

The interactions of nitric oxide and adenosine on penicillin-induced epileptiform activity in rats

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In this study, the influence of nitric oxide (NO) and adenosine systems on penicillin-induced epileptiform activity was examined in rats. NO donor, sodium nitroprusside (SNP, 50 µg/rat, i.c.v.) reduced the frequency but not the amplitude of epileptiform discharges. Non-selective NOS inhibitor, N-omega-nitro-L-arginine methyl ester (L-NAME, 100 µg/rat, i.c.v.) practically did not exert any effect on the spike frequency and amplitude. Adenosine (100 µg/rat, i.c.v.) reduced spike frequency but not the amplitude, whereas theophylline (100 µg/rat, i.c.v.) increased the mean spike frequency and amplitude of penicillin-induced epileptiform discharges. Co-injection of theophylline and L-NAME did not cause a further increase in the epileptiform activity compared with theophylline. When NO production was blocked with L-NAME, the inhibitory effects of adenosine were lost. The obtained results suggest that NO and adenosine may decrease penicillin-induced epileptiform activity in rats and that NO, at least in part, may mediate the anticonvulsant effect of adenosine.

Key words: adenosine, ECoG, epileptiform activity, nitric oxide, rat

INTRODUCTION

Nitric oxide (NO) is a messenger molecule which is synthesized from L-arginine by the nitric oxide synthase (NOS) in different cell types (Bredt and Snyder 1992, Prast and Philippu 2001). There are three isoforms of NOS named according to their activity or the tissue type. Neuronal NOS (nNOS) and endothelial NOS (eNOS) are Ca²⁺-dependent and both enzymes are widely distributed throughout the hippocampus and other regions of the brain (Dinerman et al. 1994). Besides the normal physiological processes like vasodilatation, neurotransmitter releasing, learning and memory formation, NO has also taken a role in the neurodegenerative diseases, like Alzheimer's dementia, Parkinson disease and Huntington chorea (Prast and Philippu 2001, Yildirim and Marangoz 2004). The relationship between NO and epilepsy has been studied in many different models of the experimental epi-

lepsy (Marangoz et al. 1994, Wang et al. 1994, Ayyildiz et al. 2007, Yildirim et al. 2010). However, the role of NO in seizures is still not fully understood.

Adenosine is an endogenous neuromodulator with inhibitory effects and regulates many physiological processes, particularly in excitable tissues (Dunwiddie and Masino 2001). Adenosine exerts its modulatory effects through four G-protein coupled receptors namely A₁, A_{2A}, A_{2B} and A₃ (Fredholm et al. 2001). It depresses neuronal activity in the central nervous system (CNS) by decreasing membrane excitability and/or neurotransmitter release (Fredholm and Dunwiddie 1998). Adenosine not only takes part in physiological processes but also acts in pathophysiological events, like epilepsy. It has been suggested that adenosine may act as an endogenous anticonvulsant (Dragunow 1986). This effect of adenosine has been observed in various experimental epilepsy models (Dragunow and Goddard 1984, Huber et al. 2002, Fedele et al. 2006).

Interactions of adenosine and NO have been especially studied on the cardiovascular and gastrointestinal systems. It has been reported that the effects of adenosine receptor agonists CGS-21680 and NECA are

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partially dependent on endothelium-derived NO released in porcine coronary artery endothelium (Abebe et al. 1995). Furthermore, it has been shown that adenosine agonists increase cyclic guanosine monophosphate (cGMP) through NO production in the cultured porcine coronary artery endothelial cells, and adenosine A_{2A} and A_{2B} receptors mediate this effect (Olanrewaju and Mustafa 2000). In another study, it has been demonstrated that NO donors S-nitroso-N-acetylpenicillamine (SNAP) and sodium nitroprusside (SNP) enhance the basal adenosine release in hippocampal slices (Fallahi et al. 1996).

There are a few studies about the interaction of both substances in the CNS (Burnstock 2007, Bracciali et al. 2008). Akula and coauthors (2008) suggested a functional relationship between the L-arginine-NO-cGMP signaling pathway and the anticonvulsant effect of adenosine. However, there is no investigation that evaluates the interaction between adenosine and NO in the epileptiform activity induced by penicillin. In the present study, we aimed to investigate the interactions between adenosine and NO on the penicillin-induced epileptiform activity in rats.

METHODS

Animals

Experiments were performed on the ninety-six adult male Wistar rats with a mean body weight of 230±15 g obtained from University of Ondokuz Mayıs Experimental Research Center. The animals were housed four or five per cage in plastic cages under stable conditions of humidity (60±5%) and temperature (21±2°C). They were permitted access to food and water *ad libitum*. The vivarium maintained under 12:12 h light-dark cycles. All experiments were conducted with governmental approval according to local guidelines for the care and use of laboratory animals and the guidelines of the European Community Council for experimental animal care. The experimental protocol was approved by the Animal Ethics Committee at Ondokuz Mayıs University.

