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Nitric oxide and convulsions in 4-aminopyridine-treated mice

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Abstract

We studied whether N^G-nitro-L-arginine (NNA), an inhibitor of nitric oxide (NO) synthase as well as L-arginine and molsidomine, two agents elevating NO, influenced convulsions caused by 4-aminopyridine, a K^+ channel blocker in mice. NNA, in a dose known to decrease level of NO (40 mg kg^{-1}), enhanced the seizure susceptibility to intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) 4-aminopyridine. L-arginine (500 mg kg^{-1}) and molsidomine (20 mg kg^{-1}) alone did not influence 4-aminopyridine-induced seizure activity. Surprisingly, the proconvulsant effect of NNA upon clonic and tonic seizures was potentiated by molsidomine (20 mg kg^{-1}). No influence of L-arginine on the proconvulsant effect of NNA was found. Taking into account the proconvulsant effect of NNA, an involvement of NO-mediated events in the mechanism of convulsive activity of 4-aminopyridine might be postulated. However, the ineffectiveness of L-arginine and molsidomine to suppress the convulsive activity of 4-aminopyridine as well as a paradoxical potentiation of the proconvulsant effect of NNA by molsidomine seem to exclude the impact of NO pathway on 4-aminopyridine-induced convulsions in mice. Our data suggest that the proconvulsant effect of NNA in this seizure model is caused by other, not related to NO, mechanisms. © 2002 Published by Elsevier Science B.V.

Keywords: Nitric oxide (NO); 4-Aminopyridine; N^G-nitro-L-arginine; Molsidomine; Seizure; Epilepsy

1. Introduction

Recent advances in neurophysiology, neuropharmacology and genetics of human epilepsy have elucidated many of cellular and metabolic abnormalities and pathological mechanisms leading to seizure initiation and propagation. Despite considerable progress in the understanding of epileptogenesis, current therapies are unsatisfactory as they provide only symptomatic relief (McNamara, 1999) and the search for new therapeutic strategies is of importance.

Treatment of the seizure disorders might benefit a more thorough understanding of the role of nitric oxide (NO), a second messenger and a neurotransmitter, in neuronal hyperexcitability and thus in epilepsy. However, it is difficult to make a clear conclusion on the involvement of NO in epileptiform activity. A number of studies have shown that the modification of NO synthesis exerts contrasting effects upon animal convulsions, depending on the model of seizures and/or the dose, on pretreatment time or type of NO pathway modulators used (Rundfeldt et al., 1995), and on brain structures and age of animals (Libri et al., 1997; Pereira de Vasconcelos et al., 2000).

Many reports have suggested the anticonvulsive action of NO (Haberny et al., 1992; Przegalinski et al., 1994), although several authors have demonstrated NO to have the proconvulsive nature (Moncada et al., 1992; De Sarro et al., 1993). Using animal models of epilepsy, it has been shown that NO may be involved in the mediation of seizure activity induced by excitatory amino acids. N^G-nitro-L-arginine (NNA), an inhibitor of NO synthase potentiated convulsions evoked by kainate in mice (Tutka et al., 1996). Similar findings has been reported by Przegalinski et al. (1994), who have found the proconvulsive effect of NNA in kainate-induced seizures that could be reversed by L-arginine, a substrate for NO formation. Haberny et al. (1992) demonstrated that NNA potentiated convulsions and neurotoxicity in the rat hippocampus following the administration of the endogenous *N*-methyl-D-aspartate agonist quinolinate. NNA suppressed, in contrast to kainate- and quinolinate-induced convulsions, the convulsive activity of intracerebroventricular glutamate in mice (Tutka et al., 1996). It has been reported that NNA and its methyl ester, two nonselective

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inhibitors, and 7-nitroindazole, a selective inhibitor of NO synthase, modulated the anticonvulsive action of conventional antiepileptic drugs and compounds in electrical, chemical, and audiogenic models of epilepsy in mice (Borowicz et al., 1997, 1998; Czuczwar et al., 1999; De Sarro et al., 2000).

