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Dose-dependent anticonvulsant and proconvulsant effects of nitric oxide synthase inhibitors on seizure threshold in a cortical stimulation model in rats

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Abstract

In the central nervous system, nitric oxide (NO) is increasingly being considered as a trans-synaptic retrograde messenger, being involved for instance in cellular responses to stimulation of glutamate receptors of the NMDA subtype. Thus, compounds that modify NO production, such as NO synthase inhibitors, may provide a means of altering NMDA receptor function. The functional consequences of NO synthase inhibition are, however, complicated by the fact that NO not only serves as a messenger to activate guanylyl cyclase and so to raise cGMP in target cells in response to NMDA receptor stimulation but also to induce feedback inhibition of the NMDA receptor via a redox modulatory site on the receptor complex. This may explain the contrasting results obtained previously with NO synthase inhibitors in animal models of ischaemia and seizures. In the present study, we tried to resolve the reported discrepancies about the effects of NO synthase inhibitors in seizure models by studying such drugs at various doses in a novel model of cortical seizure threshold. In this model, the threshold for seizures in rats is determined at short time intervals by applying ramp-shaped electrical pulse-trains directly to the cerebral cortex, allowing one to determine the time course of anti- or proconvulsant drug effects in individual rats. Two NO synthase inhibitors, N^G-nitro-L-arginine and N^G-nitro-L-arginine methyl ester, were compared with a clinically effective antiepileptic drug, i.e. valproate. Whereas N^G-nitro-L-arginine methyl ester, 1–40 mg/kg i.p., did not exert any marked effects on seizure threshold, N^G-nitro-L-arginine, 1–10 mg/kg, induced significant threshold increases, which reached about 50% of the increases seen with valproate, 200 mg/kg. At 40 mg/kg N^G-nitro-L-arginine, however, a significant and long-lasting decrease in seizure threshold was observed, presumably induced by blockade of the negative feedback exerted by NO on the NMDA receptor. The data demonstrate that a NO synthase inhibitor can produce both anti- and proconvulsant effects in the same model, depending on the dose administered. Similar observations have previously been reported for NMDA receptor antagonists and clinically established antiepileptic drugs, so that the biphasic effects of NO synthase inhibitors are not unusual for drugs with anticonvulsant activity.

Keywords: Glutamate; Nitric oxide (NO); NMDA (*N*-methyl-D-aspartate); Epilepsy; Ischemia

1. Introduction

Nitric oxide (NO), a small diffusible messenger molecule synthesized from the amino acid L-arginine by NO synthase, appears to play a crucial role in a number of physiological and pathophysiological processes (Moncada et al., 1991; Lowenstein et al., 1994). NO acts as an endogenous activator of guanylyl cyclase and thereby increases cyclic GMP (Moncada et al., 1991). In the brain, increased activity in excitatory pathways has long been known to cause increases in the levels of cyclic GMP, and more recently it has been shown that one subtype of excitatory amino acid receptor, the NMDA receptor, is coupled to NO synthesis by NO synthase, so that activation of this glutamate receptor subtype leads to elevation of cyclic GMP through stimulation of NO synthase (Garthwaite, 1991; Vincent and Hope, 1992). The NO synthase in the brain has been characterized and shown to be inhibited by L-arginine analogues such as N^G-nitro-L-arginine, N^G-nitro-L-arginine methyl ester or N^G-monomethyl-L-