Surgical procedure

Animals were anesthetized with urethane (1.2 g/kg, intraperitoneally; i.p.). Additional doses of urethane (0.2-0.3 mg/kg) were given when required. The left

cerebral cortex was carefully exposed by craniotomy. After incision of the skull, the head of the animal was placed in the stereotaxic apparatus (Harvard Instruments, South Natick, MA, USA). Four different corners of the scalp were stitched by surgical threads and stretched in order to form a liquid vaseline pool (37°C). Body temperature was monitored using a rectal probe and maintained at 37°C with a homeothermic blanket system (Harvard Homoeothermic Blanket, USA). All contact and incision points were infiltrated with procaine hydrochloride to minimize possible sources of pain.

Induction of epileptiform activity

The epileptiform activity was produced by the administration of penicillin (200 IU/ 1 µl) intracortically (i.c.). It was injected into the left sensorimotor cortex by using a Hamilton microsyringe at 1 mm beneath the brain surface (type 701N, Hamilton Co., Reno, NV, USA). Taking Bregma as a reference point, all intracortical injections were made at AP -2 mm and L +3 mm. Focal doses of adenosine were also administered intracortically.

Electrophysiological recordings

Ag-AgCl electrode was used to record electrocorticogram (ECoG). Two ball electrodes were placed over the left somatomotor cortex with the common reference electrode being fixed on the right pinna. The stereotaxic coordinates of recording sites were set as follows: first electrode, 2 mm lateral to the sagittal suture and 1 mm anterior to the bregma; second electrode, 2 mm lateral to the sagittal suture and 5 mm posterior to the bregma. The data acquisition system with multi-channel was used to record the ECoG signal from subjects (PowerLab/4SP, ADInstruments Pty Ltd, Castle Hill, NSW, Australia). The signals from the electrodes were amplified and filtered with a 0.1-50 Hz bandpass via the amplifiers (BioAmp, AD Instruments, Australia). It was digitized at a sampling rate of 1024 Hz. ECoG activity was simultaneously monitored and stored using a personal computer. The frequency and amplitude of epileptiform activity were evaluated offline. Voltage differences between the peaks of maximum positivity to maximum negativity are described as spike amplitude (Kozan et al. 2009, Tutkun et al. 2010).

Chemicals and experiment groups

SNP, N(omega)-nitro-L-arginine methyl ester (L-NAME), adenosine, theophylline and urethane were purchased from Sigma (Saint Louis, MO, USA). To produce epileptiform activity, penicillin G potassium (200 IU/ 1 μ l) volume was given intracortically. Adenosine was dissolved initially in dimethylsulfoxide to which was added sterile physiological saline (20% DMSO; final solution DMSO/saline 1:4, v/v, respectively). Thirty minutes after penicillin injection, it was intracortically injected at a dose of 100 μ g/ 5 μ l. L-NAME (100 μ g/ 5 μ l) and theophylline (100 μ g/ 5 μ l) were dissolved in saline 0.9% and then they were administered via intracerebroventricular (i.c.v.) 30 min after penicillin treatment. SNP (50 μ g/ 5 μ l) was given in the same way. Control animals were administered i.c.v. sterile saline solution (0.9%; 5 μ l) 30 min after the penicillin injection.

L-NAME, SNP, theophylline and saline were injected into the lateral ventricle. Taking Bregma as a reference point, the stereotaxic coordinates used for the lateral ventricle were: AP = -0.8 mm, L = 1.5 mm. The injection rate was 5 μ l/min and the needle was left in place for 1 min following the infusion.

Seventy animals were equally divided into ten experimental groups as followings: (1) 0.9% saline solution (2) 20% DMSO (3) adenosine (4) theophylline (5) SNP (6) L-NAME (7) adenosine+SNP (8) L-NAME+adenosine (9) theophylline+SNP (10) theophylline+L-NAME. The effective doses of above substances were determined according to previous studies (Marangoz and Bagirici 2001, Yildirim and Marangoz 2007). Furthermore, twenty rats were divided into five groups of four animals each. These animals were given only 0.9% saline (i.c.v.), adenosine (100 μ g, i.c.v.), theophylline (100 μ g, i.c.v.), SNP (50 μ g, i.c.v.) and L-NAME (100 μ g, i.c.v.) without penicillin pretreatment. One group of rats ($n=6$) was received SNP ten minutes before theophylline so that the checking of data obtained from theophylline+SNP group could be recorded.

Statistical analyses

Frequencies and amplitudes of epileptiform activity for each animal were automatically computed using the software (Chart v.5.1.1, ADInstruments Pty Ltd, Castle Hill, NSW, Australia). Epileptiform activity

was analyzed for the segments of 60 sec at every 5 min interval. Statistical procedures were performed using SPSS statistical software package (version 12.0; SPSS Inc., Chicago, IL, USA). All analyses were carried out by one-way analysis of variance (ANOVA), followed by Tamhane *post-hoc* test to correct for multiple comparisons of treatments. Data are expressed as the mean \pm the standard error of the mean (SEM). The significance level was $p<0.05$.