4-Aminopyridine, a K^+ channel blocker, has been used as a therapeutic agent in the treatment of many neurological disorders such as Alzheimer's disease, multiple sclerosis and Lambert–Eaton myasthenic syndrome, spinal cord injury, and selected cases of botulism (McEvoy et al., 1989; Bever et al., 1994; Hayes, 1994). However, slight overdose of this compound has been associated with serious side effects, including convulsions (Bever et al., 1994). A few reports have addressed the pharmacological mechanisms involved in the convulsive effect of 4-aminopyridine (Yamaguchi and Rogawski, 1992; Boda and Szente, 1996). Due to its inhibitory action on the K^+ level, 4-aminopyridine impairs the repolarization of the action potential, enhances synaptic efficacy and thus may cause hyperexcitability (Muller et al., 1999).

Epileptiform activity can be induced by 4-aminopyridine in vitro (Perreault and Avoli, 1991; Luhmann et al., 2000) and in vivo (Fragoso-Veloz et al., 1990; Morales-Villagran et al., 1996). In rat hippocampus and cerebral cortex, 4-aminopyridine has been shown to induce, in a concentration-dependent manner, intense and frequent epileptic discharges including short bursts that resemble interictal spiking, and longer polysynaptic rhythmic bursting events that resemble ictal bursts in vivo (Arvanov et al., 1995; Pena and Tapia, 1999). Biochemical data have demonstrated 4-aminopyridine to be a modulator of the nondepolarization-dependent neurotransmitters release in brain slices, synaptosomes and neuromuscular junctions (Thesleff, 1980; Tibbs et al., 1989; Hu et al., 1991). 4-Aminopyridine administered into the rat lateral cerebral ventricle stimulated the release of glutamate that is involved in the induction and development of seizure activity (Morales-Villagran et al., 1996). This effect seems to be related to the convulsant and lethal action of 4-aminopyridine (Morales-Villagran and Tapia, 1996). Accumulating reports have described the induction of γ-aminobutyric acid (GABA) release by 4-aminopyridine (Pena and Tapia, 1999). It has been hypothesized that the enhancement of extracellular GABA induced by 4-aminopyridine could be involved in neuronal hyperexcitation because it has been known that the synaptic action of GABA changes from inhibitory to excitatory (Pena and Tapia, 1999). 4-Aminopyridine stimulates the release of acetylcholine from synaptic terminals, as it has been reported in mouse brain synaptosomes (Tapia et al., 1985) and rabbit cervical ganglia (Simmons and Dun, 1984). Microdialysis infusion of 4-aminopyridine in rat motor cerebral cortex induces the release of catecholamines either through a direct effect on nerve endings or as a consequence of seizures (Morales-Villagran et al., 1999).

Recently, Bruckner and Heinemann (2000) have proposed that the activities induced by 4-aminopyridine in combined entorhinal cortex–hippocampal slices may provide an in vitro model for the development of new drugs against difficult-to-treat focal epilepsy.

Based on the facts described above, it is possible that NO synthesis and release may contribute to the generation of seizure activity in response to excessive glutamate-mediated excitation induced by 4-aminopyridine. Therefore, in the present study, we have investigated the influence of pharmacological interventions in NO pathway on the 4-aminopyridine convulsant action and lethality.

2. Materials and methods

2.1. Animals

The experiments were performed on male Swiss mice weighing 20–26 g. The mice were maintained in colony cages on standard laboratory conditions (ambient temperature 20 ± 1 °C, free access to chow pellets and tap water, natural light/dark cycle). The experimental groups, consisting of 8–10 mice, were chosen by means of a randomized schedule. All tests took place between 10:00 and 15:00. The experimental protocol was approved by the Medical University of Lublin ethics committee for the use of experimental animals and confirmed with the Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

4-Aminopyridine and N^G-nitro-L-arginine (NNA) (both from RBI, Natick, MA, USA) as well as L-arginine hydrochloride (Sigma, St. Louis, MO, USA) were dissolved in sterile saline. Molsidomine (Polfa, Warsaw, Poland) was suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria). For i.c.v. injection, the pH of 4-aminopyridine was adjusted to 7.3 with 1 N HCl or 1 N NaOH.