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arginine (Moncada et al., 1991). In addition to effects mediated by cyclic GMP, NO is a highly reactive chemical in its own right and is likely to have effects unrelated to stimulation of guanylyl cyclase (Garthwaite, 1991). Excessive NMDA receptor activation is thought to contribute to a number of neurodegenerative conditions, including those induced by cerebral ischaemia, trauma and chronic epilepsy, and it has been suggested that NO participates in these conditions (Garthwaite, 1991; Vincent and Hope, 1992). Furthermore, it has been recently suggested that NO may contribute to the genesis of seizure activity (De Sarro et al., 1991). Similar to NMDA receptor antagonists, inhibitors of NO synthase prevent glutamate-, NMDA-, ischaemia-, and hypoxia-induced neuronal injury (Cunningham et al., 1993; Hara et al., 1994), but only relatively few studies examined their effects in seizure models. Unexpectedly, the NO synthase inhibitor N^G-nitro-L-arginine was ineffective in amygdala kindling, i.e. a model of temporal lobe epilepsy thought to be associated with enhanced NMDA receptor function, but even accelerated the development of kindling in rats (Rondouin et al., 1992). In mice, the duration of NMDA-induced wild running was increased by the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (Buisson et al., 1993). Similarly, N^G-nitro-L-arginine increased the severity of kainate-induced seizures in mice and rats (Penix et al., 1994). Furthermore, N^G-nitro-L-arginine methyl ester facilitated pilocarpine-induced limbic motor seizures in the mouse (Starr and Starr, 1993). In contrast, N^G-nitro-L-arginine methyl ester had no effect on carbachol-induced limbic motor seizures in rats, but blocked generalized convulsive seizures and antagonized the proconvulsant activity of the NO precursor L-arginine (Mraovitch et al., 1993). Similarly, the NO synthase inhibitor N^G-monomethyl-L-arginine was reported to exert an anticonvulsant effect on NMDA- or kainate-induced convulsions (De Sarro et al., 1991). These paradoxical differences between experimental studies on NO synthase inhibitors in seizure models may be explained by differences in species, type of seizure and seizure induction, and dose as well as pretreatment time of the NO synthase inhibitors. This prompted us to undertake a study in which the time- and dose-dependency of effects induced by N^G-nitro-L-arginine and N^G-nitro-L-arginine methyl ester were examined in a new seizure threshold model in rats. By applying ramp-shaped pulse trains directly to the cerebral cortex, this model allows one to determine seizure thresholds at various times after drug application in individual rats so that time- and dose-effect relationships can be studied without using separate groups of animals per time and dose (Voskuyl et al., 1989, 1992). An added advantage of this model is that both pro- and anticonvulsant drug activities can be detected in the same individual rat. For comparison with the effects of NO synthase inhibitors, the antiepileptic valproate was included in the experiments.

2. Materials and methods

2.1. Animals

Female Wistar rats (Harlan-Winkelmann, Borchen, Germany), weighing 200–280 g, were used. The animals were purchased from the breeder at a body weight of about 200 g. Following arrival in the animal colony, the rats were kept in a vivarium under controlled environmental conditions (ambient temperature 24–25°C, humidity 50–60%, 12/12 h light/dark cycle, artificial light on at 7:00 a.m.) for at least one week before being used in the experiments. Standard laboratory chow (Altromin 1324 standard diet) and tap water were allowed ad libitum. All experiments were done in a laboratory with the same environmental conditions as the vivarium. Temperature and humidity were continuously controlled in both vivarium and laboratory. All drug or vehicle applications were done at the same time in the morning (between 8:00 and 9:00 a.m.) to avoid circadian influences.

2.2. Electrode implantation

For implantation of electrodes, 12 rats were anaesthetized with chloral hydrate (360 mg/kg i.p.) and placed in a stereotaxic frame according to the method of Paxinos and Watson (1986). The skull surface was exposed and after trepanation two stainless steel screw electrodes were implanted bilaterally over the frontoparietal cortex at the following coordinates (relative to bregma in mm): AP −1.0, L ±3.5. The screw electrodes were lowered approximately 1 mm below the surface of the bone to penetrate the dura without lesioning the cortex. To form the screw electrodes, a 0.1 mm Teflon insulated stainless steel wire with a standard microelectronic connector was soldered to the head of M1 screws. An additional grounding electrode was implanted over the olfactory bulb. The electrode assembly was combined to form a female connector and was affixed to the skull with dental acrylic cement. Determination of cortical electrical threshold was initiated following 2 weeks of recovery after surgery. The integrity of the cortical surface was controlled after the experiments using standard histological techniques.