RESULTS

Baseline activity of each animal were recorded before the administration of substances, and it was confirmed that none of the animals had spontaneous spikes (Fig. 1A). Intracortical injection of penicillin (200 IU) induced epileptiform activity characterized by bilateral spikes in the all experimental animals (Fig. 1B). This activity began within 3-5 min after penicillin application and lasted for 4-5 h. It reached a stable level as to frequency and amplitude in 30 min. The mean spike frequency and amplitude of ECoG activity were 22 ± 2 spike/min, 1285 ± 630 μ V in the control group after 15 min 0.9% saline solution injection (i.c.v.), respectively (Fig. 1B).

The effects of nitric oxide on the spike frequency and amplitude

SNP (50 μ g, i.c.v.) and L-NAME (100 μ g, i.c.v.) were administered 30 min after penicillin injection. The mean frequency and amplitude of epileptiform ECoG activity was 23 ± 3 spike/min, 1838 ± 426 μ V just before SNP administration, respectively. The frequency of epileptiform ECoG activity was decreased to 8 ± 4 spike/min in the fifth min after SNP injection ($p<0.01$; Fig. 1C and 2A). The significant effect of SNP was continued throughout the experiment (Fig. 2A and 2B). There was no significant difference in the mean amplitude of epileptiform activity in the SNP group compared with the control group. The mean frequency and amplitude of epileptiform ECoG activity was 25 ± 3 spike/min and 1977 ± 509 μ V just before L-NAME administration, respectively. L-NAME injection significantly increased the mean frequency of epileptiform activity at only two time points (at the 10th and 50th min; $p<0.05$ and $p<0.01$; Fig. 2A and 2B) and spike amplitude at only one timing point compared with the

control group (at the 10th min; $p < 0.05$). NO synthase inhibitor L-NAME (100 μg) practically did not exert any effect on the spike frequency (except that two time-points) and amplitude (except that one time-point) of epileptiform activity.

The effects of adenosine on the spike frequency and amplitude

Adenosine (100 μg , i.c.) reduced the mean spike frequency from 26 ± 10 to 8 ± 5 spike/min in the 15 min