2.3. 4-Aminopyridine-induced convulsions and lethality

Clonic and tonic convulsions were induced in mice by the administration of 4-aminopyridine. 4-Aminopyridine was given intraperitoneally (i.p.) in doses of 5–11 mg kg^{-1} in a volume of 10 ml kg^{-1}. After receiving 4-aminopyridine, the mice were observed for 60 min, and the number of mice convulsing out of the total number of mice tested was noted for each treatment condition. Clonic seizure was defined as clonus of all four limbs with loss of righting reflex for at least 3 s. The endpoint for tonic seizure was the tonic extension of the hind limbs (hind limbs outstretched 180° to the plane of the body axis). The convulsant action of 4-aminopyridine was evaluated as the CD_{50} (convulsive $dose_{50}$, i.e. the dose of 4-aminopyridine producing the seizure response in 50% of animals) for clonic and tonic convulsions. Lethality was assessed 60 min after the injection of 4-aminopyridine and was expressed as lethal $dose_{50}$ (LD_{50}).

Table 1
Effect of N^G-nitro-L-arginine (NNA) on clonic and tonic convulsions induced by intraperitoneal administration of 4-aminopyridine (4-AP) in mice

Treatment (mg kg^{-1})	4-AP (mg kg^{-1})	
	Clonus	Tonus
Saline	9.0 (8.7–9.4)	9.2 (8.8–9.6)
NNA 1	9.6 (8.8–10.5)	9.6 (8.9–10.3)
NNA 2.5	8.6 (7.9–9.3)	8.7 (8.0–9.4)
NNA 5	7.6 (6.9–8.4)[a]	7.7 (7.0–8.4)[b]
NNA 10	7.5 (6.8–8.2)[b]	7.5 (6.8–8.2)[b]
NNA 40	6.8 (5.8–8.0)[b]	6.9 (5.9–8.1)[b]

Table data are CD_{50} values (in mg kg^{-1}) with 95% confidence limits in parentheses. 4-AP was given i.p. 30 min after i.p. administration of NNA. The calculation of CD_{50} values and their statistical evaluation were based upon the method of Litchfield and Wilcoxon (1949), but modified in that dose–effect curves were calculated on a computer. At least 32 mice were used to calculate each CD_{50} value.

[a] $P<0.01$ vs. saline group.

[b] $P<0.001$ vs. saline group.

Convulsions and mortality were also induced when 4-aminopyridine was administered intracerebroventricularly (i.c.v.) in doses of 400–1000 ng/mouse in a volume of 5 μl into the lateral brain ventricle, according to the method of Lipman and Spencer (1980). The mice were subsequently observed for the occurrence of clonic and tonic seizures for 60 min. For both phases of seizures, the CD_{50} values were calculated, and the LD_{50} value was calculated for lethality.

2.4. Effects of L-arginine, molsidomine, and N^G-nitro-L-arginine on 4-aminopyridine-induced convulsions and lethality

The ability of L-arginine (60 min prior to the test; 500 mg kg^{-1}), molsidomine (30 min; 20 mg kg^{-1}), and NNA (30 min; 1–40 mg kg^{-1}) to affect the seizure susceptibility and lethality was evaluated by injecting L-arginine, molsidomine or NNA i.p. in a volume of 10 ml kg^{-1} before i.p. injection of 4-aminopyridine. NNA was also tested against seizures and lethality when 4-aminopyridine was administered i.c.v. In order to determine the influence of L-arginine, molsidomine, and NNA upon the convulsive action and lethality of 4-aminopyridine, the CD_{50} and LD_{50} of 4-aminopyridine were evaluated and compared with the CD_{50} and LD_{50} of 4-aminopyridine administered alone.

2.5. Statistics

The calculation of CD_{50} and LD_{50} values and statistical analysis of the data were estimated by computer probit analysis according to Litchfield and Wilcoxon (1949). Each CD_{50} and LD_{50} value was calculated from dose–response curve of tree to five data points. The CD_{50}s and LD_{50}s are accompanied by 95% confidence limits (in parentheses). For all of the statistical comparisons, $P<0.05$ was considered significant.

3. Results

3.1. 4-Aminopyridine-induced convulsions

The i.p. or i.c.v. administration of 4-aminopyridine produced a typical sequence of behavioral activation. The mice initially exhibited hyperactivity, vocalization, salivation, blinking/eye closing, chewing and rearing, followed by Straub tail and trembling. Then, clonic jerks occurred, followed by explosive running and continuous clonic convulsions. When 4-aminopyridine was administered i.p. in a dose of 9.0 mg kg^{-1}, clonic convulsions occurred within 20 min after injection. When 4-aminopyridine was applied i.c.v. in a dose of 628 ng/mouse, clonic convulsions occurred within 10 min after injection. After clonic convulsions, the majority of animals showed generalized tonic seizures and death. The CD_{50} of 4-aminopyridine administered i.p. were 8.6 (8.1–9.0) mg kg^{-1} for trembling/clonic jerks, 9.0 (8.7–9.4) mg kg^{-1} for continuous clonic seizures and 9.2 (8.8–9.6) mg kg^{-1} for tonic phase.