2.3. Determination of seizure threshold

For determination of seizure threshold for minimal convulsive motor activity, a single train of bipolar pulses (total pulse duration 2 ms, 50 pulses/s) with steadily

increasing (ramp-shaped) current amplitude was applied directly to the cortex through the two screw electrodes in freely moving rats. Stimulation was interrupted at the onset of the first sign of convulsive behaviour, and the current of the last pulse applied was defined as threshold. The individual convulsive endpoints differed from rat to rat but were strictly reproducible in the same animal upon repeated determination of seizure threshold. Neither seizure severity nor seizure duration recorded at threshold currents changed over the period of more than 100 threshold determinations (usually 14 threshold determinations per vehicle or drug experiment times at least 15 experiments) per animal. In most rats, the first sign of seizure activity (which was used as endpoint) was a short-lasting clonic seizure of one forelimb. In some rats, a twitch of the whole body, or backward or stooping movements of the body were the first behavioural signs, sometimes preceded by arrest reactions. Separate experiments with rats, in which additional electrodes for EEG recording had been implanted, demonstrated that these first behavioural seizure signs coincided with the appearance of paroxysmal alterations (spikes) in the EEG (unpublished data), confirming previous studies of Voskuyl et al. (1992). If stimulation was continued, more severe signs of seizure activity, e.g. prolonged generalized clonic activity, appeared after these initial signs, but this more severe seizure activity induced prolonged postictal threshold increases, which was the reason to stop the ramp stimulation at the initial sign of seizure activity for all experiments described in this study.

2.4. Ramp generator

Since a ramp generator as used by Voskuyl et al. (1989) was not commercially available, a digital controlled generator was custom-designed to produce the ramp-shaped pulse trains. Two line frequency triggered square-wave generators produced two consecutive monopolar rectangular pulses each of 1 ms duration. One pulse was fed into the inverting and the other into the non-inverting input of a differential amplifier. The output of this amplifier was the bipolar pulse (U_e) of 2 ms duration and a rate of 50 Hz. This signal was fed to the reference input of a multiplying digital to analog converter. The digital input of the converter was connected to a 12-bit counter (12 bit = 4096 steps), which was line triggered too. The bipolar pulses were multiplied with the counter setting (z) to produce the output signal (U_a) according to the following equation:

$$U_a = z/4096 \times U_e$$

The voltage output was amplified and converted into a bipolar current pulse using a voltage-controlled current source. Three maximal current settings (I_{max}) were provided: plus/minus 0.5, 1 and 2 mA.

When starting the ramp stimulation, the digital counter was reset to zero and the pulse train was enabled to increment the counter. The stimulation could be stopped at any time with an internal or external stop pulse. This pulse stopped the counter and shut down the pulse train – no further signal appeared at the stimulation output. The reading of the counter was preserved on a 4-digit display. The counter reading was used to calculate the peak-to-peak current of the last pulse (I_{eff}) applied to the animal using the following equation:

$$I_{eff} = z/4096 \times I_{max} \times 2$$

Maximal 4095 pulses were applied for one stimulation train. At a rate of 50 bipolar pulses each second the maximal stimulus duration was 81.9 s. Since the stimulus was stopped at the first sign of convulsive behaviour, the stimulus duration varied from animal to animal and between control and drug trial. The current setting was selected to produce a stimulus duration between 10 and 30 s for control and drug experiments.

2.5. Drug experiments

Before the first drug experiments, several control threshold determinations with vehicle application were done to test the stability of the threshold responses. The protocol used for these vehicle controls and all subsequent drug experiments was as follows. In each rat, the individual seizure threshold was determined 0.5 h, 0.25 h and immediately prior to i.p. injection as well as 0.25, 0.5, 1, 1.5, 2, 2.5, 3, and 4 h after injection. In the case of prolonged drug activity, further threshold determinations were done up to 24 h after drug (or vehicle) injection. During the first control trials, the seizure threshold tended to decrease from determination to determination, but became relatively stable thereafter without significant differences between seizure thresholds repeatedly determined on one experimental day. Drug testing was started after stabilization of the seizure threshold. During the first experiments, each drug trial was preceded by a control trial (usually 2 days prior to the drug trial) with the same fixed time intervals after injection. After several vehicle control trials were performed in this way without any evidence of significant vehicle effects, further drug experiments were undertaken without separate control trials. At least 7 days were interposed between two drug experiments in the same group of rats. All data shown in the present study are from a group of 9 rats with reproducible seizure thresholds.

