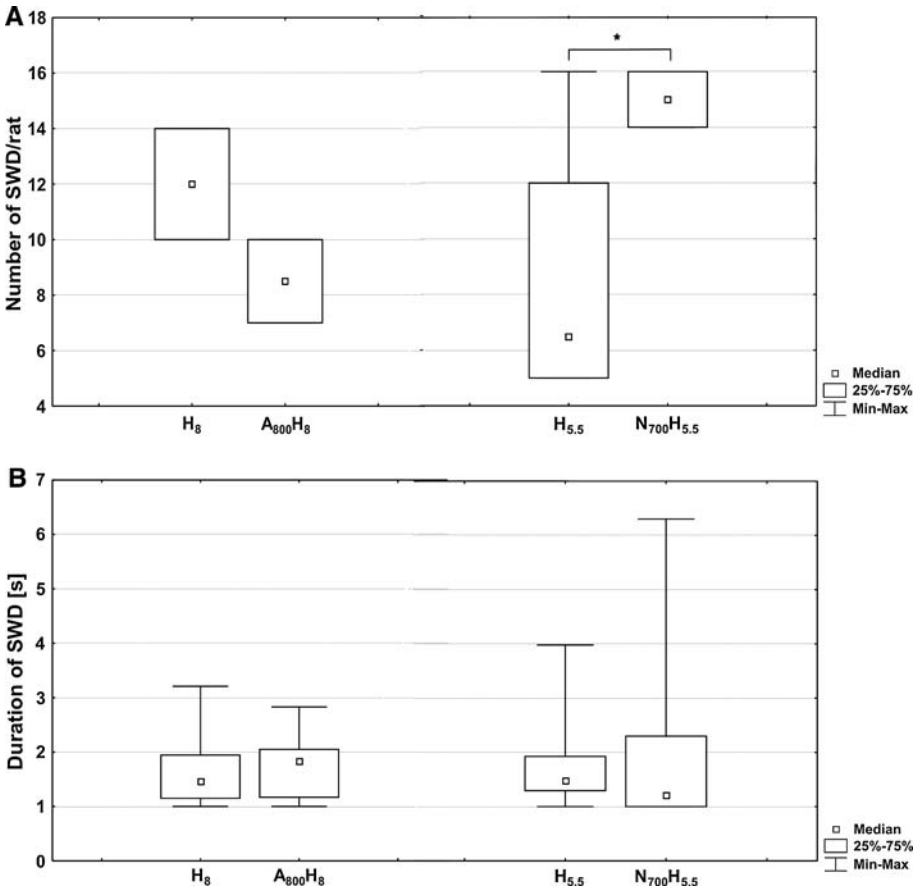


Fig. 6 A number (**a**) and duration (**b**) of SWD estimated in 90 min EEG recordings of rats from the experimental groups. The significance of the differences between the groups was estimated by Mann–Whitney *U*-test (* $P < 0.05$). For the details see the caption of Figs. 1 and 3

Moreover, NO induces reduction in glutamate by activation of glial cells (Nanri et al. 1996).

Many experimental studies have demonstrated the co-localization of NOS and GABA (Wang et al. 1997) and suggested that NO inhibited GABA transaminase (Paul and Ekambaram 2005). The basal NO levels induce depression, while high concentrations of NO increase the GABA release (Getting et al. 1996). This could explain the anti-convulsive NO properties. Opposite to homocysteine, which increases oxidative stress by production of reactive oxygen species (Ramakrishnan et al. 2006), NO can act as a neural protector, due to the formation of *S*-nitroso-L-glutathione, an antioxidant and NO-storing molecule (Rauhala et al. 1998).

It is known that homocysteine can cause neurodegeneration, synaptic dysfunction and neuronal death by promoting DNA damage and activation of apoptotic signaling (Mattson and Shea 2003), which contributes to high lethality of H_8 (85.71%). Inhibition of caspases by *S*-nitrosylation (Mannick et al. 1999) and expression of cytoprotective genes (Hao et al. 1999) by NO could explain the neuroprotective effects of NO demonstrated in our experiments by reduction of lethality. Namely, the treatment with L-arginine prior to H_8 ($A_{300}H_8$, $A_{600}H_8$, $A_{800}H_8$) decreased lethal outcome in all these groups.