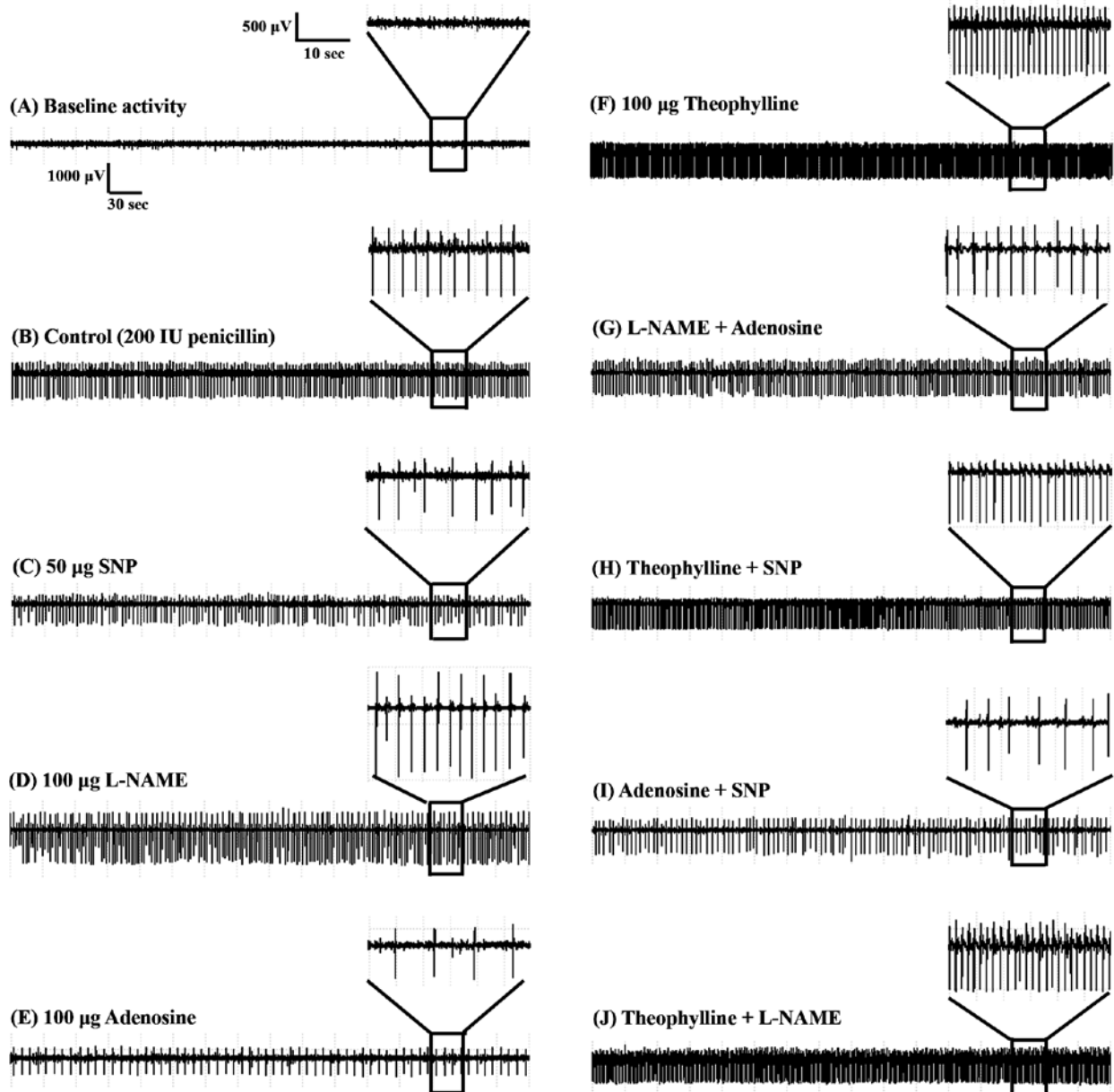


Fig. 1. Representative ECoGs are presented in the 15-23 minutes from chemicals or in the 45-53 minutes from penicillin administration. (A) Baseline ECoG activity before penicillin injection. (B) Intracortical injection of penicillin (200 IU) induced epileptiform activity on ECoG. Saline (0.9% NaCl) injection did not change the frequency of penicillin-induced epileptiform activity. (C and E) Administration of SNP (50 $\mu\text{g}/\text{rat}$, i.c.v.) and adenosine (100 $\mu\text{g}/\text{rat}$, i.c.) decreased the frequency of epileptiform activity. (D and F) Administration of L-NAME (100 $\mu\text{g}/\text{rat}$, i.c.v.) and theophylline (100 $\mu\text{g}/\text{rat}$, i.c.v.) increased the frequency of epileptiform activity. (G-J) The interactions between NO and adenosine.

after the injection (Fig. 1E). These reductions in the spike frequencies of adenosine injected animals are statistically significant ($p<0.01$; Fig. 2A and 2B). Adenosine did not affect the mean spike amplitude. Theophylline (100 μg , i.c.v.) increased the mean spike frequency of epileptiform activity from 26 ± 6 to 48 ± 5 , 53 ± 4 and 38 ± 7 in the 15, 30 and 45 min, respectively ($p<0.001$ and $p<0.05$; Fig. 2A and 2B). The mean spike amplitude was also significantly enhanced between 5 and 15 min after theophylline administration ($p<0.01$ and $p<0.001$). It was increased from 1.9 ± 0.1 mV (before the injection) to the 2.7 ± 0.1 mV in the 3rd min, 3.1 ± 0.1 mV in the 6th min after the injection. As stated above, the epileptiform activity was decreased by the administration of exogenous adenosine. We also tested whether DMSO (a solvent for adenosine) has any effect on the epileptiform activity. However, 20% DMSO administration (i.c.) influenced neither spike frequency nor spike amplitude (data not shown in the figures).

The effects of the interactions of NO and adenosine on the spike frequency and amplitude

Adenosine (100 μg) was injected ten min after the administration of L-NAME (100 μg , i.c.v.) intracortically. There are no significant differences both amplitude and frequency of epileptiform activity in the L-NAME+adenosine group compared with the control animals (Fig. 2A and 2B).

The experimental group which received SNP (50 μg , i.c.v.) ten min after the administration of theophylline (100 μg , i.c.v.) was formed to evaluate the role of NO by blocking adenosine. Before the injection of theophylline the mean spike frequency, was 28 ± 2 spike/min. Then it increased to 46.7 ± 3 spike/min ten min after the injection of theophylline ($p<0.001$), but this value decreased to 21.1 ± 1 spike/min 25 min after SNP injection (Fig. 2A). Theophylline increased the mean spike amplitude from 2.1 ± 0.6 mV to 3.2 ± 0.1 mV, and 25 min after SNP injection this value decreased to 2.5 ± 0.2 mV. There were statistically significant differences between mean spike amplitude of theophylline and control groups ($p<0.001$), but spike amplitude returned back to the control level after SNP administration.

Another group of rats was used to research the effects of increases in both adenosine and NO. Hence, SNP (50 μg , i.c.v.) was injected immediately after adenosine (100 μg , i.c.). The mean spike frequency was 24.7 ± 1 spike/min before the administrations of these substances, and

it decreased to 9.7 ± 1 spike/min after the administrations ($p<0.001$). Co-injection of these substances caused an important decrease in the spike frequency ($p<0.001$, Fig. 2A and 2B). However, differences in the spike amplitude were not statistically significant.