2.6. Drugs

N^G-Nitro-L-arginine and N^G-nitro-L-arginine methyl ester (used as HCl) were purchased from Sigma

(Munich, Germany). Valproate (used as sodium salt) was generously supplied by Desitin Arzneimittel (Hamburg, Germany). All drugs were freshly dissolved in water (N^G-nitro-L-arginine with the aid of dilute HCl) prior to each experiment and injected i.p. at a volume of 2–3 ml/kg body weight. Control experiments were done with the same injection volume of saline.

2.7. Statistics

The three seizure threshold determinations prior to each vehicle or drug injection were averaged to give a baseline value for each experiment. The difference in μA from this baseline value was used to illustrate the time course of vehicle or drug effects on seizure threshold. Individual basal control thresholds were in the range of 300–900 μA in the animals used for the present experiments. For statistical evaluation of data, each control or drug trial (including the three pre-injection values) was first subjected to analysis of variance, using the Friedman test for paired replicates. The significance of differences in seizure threshold after drug or vehicle application compared to the average pre-injection baseline value was calculated by the Wilcoxon signed rank test for paired replicates. All tests were used two-sided.

3. Results

All control experiments undertaken during the course of the present study demonstrated the lack of significant increases or decreases in seizure threshold during the course of an experiment with repeated threshold determinations at fixed post-injection intervals, so only two examples of respective control experiments are shown as examples in Figs. 1 and 2. The antiepileptic valproate, administered at a dose previously demonstrated to induce anticonvulsant effects in rodent seizure models (Löscher, 1993), rapidly and potently increased seizure threshold, but the effect was only relatively short-lasting so that pre-drug control values were reached after 3 h (Fig. 1).

The experiments with NO synthase inhibitors were started with N^G-nitro-L-arginine methyl ester, injected at 5 and 10 mg/kg, and seizure threshold was determined up to 5 h after injection. As shown in Fig. 1, no pronounced alterations in threshold were observed,