3.2. Effects of N^G-nitro-L-arginine on convulsions and lethality induced by 4-aminopyridine administered intraperitoneally

As shown in Table 1, the pretreatment of animals with NNA (5, 10 and 40 mg kg^{-1}) resulted in a significant dose-

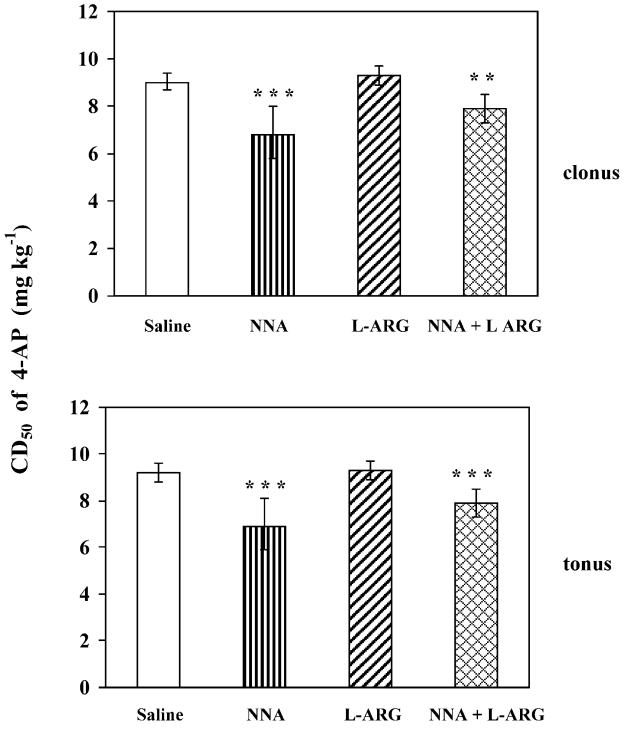

Fig. 1. Influence of L-arginine (L-ARG) on the proconvulsive effect of N^G-nitro-L-arginine (NNA) in 4-aminopyridine (4-AP)-induced clonic and tonic seizures. L-ARG was injected 60 min, and NNA was injected 30 min before 4-AP i.p. administration. ** $P<0.01$, *** $P<0.001$ vs. control group.

dependent increase of the susceptibility to clonic and tonic seizures induced by i.p. administration of 4-aminopyridine. Lower doses of NNA (1 and 2.5 mg kg^{-1}) had no effect on 4-aminopyridine-induced clonus and tonus.

4-Aminopyridine injected i.p. caused lethality with LD_{50} of 9.2 (8.8–9.6) mg kg^{-1}. NNA in a dose of 5 mg kg^{-1} increased the 4-aminopyridine-induced lethality [LD_{50} = 7.7 (7.0–8.4) mg kg^{-1}; $P < 0.001$ vs. 4-aminopyridine injected group]. For NNA administered in higher doses (10 and 40 mg kg^{-1}), the LD_{50}s were 7.5 (6.8–8.2) and 6.9 (5.9–8.1) mg kg^{-1}, respectively ($P < 0.001$ vs. 4-aminopyridine injected group).

3.3. Effects of L-arginine and molsidomine on the proconvulsant activity of N^G-nitro-L-arginine in convulsions induced by 4-aminopyridine administered intraperitoneally