The pretreatment with L-NAME exhibited an opposite effect and significantly increased lethality in the $N_{700}H_{5.5}$ group.

EEG Analyses

No epileptiform activity was recorded in EEG of the rats treated only with L-arginine (A_{800}, 800 mg/kg) or L-NAME (N_{800}, 700 mg/kg). But Ferraro et al. (1999) have shown opposite findings to ours that the inhibition of NOS evokes the epileptiform activity and appearance of spikes, polyspikes and spike and waves in the rat hippocampus and the somatosensory cortex.

SWDs, symmetrical generalized discharges of 6–8 Hz and H-induced brain bioelectrical patterns, already reported in our previous study (Stanojlović et al. 2009), were accompanied with typically absence-like seizures. This nonconvulsive behavior was characterized by a quiet immobile, hypo-reactive behavioral state with minimal myoclonic facial jerks. It is believed that the cortico-thalamo-cortical oscillatory network is primarily involved in the initiation and propagation of SWD (Sitnikova and van Luijtelaar 2007).

L-arginine (800 mg/kg) decreased, while L-NAME (700 mg/kg) increased the median SWD number per rat in

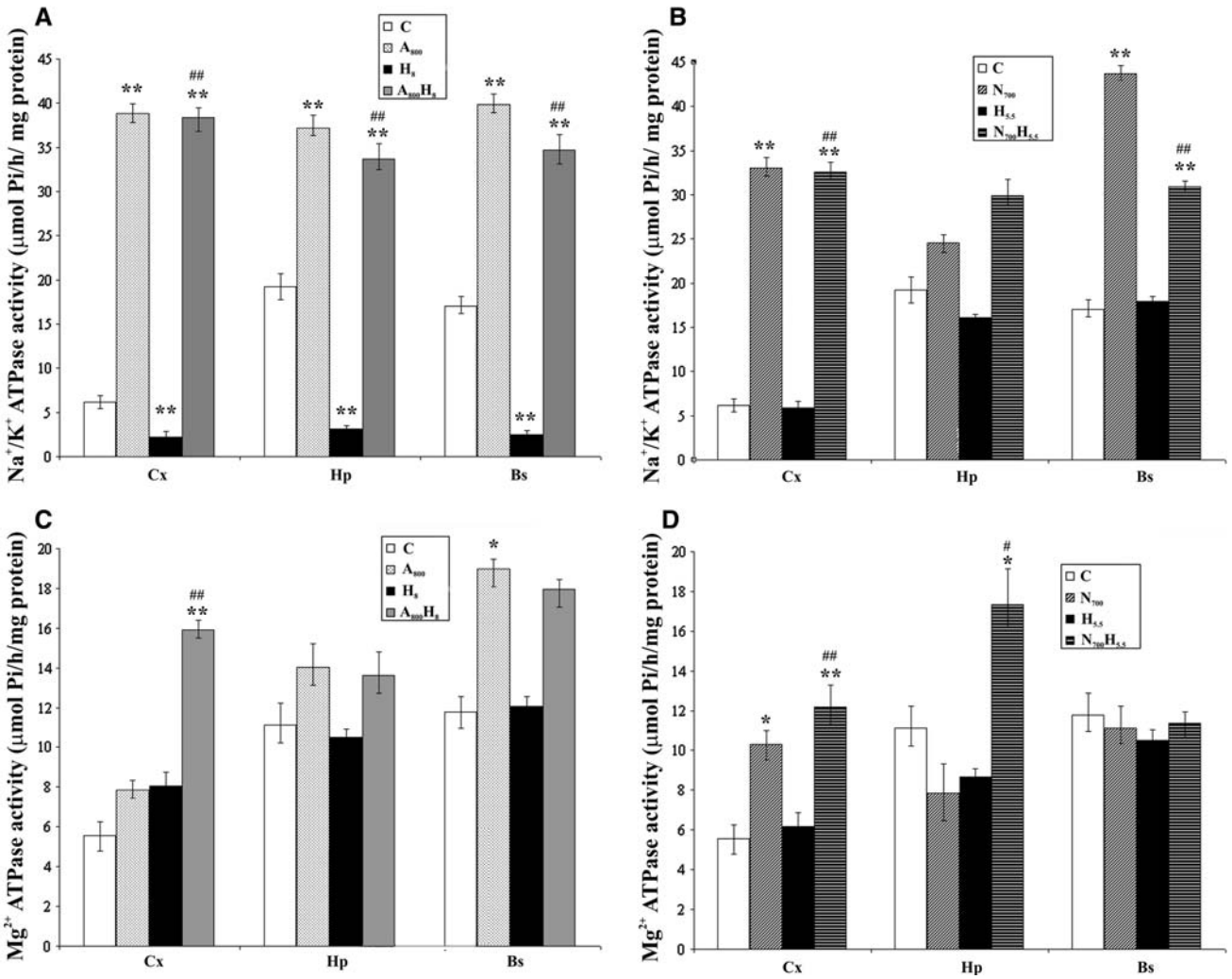


Fig. 7 The activity of Na^+/K^+-ATPase (**a**, **b**) and Mg^{2+}-ATPase (**c**, **d**) in the brain cortex (*Cx*), the hippocampus (*Hp*) and the brain stem (*Bs*) of rats from the experimental and control groups. The results are means ± SD from at least four independent experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$ vs. control and # $P < 0.05$, ## $P < 0.01$ vs. H groups. For the details see the caption of Figs. 1, 3 and 5

90-min EEG recordings. The duration of individual SWD was not altered by either of these drugs (Fig. 6). Similar to our findings, potentiations of the EEG epileptiform activity by the decreased NO levels were reported in models of aminopyridine, penicillin and methylmalonate-induced seizures (Boda and Szente 1996; Marangoz and Bagirici 2001; Ayyildiz et al. 2007; Royes et al. 2007).