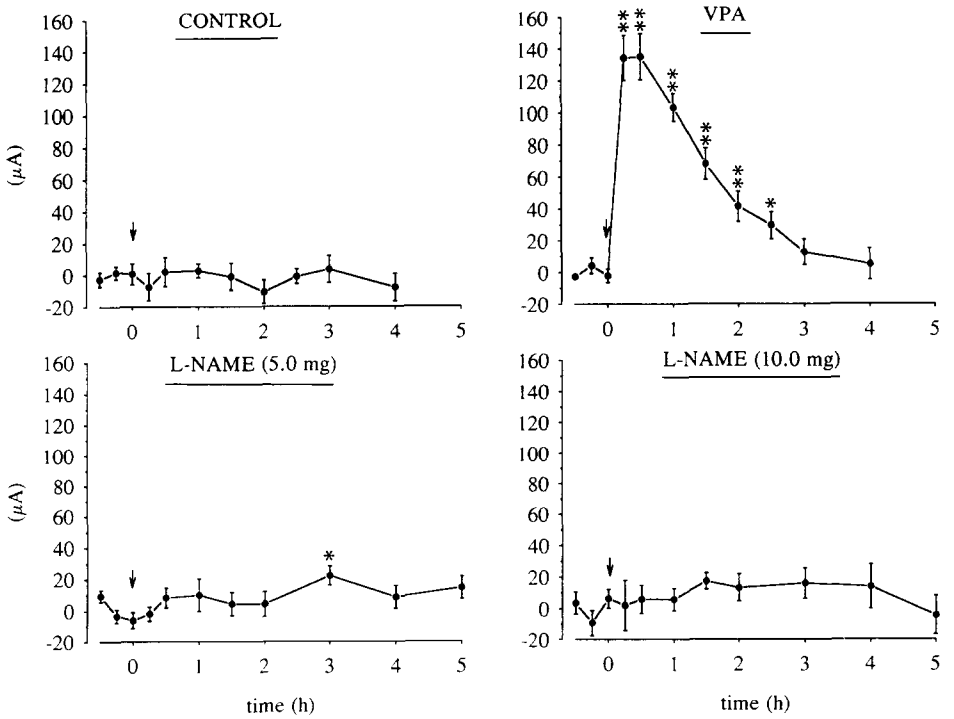

Fig. 1. Effect of vehicle (control), valproate (VPA) and N^G-nitro-L-arginine methyl ester (L-NAME) on cortical seizure threshold in rats. Data are shown as differences from pre-injection baseline threshold in μA. For each experiment, the pre-injection baseline threshold was calculated as the mean from the three separate seizure thresholds determined prior to vehicle or drug injection. Vehicle (saline) or drugs were i.p. injected immediately after the third pre-injection threshold (indicated by an arrow). Doses were 200 mg/kg in case of valproate and 5 or 10 mg/kg in case of N^G-nitro-L-arginine methyl ester. All data are shown as means ± S.E. Analysis of variance by the Friedman test indicated significant differences (P at least < 0.05) between data from the experiment with valproate and the experiment with 5 mg/kg N^G-nitro-L-arginine methyl ester. Significance of post-injection data to the average pre-injection baseline threshold of each experiment as calculated by the Wilcoxon rank test for paired replicates is indicated by asterisks (* $P < 0.01$; * * $P < 0.001$).

although there was a tendency to increased thresholds after application of both doses. In the case of 5 mg/kg, a significant threshold increase was calculated at 3 h post-drug. When 1 mg/kg of N^G-nitro-L-arginine methyl ester was injected and seizure threshold was repeatedly determined up to 24 h after injection, a moderate but significant threshold increase was seen 1 h after drug injection (Fig. 2). Following 40 mg/kg N^G-nitro-L-arginine methyl ester, however, the seizure threshold tended to decrease (Fig. 3), although not to any statistically significant extent.

Following N^G-nitro-L-arginine, 1 mg/kg, seizure threshold tended to increase after 4 h and was significantly above pre-injection control after 6 h (Fig. 2). When the dose of N^G-nitro-L-arginine was increased to 10 mg/kg, a significant increase in seizure threshold was seen already after 0.25 h and the peak threshold increase occurred after 1 h. Thereafter, the threshold slowly returned towards control. When the experiment with 10 mg/kg of N^G-nitro-L-arginine was repeated, the same time course and extent of threshold increase were obtained (not illustrated). In both experiments, the peak threshold increase was about 50% of that obtained with valproate. A further increase in the dose to 40 mg/kg, however, reversed the effect of N^G-nitro-L-arginine in that a significant and long-lasting decrease was observed (Fig. 3).

At none of the doses of NO synthase inhibitors tested were any behavioural adverse effects observed, while valproate induced wet dog shakes and ataxia at the dose administered.

4. Discussion

The present data demonstrate for the first time that the effects of NO synthase inhibitors on seizure threshold depend on the dose, pretreatment time and type of drug used. Thus, while N^G-nitro-L-arginine methyl ester was almost ineffective in the present model, the more potent NO synthase inhibitor N^G-nitro-L-arginine increased seizure threshold at 1 and 10 mg/kg, but induced a proconvulsant effect at 40 mg/kg. At 10 mg/kg, N^G-nitro-L-arginine produced about 50% of the seizure threshold increase elicited by 200 mg/kg valproate, but, in contrast to valproate, N^G-nitro-L-arginine did not exert any behavioural adverse effects. The time course of anticonvulsant activity of N^G-nitro-