L-arginine, in a dose of 500 mg kg^{-1}, which alone is ineffective against clonic and tonic seizures, did not reverse the proconvulsant effect of NNA (40 mg kg^{-1}) (Fig. 1). Molsidomine administered alone in a dose of 20 mg kg^{-1} did not influence clonic or tonic seizures. However, as shown in Fig. 2, the proconvulsive activity of NNA given at 40 mg kg^{-1} was significantly potentiated by molsidomine (20 mg kg^{-1}). Molsidomine combined with NNA decreased the CD_{50} of 4-aminopyridine from 7.6 (6.8–8.5) to 5.4 (4.6–6.2) and from 7.7 (6.9–8.7) to 5.5 (4.7–5.4) mg kg^{-1} for clonus and tonus, respectively. The LD_{50}s of L-arginine and molsidomine given alone or combined with NNA (40 mg kg^{-1}) were identical to respective CD_{50}s for tonic seizures (Figs. 1 and 2). Statistically significant differences were found between the LD_{50} of 4-aminopyridine in molsidomine-treated mice and LD_{50} of 4-aminopyridine in molsidomine + NNA-treated mice ($P < 0.001$). No significant differences were found between the LD_{50} of 4-aminopyridine in L-arginine- and L-arginine + NNA-treated animals.

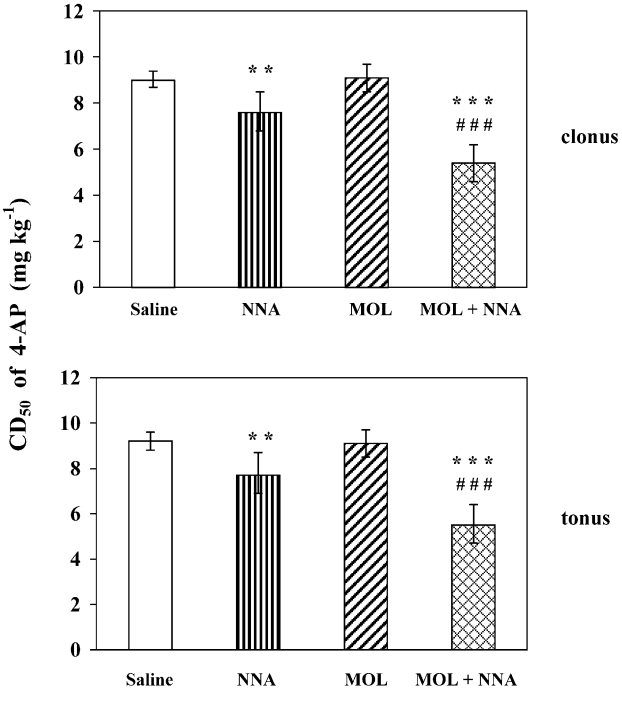

Fig. 2. Influence of molsidomine (MOL) on the proconvulsant effect of N^G-nitro-L-arginine (NNA) in 4-aminopyridine (4-AP)-induced clonic and tonic seizures in mice. MOL and NNA were injected 30 min before i.p. administration of 4-AP. ** $P < 0.01$, *** $P < 0.001$ vs. control group, ### $P < 0.001$ vs. NNA. See also Table 1.

Table 2
Influence of N^G-nitro-L-arginine (NNA) on convulsions induced by intracerebroventricular administration of 4-aminopyridine (4-AP) in mice

Treatment (mg kg^{-1})	4-AP (ng/mouse)	
	Clonus	Tonus
Saline	628 (516–763)	716 (546–940)
NNA 10	540 (356–820)	601 (358–974)
NNA 40	497 (456–541)[a]	525 (469–587)[a]

Table data are CD_{50} values (in ng/mouse) with 95% confidence limits in parentheses. NNA was injected i.p. 30 min before i.c.v. administration of 4-AP. See also Table 1.
[a] $P < 0.05$ vs. control group.

3.4. Effect of N^G-nitro-L-arginine on convulsions and lethality induced by 4-aminopyridine administered intracerebroventricularly

NNA, in a dose of 40 mg kg^{-1}, but not in a lower dose of 10 mg kg^{-1}, had a proconvulsant effect on clonic and tonic seizures induced by 4-aminopyridine injected i.c.v. (Table 2). The LD_{50}s were 716 (546–940) (4-aminopyridine alone), 601 (358–974) (4-aminopyridine + NNA 10 mg kg^{-1}), and 525 (469–587) (4-aminopyridine + NNA 40 mg kg^{-1}; $P < 0.05$ vs. 4-aminopyridine alone group) ng/mouse.