Analyses of ATPase Activity

The maintenance of the Na^+/K^+-ATPase activity is critical for normal brain functioning and reduction in this enzyme activity is associated with neuronal hyperexcitability (Matte et al. 2004) and selective neuronal damage of rat and human brains (Cousin et al. 1995; Lees and Leong 1994). The decreased Na^+/K^+-ATPase activity in acute and chronic lesions of experimental and human epilepsy was found by Streck et al. (2002).

Numerous in vivo and in vitro investigations, like our results, demonstrated a strong inhibitory effect of homocysteine on the Na^+/K^+-ATPase activity in SPM of the rat hippocampus and the parietal cortex of rats without affecting the Mg^{2+}-ATPase activity (Streck et al. 2002; Wyse et al. 2002). Silva et al. (1999) have demonstrated that L-arginine per se does not have a direct effect on the enzymes in the in vitro study. On the other hand, in vivo studies have shown that L-arginine produces a significant reduction in the Na^+/K^+-ATPase activity in the rat hippocampus (dos Reis et al. 2002) and the midbrain (Wyse et al. 2001).

L-arginine, when applied alone, significantly increases the activity of Na^+/K^+-ATPase activity in the hippocampus, the cortex and the brain stem and when applied prior

to H (8 mmol/kg) completely reversed the inhibitory effect of H. The same holds true with Mg^{2+}-ATPase in the rat cortex and the brain stem. L-NAME per se increases the Na^+/K^+-ATPase activity in the cortex and the brain stem, but not in the hippocampus. When L-NAME was administered prior to H (5.5 mmol/kg), it increased the activity of both the Na^+/K^+-ATPase (in the cortex and the brain stem, but not in the hippocampus) and Mg^{2+}-ATPase (in the rat cortex and the hippocampus) activity.