To find out the effects of both adenosine and NO antagonism, L-NAME (100 μg , i.c.v.) was injected immediately after theophylline (100 μg , i.c.v.). Before the treatments mean spike frequency and amplitude were 27.2 ± 2 spike/min and 1.6 ± 0.2 mV, respectively. Ten minutes following the treatments these values increased to 44.2 ± 2 spike/min ($p<0.001$) and 1.9 ± 0.2 mV respectively. Co-administration of theophylline and L-NAME caused an important increase in the spike frequency throughout the experiment ($p<0.001$, Fig. 2A and 2B). However, the increase in the spike amplitude was not statistically significant.

Injections of 0.9% saline, adenosine, theophylline, SNP and L-NAME did not cause any change of the frequency or amplitude of ECoG activity in non-penicillin injected animals. No spontaneous epileptiform activity was observed after administration of these substances. There were no differences between SNP+theophylline and SNP groups in terms of the frequency and amplitude of epileptiform activity.

DISCUSSION

The present study demonstrates for the first time that NO may mediate, at least in part, the anticonvulsant effect of adenosine in epileptiform activity induced by penicillin. The NO donor SNP (50 μg) decreased the spike frequency during the experiment but did not cause any significant changes in the amplitude. NO synthase inhibitor L-NAME (100 μg) practically did not exert any influence upon the spike frequency (except that two time-points) and amplitude (except that one time-point) of epileptiform activity. This result shows that an increase in the amount of NO reduces the epileptiform activity. On the other hand, it was shown that exogenous adenosine (100 μg) decreases the spike frequency, and adenosine receptor antagonist theophylline (100 μg) increases both spike frequency and amplitude. When NO production is blocked, the inhibitory effect of adenosine was lost, so it may be thought that NO mediates, at least in part, the inhibitory effect of adenosine. Furthermore, the blockade of adenosine receptors by theophylline caused an increase in epileptic activity, but this augmentation was disap-