4. Discussion

The results of the present study demonstrate that systemic and intracerebroventricular administration of 4-aminopyridine induces generalized tonic–clonic convulsions and death in mice. The potent convulsant effect of 4-aminopyridine has been described in rodents (Morales-Villagran et al., 1996), and the epileptogenic action of 4-aminopyridine has been useful as valuable chemical model of seizures (Bruckner and Heinemann, 2000). However, the precise mechanism of the 4-aminopyridine convulsant effect has not been clearly determined. As it was mentioned in the introduction, the convulsant effect of 4-aminopyridine can be correlated to its blocking effect on voltage-dependent K^+ channels (Muller et al., 1999) and/or can correlated with its releasing action on glutamate and other excitatory amino acids (Morales-Villagran and Tapia, 1996; Pena and Tapia, 2000). However, the role of glutamate in 4-aminopyridine-induced seizures is uncertain. It has been reported that (+)-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)-cyclohepten-5,10-imine maleate

(MK-801), 3-[(±)-2-carboxypiperazine-4-yl]propyl-1-phosphonic acid ((±)-CPP), and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline (NBQX), the glutamate receptor antagonists, were unable to block seizures induced by 4-aminopyridine (Rogawski et al., 1991; Yamaguchi and Rogawski, 1992; Yamaguchi et al., 1993). On the other hand, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride (GYKI 52466), and (±)-5-aminocarbonyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (ADCI), two other glutamate receptor antagonists were protective against seizures and lethality induced by 4-aminopyridine (Rogawski et al., 1991; Yamaguchi et al., 1993).

With the use of glutamate as a convulsant, we provided evidence that NNA differentially affected seizures arising from a diffuse stimulation of glutamate receptors and seizures resulting from an activation of an individual subtype of these receptors (Tutka et al., 1996). Bearing in mind the relationship between NO formation and the genesis of seizures produced by excitatory amino acids, we attempted to define the role of NO in the convulsant action of 4-aminopyridine.

To decrease the level of endogenous NO, we used NNA, which, in doses of 5–40 mg kg^{-1}, potentiated both clonic and tonic seizures as well as lethality induced by i.p. administration of 4-aminopyridine. The lower doses of NNA, effective when 4-aminopyridine were administered i.p., were without effect on seizures when 4-aminopyridine was administered i.c.v. After i.c.v. injection of 4-aminopyridine, only the highest dose of NNA (40 mg kg^{-1}) was significantly effective. Thus, an influence of NNA on the seizure activity was weaker after i.c.v. than i.p. injection of the convulsant. A cause of the difference in a potency of the proconvulsive action of NNA is unclear. Possibly, the convulsive action of 4-aminopyridine could be affected by the vascular effects of i.p.-administered NNA. It is known that systemically administered nonselective inhibitors of NO, like NNA, may modify the disposition of convulsants by the vascular effects due to the inhibition of endothelial NO synthase (Penix et al., 1994; Urbańska et al., 1996; Takei et al., 1999).

Does the proconvulsive effect of NNA indicate that endogenous NO acts as an anticonvulsant? Boda and Szente (1996) have hypothesized that convulsions produced by 4-aminopyridine could be related to the decreased level of NO. This hypothesis has been based on the fact that NNA administered i.p. or i.c.v. considerably facilitated induction and propagation of focal-ictal-like seizures induced by aminopyridine in rat neocortex (Boda and Szente, 1996). The facilitation of seizures by NNA had an origin different from *N*-methyl-D-aspartate receptors because D(−)2-amino-5-phosphonovaleric acid, an antagonist of *N*-methyl-D-aspartate receptors, did not modify the proconvulsive effect of NNA (Boda and Szente, 1996).

In our opinion, however, the hypothesis attributing the 4-aminopyridine seizures to endogenous NO might be questionable. First, L-arginine, a precursor of NO, in a large dose of 500 mg kg^{-1}, did not affect the susceptibility of mice to 4-aminopyridine. Second, the observed difference in a potency of the effect of NNA on seizures induced by i.p.- and i.c.v.-administered 4-aminopyridine suggests that not changes in NO pathway but the vascular effects of NNA could influence the convulsive activity of 4-aminopyridine. Finally, our attempt to reverse the proconvulsant effect of NNA with L-arginine was unsuccessful. Thus, our results may indicate that NNA acts as proconvulsant in 4-aminopyridine-induced seizures in a nonspecific manner. For example, pharmacokinetic mechanisms may be responsible for this effect of NNA. In the study of Urbańska et al. (1996), NNA enhanced the convulsive properties of aminophylline by a significant increase in the effective level of convulsant in the plasma. The results suggest that proconvulsant effects of NNA can be associated with a pharmacokinetic interaction between NNA and convulsant, so we cannot exclude that such interaction exists also in the case of NNA and 4-aminopyridine.