Na^+/K^+-ATPase in all the examined brain structures was not affected by D,L-homocysteine thiolactone in the subconvulsive dose (H, 5.5).

The altered Na^+/K^+-ATPase activity affected the release of various neurotransmitters including GABA and glutamate (Vizi 1979). The primary mechanism of hyperexcitability associated with the impaired activity of Na^+–K^+ ATPase was noted from Vaillend et al. (2002).

The compounds influencing the NO pathway have been tested as potential adjunctive anticonvulsive agents, and they have been found to affect the anticonvulsant activity of several antiepileptic drugs (Wojtal et al. 2003), which is the base for pharmacological strategies and further development of potential interventions in seizure disorders.

In summary, the current study reports that NO acts as an anticonvulsant in H-induced seizures and prevents the H-induced inhibition of the Na^+–K^+ ATPase activity. Further studies are required to elucidate detailed mechanisms of these findings.

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References

Alabadí JA, Thibault JL, Pinard E, Seylaz J, Lasbennes F (1999) 7-Nitroindazole, a selective inhibitor of nNOS, increases hippocampal extracellular glutamate concentration in status epilepticus induced by kainic acid in rats. Brain Res 839:305–312

Avrova NF, Shestak KI, Zakharova IO, Sokolova TV, Leontev VG (1999) The difference in the effect of glutamate and NO synthase inhibitor on free calcium concentration and Na^+, K^+-ATPase activity in synaptosomes from various brain regions. Neurochem Res 24:1101–1106

Ayyildiz M, Yildirim M, Agar E (2007) The involvement of nitric oxide in the anticonvulsant effects of alpha-tocopherol on penicillin-induced epileptiform activity in rats. Epilepsy Res 73:166–172

Bashkatova V, Vitskova G, Narkevich V, Vanin A, Mikoyan V, Rayevsky K (2000) Nitric oxide content measured by ESR-spectroscopy in the rat brain is increased during pentylenetetrazole-induced seizures. J Mol Neurosci 14:183–190

Bignami A, Palladini C, Venturini G (1966) Effect of cardiazol on sodium–potassium adenosine triphosphatase of the rat brain in vivo. Brain Res 1:413–414

Boda B, Szente M (1996) Nitric oxide synthase inhibitor facilitates focal seizures induced by aminopyridine in rat. Neurosci Lett 209:37–40

Buisson A, Lakhmeche N, Verrecchia C, Plotkine M, Boulu RG (1993) Nitric oxide: and endogenous anticonvulsant substance. NeuroReport 4:444–446

Cohen RS, Blomber F, Berzins K, Siekevitz P (1977) The structure of postsynaptic densities isolated from dog cerebral cortex: I. Overall morphology and protein composition. J Cell Biol 74:181–203

Cousin MA, Nicholls DG, Pocock JM (1995) Modulation of ion gradients and glutamate release in cultured cerebellar granule cells by ouabain. J Neuroscem 64:2097–2104

Del-Bel EA, Oliveira PR, Oliveira JA, Mishra PK, Jobe PC, Garcia-Cairasco N (1997) Anticonvulsant and proconvulsant roles of nitric oxide in experimental epilepsy models. Braz J Med Biol Res 30:971–979

Djurić D, Jakovljević V, Rašić-Marković A, Djurić A, Stanojlović O (2008) Homocysteine, folic acid and coronary artery disease: possible impact on prognosis and therapy. Indian J Chest Dis Allied Sci 50:39–48

dos Reis EA, de Oliveira LS, Lamers ML, Netto CA, Wyse AT (2002) Arginine administration inhibits hippocampal Na^+, K^+-ATPase activity and impairs retention of an inhibitory avoidance task in rats. Brain Res 951:151–157

Ferraro G, Montalbano ME, La Grutta V (1999) Nitric oxide and glutamate interaction in the control of cortical and hippocampal excitability. Epilepsia 40:830–836

Ferraro G, Sardo P (2004) Nitric oxide and brain hyperexcitability. In vivo 18:357–366