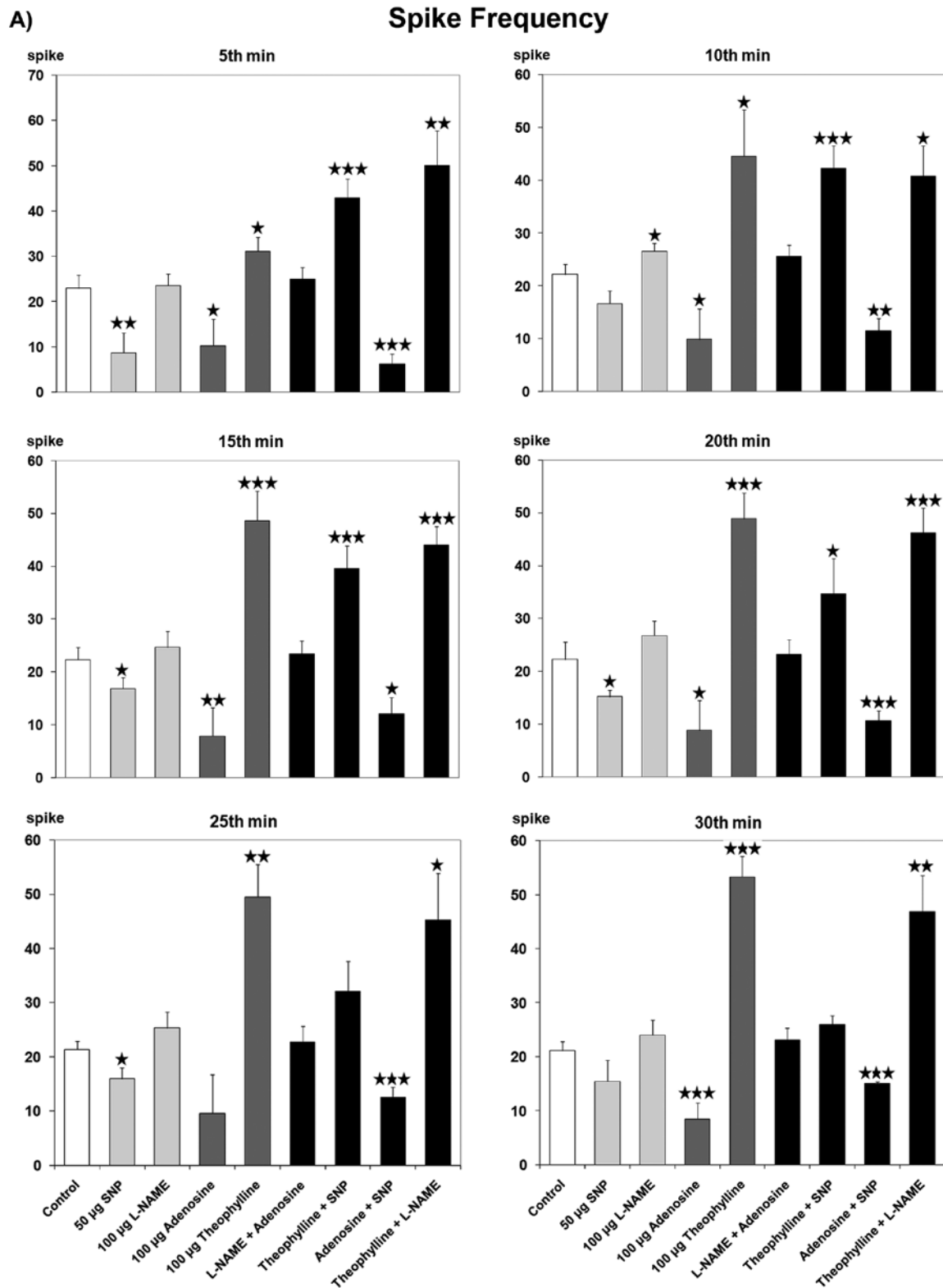


Fig. 2A. The mean spike frequencies of epileptiform activity in the 5-30 minutes from chemicals or in the 35-60 minutes from penicillin administration. Values are mean \pm S.E.M. * p <0.05, ** p <0.01, *** p <0.001, as compared to control group by Tamhane *post-hoc* test (n =7 animals per group).

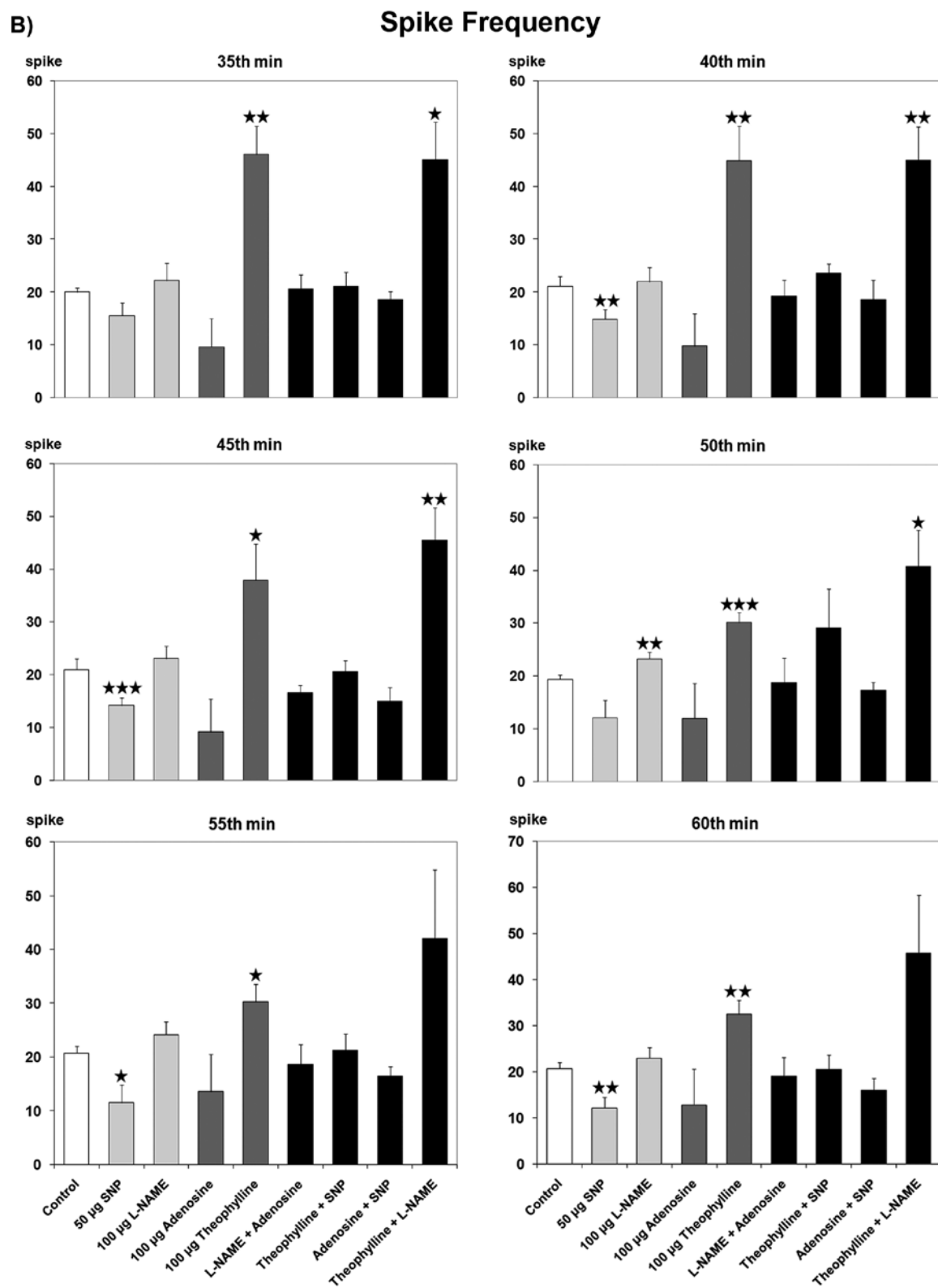


Fig. 2B. The mean spike frequencies of epileptiform activity in the 35-60 minutes from chemicals or in the 65-90 minutes from penicillin administration. Values are mean \pm S.E.M. * p <0.05, ** p <0.01, *** p <0.001, as compared to control group by Tamhane *post-hoc* test (n =7 animals per group).