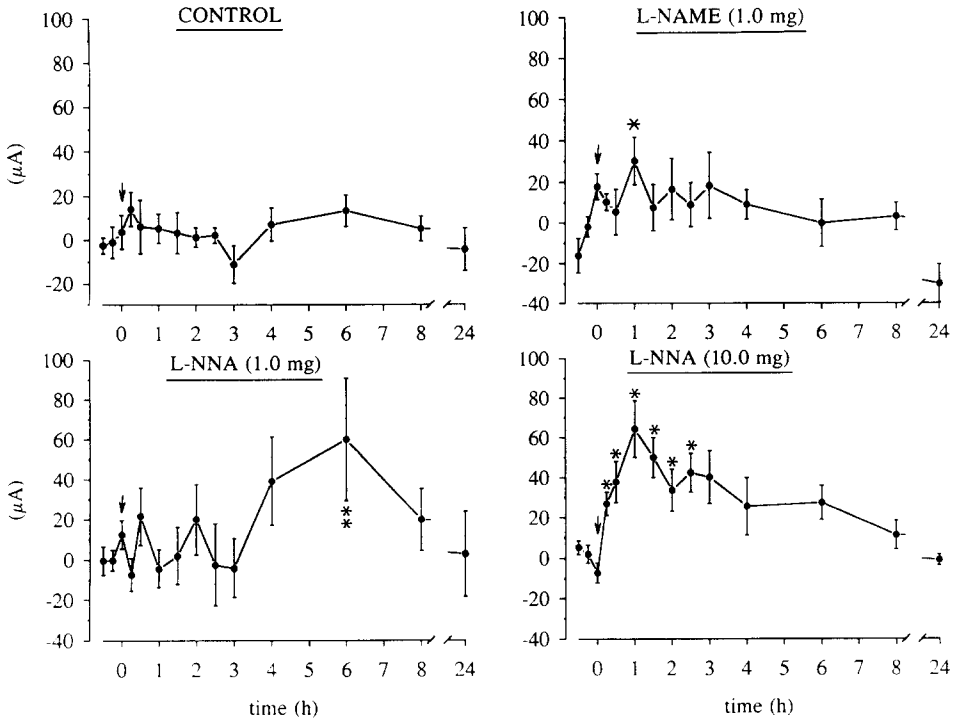

Fig. 2. Effect of vehicle (control), N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NNA) on cortical seizure threshold in rats. Data are shown as differences from pre-injection baseline threshold in μA. For each experiment, the pre-injection baseline threshold was calculated as the mean from the three separate seizure thresholds determined prior to vehicle or drug injection. Vehicle (saline) or drugs were i.p. injected immediately after the third pre-injection threshold (indicated by an arrow). Doses were 1 mg/kg in case of N^G-nitro-L-arginine methyl ester and 1 or 10 mg/kg in case of N^G-nitro-L-arginine. All data are shown as means ± S.E. Analysis of variance by the Friedman test indicated significant differences (P at least < 0.05) between data from all experiments except control. Significance of post-injection data to the average pre-injection baseline threshold of each experiment as calculated by the Wilcoxon rank test for paired replicates is indicated by asterisks (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

L-arginine markedly differed at 1 and 10 mg/kg in that a retarded effect was observed at the lower dose but an immediate and relatively short-lasting effect occurred at the higher dose. Thus, by using only one time point after injection, as is usually done in studies on NO synthase inhibitors, the anticonvulsant activity of N^G-nitro-L-arginine could have been missed.