Folbergrova J (1997) Anticonvulsant action of both NMDA and non-NMDA receptor antagonists against seizures induced by homocysteine in immature rats. Exp Neurol 145:442–450

Getting SJ, Segieth J, Ahmad S, Biggs CS, Whitton PS (1996) Biphasic modulation of GABA release by nitric oxide in the hippocampus of freely moving rats in vivo. Brain Res 717:196–199

Grisar T, Guillaume D, Delgado-Escueta AV (1992) Contribution of Na^+/K^+-ATPase to focal epilepsy: a brief review. Epilepsy Res 12:141–149

Guix FX, Uribesalgo I, Coma M, Muñoz FJ (2005) The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 76:126–152

Haberny KA, Pou S, Eccles CU (1992) Potentiation of quinolinate-induced hippocampal lesions by inhibition of NO synthesis. Neurosci Lett 146:187–190

Han D, Yamada K, Senzaki K, Xiong H, Nawa H, Nabeshima T (2000) Involvement of nitric oxide in pentylenetetrazole-induced kindling in rats. J Neurochem 74:792–798

Hao W, Myhre AP, Palmer JP (1999) Nitric oxide mediates IL-1beta stimulation of heat shock protein but not IL-1beta inhibition of glutamic acid decarboxylase. Autoimmunity 29:93–101

Horvat A, Nikezić G, Martinović JV (1995) Estradiol binding to synaptosomal plasma membranes of rat brain regions. Experientia 51:11–15

Itoh K, Watanabe M (2009) Paradoxical facilitation of pentylenetetrazole-induced convulsion susceptibility in mice lacking neuronal nitric oxide synthase. Neuroscience 159:735–743

Jakubowski H (2004) Molecular basis of homocysteine toxicity in humans. Cell Mol Life Sci 61:470–487

Kim WK (1999) S-nitrosation ameliorates homocysteine-induced neurotoxicity and calcium responses in primary culture of rat cortical neurons. Neurosci Lett 265:99–102

Kubova H, Folbergova J, Mareš P (1995) Seizures induced by homocysteine in rats during ontogenesis. Epilepsia 36:750–756

Lees GJ (1993) Contributory mechanisms in the causation of neurodegenerative disorders. Neuroscience 54:287–322

Lees GJ, Leong W (1994) Brain lesions induced by specific and non-specific inhibitors of sodium–potassium ATPase. Brain Res 649:225–233

Lipton SA, Choi Y, Pan Z, Lei SZ, Chen HV, Sucher NJ, Loscalzo J, Singel DJ, Stamler JS (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364:626–632

Mannick JB, Hausladen A, Liu L, Hess DT, Zeng M, Miao QX, Kane LS, Gow AJ, Stamler JS (1999) Fas-induced caspase denitrosylation. Science 284:651–654

Marangoz C, Bağirici F (2001) Effects of L-arginine on penicillin-induced epileptiform activity in rats. Jpn J Pharmacol 86:297–301

Mareš P, Haugvicova R, Kubova H (2002) Interaction of excitatory amino acid agonists with cortical after discharges in developing rats. Epilepsia 43:61–67

Matte C, Monteiro SC, Calcagnotto T, Bavaresco CS, Netto CA, Wyse AT (2004) In vivo and in vitro effects of homocysteine on Na^+, K^+-ATPase activity in parietal, prefrontal and cingulated cortex of young rats. Int J Dev Neurosci 22:185–190

Matte C, Scherer EBS, Stefanello FM, Barschak AG, Vargas CR, Netto CA, Wyse AT (2007) Concurrent folate treatment prevents Na^+, K^+-ATPase activity inhibition and memory impairments caused by chronic hyperhomocysteinemia during rat development. Int J Dev Neurosci 25:545–552

Mattson PM, Shea T (2003) Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci 26:137–146

Nanri K, Takizawa S, Fujita H, Ogawa S, Shinoara Y (1996) Modulation of extracellular glutamate concentration by nitric oxide synthase inhibitor in rat transient forebrain ischemia. Brain Res 738:243–248