Table I

The doses and timings of chemicals used in each experimental group.				
Experiment Groups	Chemicals			
	Name	Dose/Volume	Injection Route	Administration Timing
Control	Penicillin	200 IU / 1µl	i.c.	First injection (FI) 30 min after FI
	0.9 % NaCl	0.9 % / 5µl	i.c.v.	
DMSO	Penicillin	200 IU / 1µl	i.c.	First injection 30 min after FI
	DMSO	20% / 5µl	i.c.	
Adenosine	Penicillin	200 IU / 1µl	i.c.	First injection 30 min after FI
	Adenosine	100µg / 5µl	i.c.	
Theophylline	Penicillin	200 IU/1 µl	i.c.	First injection 30 min after FI
	Theophylline	100µg / 5µl	i.c.v.	
SNP	Penicillin	200 IU / 1µl	i.c.	First injection 30 min after FI
	SNP	50µg / 5µl	i.c.v.	
L-NAME	Penicillin	200 IU / 1µl	i.c.	First injection 30 min after FI
	L-NAME	100µg / 5µl	i.c.v.	
Adenosine + SNP	Penicillin	200 IU / 1µl	i.c.	First injection 30 min after FI (~) 30 min after FI
	Adenosine	100µg / 5µl	i.c.	
	SNP	50µg / 5µl	i.c.v.	
L-NAME + Adenosine	Penicillin	200 IU / 1µl	i.c.	First injection 30 min after FI 40 min after FI
	L-NAME	100µg / 5µl	i.c.v.	
	Adenosine	100µg / 5µl	i.c.	
Theophylline + SNP	Penicillin	200 IU / 1µl	i.c.	First injection 30 min after FI 40 min after FI
	Theophylline	100µg / 5µl	i.c.v.	
	SNP	50µg / 5µl	i.c.v.	
Theophylline + L-NAME	Penicillin	200 IU / 1µl	i.c.	First injection 30 min after FI (~) 30 min after FI
	Theophylline	100µg / 5µl	i.c.v.	
	L-NAME	100µg / 5µl	i.c.v.	

peared after SNP treatment. These findings support the idea of that NO may mediate the anticonvulsant effect of adenosine. In addition, the epileptiform activity was increased in the theophylline+L-NAME group while it was decreased in the adenosine+SNP group. The effects obtained by the co-administration of these substances did not differ from their separate effects.

Therefore, it may be suggested that NO mediates the anticonvulsant effect of adenosine, and each system may share the same mechanistic pathway. We also demonstrated that injections of saline (0.9 % NaCl), adenosine, theophylline, SNP and L-NAME did not cause any change of the frequency or amplitude of ECoG activity in the non-penicillin injected animals.

In a previous study, it has been found that at higher doses (50-300 mg/kg) theophylline can independently induce seizures in rats (Ray et al. 2005). However, the dose of theophylline used in this study was not sufficient to initiate the epileptiform activity independently.

The precise role of endogenous NO in the pathophysiology of epilepsy remained unclear and debatable. Although some studies suggest that NO may act as an endogenous anticonvulsant (Rondoin et al. 1992, Marangoz et al. 1994, Przegalinski et al. 1994, Wang et al. 1994, Prast and Philippu 2001, Bosnak et al. 2007), the others reported that this molecule may be proconvulsant (Mollace et al. 1991, Yamamota 1995, Murashima et al. 2002, Zandieh et al. 2010). It has been shown that N-omega-nitro-L-arginine (L-NNA), a general NOS inhibitor, delayed the beginning of the clonic convulsions induced by pentylenetetrazole (Bashkatova et al. 2000). 7-nitroindazole (7-NI), a specific nNOS inhibitor, strengthened the effects of anti-epileptic drugs in the picrotoxin induced seizures in rats. In the light of these findings, it has been claimed that NO may have a role in triggering seizures (Rajasekaran et al. 2003). On the other hand, nitric oxide seems to have anticonvulsant properties in many studies. NO reduced the convulsions induced by picrotoxin (Jayakumar et al. 1999, Paul and Ekambaram 2004, Paul and Subramanian 2002). It was reported that intracortical injection of SNP decreased epileptiform activity elicited by penicillin. The NO donor SNP significantly depressed epileptiform activity in anesthetized rats, methylene blue (NOS inhibitory) and hemoglobin prevented this effect of SNP (Marangoz et al. 1994). In addition, it has been reported that L-arginine and SNP depressed the epileptic activity while 7-NI, administered 30 min before penicillin, and causes a significant decrease the latency in the penicillin-induced epileptiform activity (Marangoz and Bagirici 2001). The results of present study are consistent with above mentioned studies which are reported that NO is an anticonvulsant substance.