The possibility to repeatedly determine seizure threshold before and after drug administration in the same group of animals on the same experimental day is one of the major advantages of the novel rat model used in this study. In conventional seizure threshold models, such as the timed i.v. pentylenetetrazole infusion method or the maximal electroshock seizure threshold determined via transcorneal or transauricular stimulation, it is not possible to use repeated determination of seizure threshold at short time intervals in the same group of animals for time-course studies on drug effects because of long-lasting postictal threshold increases and ethical restraints, particularly with respect to repeated transcorneal stimulation (Green, 1986; Löscher and Schmidt, 1988). Furthermore, a kindling-like effect with increased severity and duration of seizures occurs upon repeated induction of seizures in such models (Sangdee et al., 1982; Wasterlain et al., 1989). Thus, separate groups of animals have to be used in such models (and various other seizure models) for time-course studies on anticonvulsant drugs, which is one of the reasons why many scientists test drugs only at one fixed time interval (e.g. 0.5 or 1 h) after drug application. The present model, which uses direct cortical, ramp-shaped electrical stimulation, was developed by Voskuyl and colleagues (Voskuyl et al., 1989). If the ramp stimulation is stopped at the first sign of convulsive activity, no postictal threshold increase is seen and the next threshold can be determined at an interval as short as 1 min (Voskuyl et al., 1992). Although seizure threshold decreases upon repeated stimulation during the first experiments after cortical electrode implantation, it stabilizes thereafter and there is no kindling-like increase in seizure severity and duration (Voskuyl et al., 1989, 1992; present experiments). Standard antiepileptic drugs such as benzodiazepines (Voskuyl et al., 1989) or valproate (present study) increase the cortical seizure threshold after acute administration at doses similar to those effective in traditional seizure threshold models with electrical stimulation (Löscher et al., 1991). As demonstrated by the present data on NO synthase inhibitors, the new seizure threshold model is quite useful for characterizing the time course and potency of anti- or proconvulsant effects of novel drugs.

At least in part, the present finding that the effects of NO synthase inhibitors on seizure threshold depend on the dose and pretreatment time might explain the discrepancies in previous studies on the effect of such

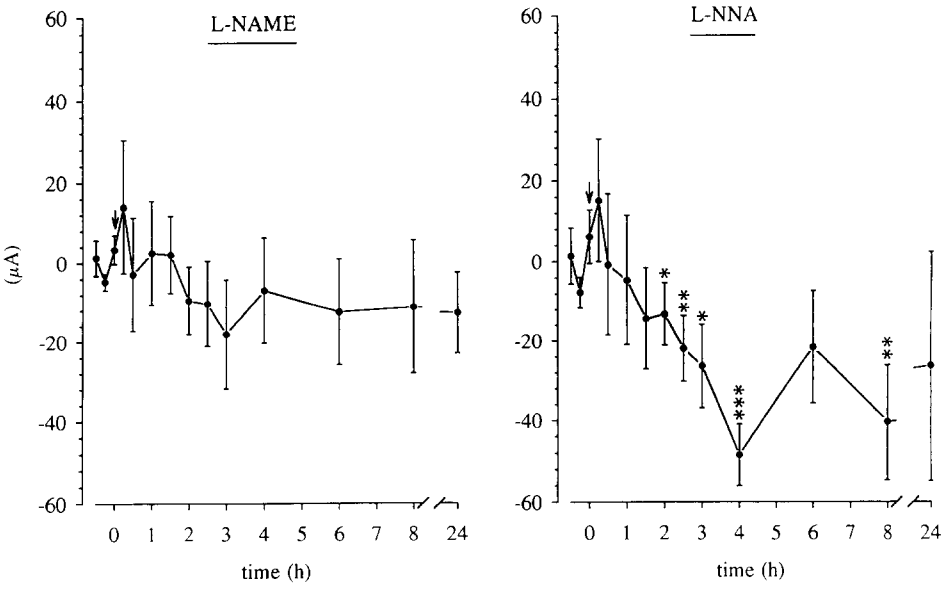

Fig. 3. Effect of N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NNA) on cortical seizure threshold in rats. Data are shown as differences between pre-injection baseline threshold in μA. For each experiment, the pre-injection baseline threshold was calculated as the mean from the three separate seizure thresholds determined prior to drug injection. Drugs were i.p. injected immediately after the third pre-injection threshold (indicated by an arrow). Doses were 40 mg/kg in case of both drugs. All data are shown as means ± S.E. Analysis of variance by the Friedman test indicated significant differences ($P < 0.001$) between data from the experiment with N^G-nitro-L-arginine. Significance of post-injection data to the average pre-injection baseline threshold of this experiment as calculated by the Wilcoxon rank test for paired replicates is indicated by asterisks (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