Noyan B, Gulec G (2000) Effects of L-arginine on prevention and treatment of lithiumpilocarpine- induced status epilepticus. Physiol Res 49:379–385

Noyan B, Jensen MS, Danscher G (2007) The lack of effects of zinc and nitric oxide in initial state of pilocarpine-induced seizures. Seizure 16:410–416

Paul V, Ekambaram P (2005) Effects of sodium nitroprusside, a nitric oxide donor, on γ-aminobutyric acid concentration in the brain and on picrotoxin-induced convulsions in combination with phenobarbitone in rats. Pharmacol Biochem Behav 80:363–370

Perla-Kajan J, Twardowski T, Jakubowski H (2007) Mechanisms of homocysteine toxicity in humans. Amino Acids 32:561–572

Przegaliński E, Baran L, Siwanowicz J (1994) The role of nitric oxide in the kainate-induced seizures in mice. Neurosci Lett 170:74–76

Przegaliński E, Baran L, Siwanowicz J (1996) The role of nitric oxide in chemically- and electrically-induced seizures in mice. Neurosci Lett 217:145–148

Ramakrishnan S, Sulochana KN, Lakshmi S, Selvi R, Angayarkanni N (2006) Biochemistry of homocysteine in health and diseases. Indian J Biochem Biophys 43:275–283

Rašić-Marković A, Djurić D, Hrnčić D, Lončar-Stevanović H, Vučević D, Mladenović D, Brkić P, Djuro M, Stanojlović O (2009a) High dose of ethanol decreases EEG total spectral power density in seizures induced by D,L-homocysteine thiolactone in adult rats. Gen Physiol Biophys 28:S23–S30

Rasić-Marković A, Stanojlović O, Hrncić D, Krstić D, Colović M, Susić V, Radosavljević T, Djuric D (2009b) The activity of erythrocyte and brain Na^+/K^+ and Mg^{2+}-ATPases in rats subjected to acute homocysteine and homocysteine thiolactone administration. Mol Cell Biochem 327(1-2):39–45

Rauhala P, Lin AM, Chiueh CC (1998) Neuroprotection by S-nitrosoglutathione of brain dopamine neurons from oxidative stress. FASEB J 12:165–173

Royes LF, Fighera MR, Furian AF, Oliveira MS, Fiorenza NG, Petry JC, Coelho RC, Mello CF (2007) The role of nitric oxide on the convulsive behavior and oxidative stress induced by methylmalonate: an electroencephalographic and neurochemical study. Epilepsy Res 73:228–237

Sachdev PS (2005) Homocysteine and brain atrophy. Prog Neuropsychopharmacol Biol Psychiatry 29:1152–1161

Sato T, Kamata Y, Irifune M, Nishikawa T (1995) Inhibition of purified (Na^+, K^+)-ATPase activity from porcine cerebral cortex by NO generating drugs. Brain Res 704:117–120

Sener U, Zorlu Y, Karaguzel O, Ozdamar O, Coker I, Topbas M (2006) Effects of common anti-epileptic drug monotherapy on serum levels of homocysteine, vitamin B12, folic acid and vitamin B6. Seizure 15:79–85

Silva CG, Parolo E, Streck EL, Wajner M, Wanmacher CMD, Wuse ATS (1999) In vitro inhibition of Na^+, K^+-ATPase activity form rat cerebral cortex by guanidine compounds accumulating in hyperargininemia. Brain Res 838:78–84

Sitnikova E, van Luijtelaar G (2007) Electroencephalographic characterization of spike-wave discharges in cortex and thalamus in WAG/Rij rats. Epilepsia 48:2296–2311

Stanojlović O, Rašić-Marković A, Hrnčić D, Šušić V, Macut D, Radosavljević T, Djurić D (2009) Two types of seizures in homocysteine thiolactone-treated adult rats, behavioral and electroencephalographic study. Cell Mol Neurobiol 29:329–339