Molsidomine is an agent widely used either orally or intravenously for the treatment of coronary artery disease (Anderson et al., 1994). Its therapeutic effects are the consequences of NO formation, similar to organic nitrates (Anderson et al., 1994). In the present study, molsidomine, similarly to L-arginine, did not influence the convulsant and lethal effects of 4-aminopyridine. Thus, the generation of NO was not involved in the occurrence of 4-aminopyridine-induced seizures. Consequently, it could be also expected the molsidomine would not reverse the proconvulsant effect of NNA. Surprisingly, molsidomine acted in an opposite way strongly increasing the proconvulsant actions of NNA following i.p. 4-aminopyridine. This unexpected action of molsidomine may suggest that convulsant and lethal effects of 4-aminopyridine are independent of central NO. On the other hand, numerous compounds exert different effects in electrical- and 4-aminopyridine-induced seizures and it is very difficult to compare these two models of seizures (Yamaguchi et al., 1993).

It has been reported that NO played an important role in the regulation of cerebral blood flow in physiological and pathological conditions (Iadecola, 1997). It is likely that an explanation of this surprising effect of molsidomine might be provided by studying the role of NO level modulators in vascular changes during convulsions triggered by 4-aminopyridine. It has been reported that systemically administered inhibitors of NO synthase could disturb cerebrovascular autoregulation (Penix et al., 1994). Nonselective inhibition of NO synthesis considerably attenuated the increase in hippocampal blood flow during seizures induced by systemic kainate in rats (Montecot et al., 1998). Nonselective inhibitors of NO, like NNA, may modify the disposition of convulsant by inhibiting endothelial NO synthase. Therefore, as mentioned above, it cannot be excluded that the changes in blood flow caused by NNA might be responsible for the proconvulsant effect of NNA observed in the present study. It is known that NO-generating agents, like molsidomine, may also play an important role in controlling blood flow (Ander-

son et al., 1994; Unger et al., 1994). Hence, it is probable that the potentiation of the proconvulsant effect of NNA by molsidomine could be caused by changes in blood flow in cerebral arteries. On the other hand, molsidomine given alone did not impair the convulsant action of 4-aminopyridine. Therefore, the vascular impact of molsidomine in the potentiation of the proconvulsant effect of NNA is uncertain.

Although many reports have supported a similar role for selective and nonselective NO synthase inhibitors in chemically induced seizures, some studies have demonstrated the differences in action among various NO inhibitors during seizures in various models of epilepsy (Rundfeldt et al., 1995). For example, there is a report that 7-nitroindazole (a selective inhibitor of neuronal NO synthase) had protective activity against pilocarpine-induced seizures in mice (Van Leeuwen et al., 1995) and there are reports indicating that pilocarpine-induced seizures were not affected by NNA (Tutka et al., 1996). Furthermore, it has been demonstrated that 7-nitroindazole, unlike NNA, attenuated kainate-elicited convulsions (Przegalinski et al., 1994; Tutka et al., 1996). We have found that systemically administered 7-nitroindazole, in contrast to NNA, did not exert any significant effect on 4-aminopyridine-induced seizures (unpublished data). Taken together, the results have shown the differences in pharmacological actions among NO synthase inhibitors. These differences could result from the actions of NO synthase inhibitors that were unrelated to brain NO synthase inhibition (De Sarro et al., 2000). For instance, Smith et al. (1996) have suggested that 7-nitroindazole may alter convulsions NO-independently. Desvignes et al. (1999), in the study on extracellular striatal dopamine and its metabolite 3,4-dihydroxyphenylacetic acid efflux in vivo, have shown that 7-nitroindazole differently to NNA increased the dopamine and 3,4-dihydroxyphenylacetic acid striatal levels. It is likely that such an effect could influence the different response to convulsant stimuli.

In summary, our findings suggest that NO formation does not seem to be involved in the mechanisms underlying the development of seizures and lethality induced by 4-aminopyridine. The observed proconvulsant action of NNA and the potentiation of this action by molsidomine are most probably due to other, unrelated to NO, mechanisms. At present, we are studying an effect of 7-nitroindazole on 4-aminopyridine-induced seizures and results of the study should provide more information.

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