The inhibitory effects of NO on the epileptiform activity may occur via at least three different mechanisms: (1) Competitive inhibition on N-methyl-D-aspartate (NMDA) receptors (Manzoni et al. 1992). (2) NO may exert its neuroprotective and antiseizure effects by interacting with the redox regulator domain of the NMDA receptor (Lei et al. 1992). (3) Results from in vitro studies suggest that the guanine nucle-

otides (cGMP) may cause NMDA-receptor inhibition by interacting with the recognition domain in a competitive fashion (Manzoni et al. 1992).

In the experimental and clinical studies, it has been shown that adenosine has an anticonvulsant effect. It has been reported that the developing and spreading of seizures in a healthy person's brain may be prevented by the tonic effect of endogenous adenosine (Dunwiddie and Masino 2001, Fredholm et al. 2001). Furthermore, it has been displayed that in patients who suffer from complex partial epilepsy, which is hard to keep under control, extracellular adenosine is increased up to 6-31 times during epileptic activity (During and Spencer 1992). Adenosine has been displayed to have an anticonvulsant effect which is mediated primarily by A₁ receptor subtype (Malhotra and Gupta 1997). Our results are consistent with the findings concluding that adenosine is an anticonvulsant (Ekonomou et al. 2000, Rebola et al. 2003).

The interaction between NO and adenosine, in particular, was investigated in the peripheral tissues and systems. In the endothelial cell culture obtained from a pig's coronary artery, it has been found that the adenosine agonists increase the production of nitrite (Olanrewaju et al. 2000, Olanrewaju and Mustafa 2000). In a different study in the same laboratory, it has been shown that the adenosine agonists increase cGMP through NO production. It has been found that there may be a direct connection between NO production and A_{2B} receptor (Olanrewaju and Mustafa 2000). In another study, the microinjection of adenosine into the nucleus tractus solitarius (NTS) caused bradycardic effects and depression of the cardiovascular system. The adenosine receptor antagonist 1,3-dipropyl-8-sulphophenylxanthine (DPSPX) and NOS inhibitors NG-monomethyl-L-arginine (L-NMMA)/L-NAME administration before adenosine injection decreased the cardiovascular effects of adenosine. According to these results, it has been claimed that NO can mediate the effects of adenosine in the NTS (Lo et al. 1998). The interactions of these neuroactive molecules have also been investigated in the CNS. It has been reported that the NO donors, SNAP and SNP, may cause the purine release in a dose-dependent manner (Fallahi et al. 1996). It is known that NO provokes guanylate cyclase and increases the amount of intracellular cGMP. This situation can change the amount of neuroactive substances, which are released from nerve endings (Garthwaite 1991). The stimulation capacity of

SNAP to the release of neurotransmitter from the cortical neurons of brain is blocked by free oxygen radicals, which is probably superoxide dismutase connected, with the production of peroxynitrite anion (Ohkuma et al. 1995). Peroxynitrite may cause the increase of purine release through NO, interacting a number of cellular components (Ohkuma et al. 1995). Shahraki and Stone (2004) demonstrated that the inhibitory effect of adenosine at presynaptic sites in hippocampal slices can be prevented by NO or superoxide. In contrast, L-NAME prevented the reduction of adenosine responses by electrically-induced LTP, suggesting that NO mediates this effect.

Interestingly, a recent investigation by Akula and coauthors (2008) demonstrated that the anticonvulsant effect of adenosine in the pentylenetetrazol seizure threshold may perhaps involve an interaction with the L-arginine-NO-cGMP pathway. The anticonvulsant effect of adenosine was reduced by pretreatment with L-arginine or SNP. Furthermore, pretreatment with L-NAME or 7-NI with the *per se* non-effective dose of adenosine induced a potent anticonvulsant effect (Akula et al. 2008). On the other hand, we found that the inhibition of NO production by L-NAME prevented the anticonvulsant effect of adenosine on the epileptiform activity. When NO production is blocked, the inhibitory effects of adenosine were lost, so it may be thought that NO mediates, at least in part, the inhibitory effect of adenosine. Akula and others (2008) results are not compatible with our findings.

The findings of the present study were consistent with previous research, which investigated that the interaction between NO and adenosine in the cardiovascular system. Olanrewaju and colleagues (2000) reported that adenosine increased the nitrite production in the endothelial cell culture. They also noted that its agonists raised the amount of cGMP through NO production and, A_{2A} and A_{2B} receptors mediated this effect (Olanrewaju and Mustafa 2000).

CONCLUSION

The findings of the present study clearly indicate that both NO and adenosine exhibited an anticonvulsant effect on the epileptiform activity induced by penicillin in the Wistar rat. When NO production is blocked, the inhibitory effects of adenosine were lost, so it may be thought that NO mediates, at least in part, the inhibitory effect of adenosine. The effects obtained

by the co-administration of adenosine and SNP did not differ from their separate effects. Co-injection of theophylline and L-NAME did not cause a further increase in the epileptiform activity compared with theophylline. These findings support the hypothesis that adenosine and NO may share a common signaling pathway. Further studies are needed to clarify the relationship between NO and adenosine.

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