Streck EL, Matte C, Vieira PS, Rombaldi F, Wannmacher CM, Wajner M, Wyse AT (2002) Reduction of Na(+),K(+)-ATPase activity in hippocampus of rats subjected to chemically induced hyperhomocysteinemia. Neurochem Res 27:1593–1598

Streck EL, Matte C, Vieira PS, Calcagnotto T, Wannmacher CM, Wajner M, Wyse AT (2003) Impairment of energy metabolism in hippocampus of rats subjected to chemically-induced hyperhomocysteinemia. Biochem Biophys Acta 1637:187–192

Towle AC, Sze PY (1983) Steroid binding to synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. J Steroid Biochem 18:135–143

Troen AM (2005) The central nervous system in animal models of hyperhomocysteinemia. Prog Neuropsychopharmacol Biol Psychiatry 29:1140–1151

Tutka P, Barczyñski B, Arent K, Mosiewicz J, Mróz T, Wielosz M (2007) Different effects of nitric oxide synthase inhibitors on convulsions induced by nicotine in mice. Pharmacol Rep 59:259–267

Urbanska EM, Drelewska E, Borowicz KK, Blaszczak P, Kleinrok Z, Czuczwar SJ (1996) NG-nitro-L-arginine, a nitric oxide synthase inhibitor, and seizure susceptibility in four seizure models in mice. J Neural Transm 103:1145–1152

Vaillend C, Mason SE, Cuttle MF, Alger BE (2002) Mechanisms of neuronal hyperexcitability caused by partial inhibition of Na^+/K^+-ATPases in the rat CA1 hippocampal region. J Neurophysiol 88:2963–2978

Van den Berg M, van der Knaap MS, Boers GH, Stehouwer CD, Rauweda JA, Valk J (1995) Hyperhomocysteinemia; with reference to its neuroradiological aspects. Neuroradiology 37:403–411

Van Leeuwen R, De Vries R, Dzoljic MR (1995) 7-Nitro indazole, an inhibitor of neuronal nitric oxide synthase, attenuates pilocarpine- induced seizures. Eur J Pharmacol 287:211–213

Vasić V, Jovanović D, Krstić D, Nikezić G, Horvat A, Vujisić L, Nedeljković N (1999) Prevention and recovery of $CuSO_4$-induced inhibition of Na?/K?-ATPase in rat brain synaptosomes by EDTA. Toxicol Lett 110:95–104

Vizi ES (1979) Presynaptic modulation of neurochemical transmission. Prog Neurobiol 12:181–290

Vizi ES (2000) Role of high-affinity receptors and membrane transporters in non synaptic communication and drug action in the central nervous system. Pharmacol Rev 52:63–90

Walton NY, Jaing Q, Hyun B, Treiman DM (1996) Lamotrigine vs. phenytoin for treatment of status epilepticus: comparison in an experimental model. Epilepsy Res 24:19–28

Wang QP, Guan JL, Nakai Y (1997) Electron microscopic study of GABA-ergic synaptic innervation of nitric oxide synthase

immunoreactive neurons in the dorsal raphe nucleus in the rat. Synapse 25:24–29

Wojtal K, Gniatkowska-Nowakowska A, Czuczwar SJ (2003) Is nitric oxide involved in the anticonvulsant action of antiepileptic drugs? Pol J Pharmacol 55:535–542

Wyse AT, Bavaresco CS, Bandinelli C, Streck EL, Franzon R, Dutra-Filho CS, Wajner M (2001) Nitric oxide synthase inhibition by L-NAME prevents the decrease of Na^+, K^+-ATPase activity in midbrain of rats subjected to arginine administration. Neurochem Res 26:515–520

Wyse AT, Zugno AI, Streck EL, Matté C, Calcagnotto T, Wannmacher CM, Wajner M (2002) Inhibition of Na^+, K^+-ATPase activity in hippocampus of rats subjected to acute administration of homocysteine is prevented by vitamins E and C treatment. Neurochem Res 27:1685–1689