drugs in seizure models. Rondouin et al. (1992) found no effect on generalized kindled seizures in rats with 25 mg/kg N^G-nitro-L-arginine (pretreatment time not specified). The same high dose chronically administered accelerated kindling development (Rondouin et al., 1992). In contrast, Mraovitch et al. (1993) reported that 3 mg/kg of N^G-nitro-L-arginine methyl ester abolished generalized seizures induced by microinjection of carbachol into the ventroposterior thalamus of rats, while L-arginine potentiated the seizures. In mice, however, a similar dose of N^G-nitro-L-arginine methyl ester potentiated seizures induced by systemic application of pilocarpine, which like carbachol acts by stimulating cholinergic receptors (Starr and Starr, 1993). The contrasting effect of comparable doses of N^G-nitro-L-arginine methyl ester in similar seizure models could either be explained by species differences or by the different mode of application (microinjection into a brain region versus systemic injection) of the cholinergic agonist. Similar opposing effects of NO synthase inhibitors were also found when NMDA or kainate was used to induce seizures. Thus, by using wild running induced by intracerebroventricular injection of NMDA in mice as a seizure endpoint, Buisson et al. (1993) reported that N^G-nitro-L-arginine methyl ester, 1–10 mg/kg i.p. 30 min prior to NMDA, dose dependently increased the duration of wild running, which was antagonized by L-arginine, while L-arginine alone had no effect on wild running. Similarly, Penix et al. (1994) found that N^G-nitro-L-arginine, 50 mg/kg injected i.p. 24 h and again 2 h before i.p. injection of kainate in rats or 0.5–15 mg/kg injected i.p. 0.5 h before i.p. injection of kainate in mice, increased the severity of seizures. In contrast, De Sarro et al. (1991, 1993) observed an anticonvulsant effect on NMDA- or kainate-induced seizures when N^G-monomethyl-L-arginine was microinjected into the prepiriform cortex 15 min prior to application of the convulsants, while L-arginine exerted a proconvulsant effect. Thus, the mode of seizure induction and route of drug administration seem to play an important role in results obtained with NO synthase inhibitors. Interestingly, intracerebellar injection of N^G-monomethyl-L-arginine was shown to antagonize the cerebellar increase in cGMP generated by the glutamate receptor agonists D-serine, quisqualate and kainate and the chemical convulsant pentylenetetrazole (Wood et al., 1990). A similar effect on the pentylenetetrazole-induced cGMP increase has previously been demonstrated for antiepileptics such as valproate (cf., Löscher, 1993).

In addition to being involved in the NMDA receptor-mediated increase in cGMP, it has recently been shown that NO can regulate NMDA receptor activation (Izumi et al., 1992), presumably via a feedback effect on the redox modulatory site of the NMDA receptor, which results in decreased NMDA receptor activity and protects neurons from excessive stimulation of the receptor (Lipton, 1993). Thus, NO may play a positive (e.g., via activation of guanylyl cyclase) or negative (e.g. via feedback effects on the NMDA receptor) modulatory role in NMDA receptor-mediated events depending on the functional state of the NMDA receptor and the ambient concentration of excitatory amino acids (Izumi et al., 1992; Lipton, 1993). Based on this concept, NO can ameliorate the effects of excitatory amino acids mediated by excessive activation of NMDA receptors, as e.g. in response to application of NMDA. This would explain the finding that relatively low doses of N^G-nitro-L-arginine methyl ester exert proconvulsant effects on seizures induced by NMDA (Buisson et al., 1993) but anticonvulsant effects in models which are not directly related to increased NMDA receptor function (Mraovitch et al., 1993 and present study). Such opposing effects of NO would also explain why NO synthase inhibitors can exert neuroprotective as well as neurotoxic effects in ischaemia models, depending on the state of the NMDA receptor (Hara et al., 1994).

With respect to the doses of NO synthase inhibitors used in the present and previous studies in seizure models in rodents, it is important to note that systemically administered N^G-nitro-L-arginine methyl ester leads to a partial but long-lasting inhibition of NO synthase activity in vivo since N^G-nitro-L-arginine, the active principle of N^G-nitro-L-arginine methyl ester, binds to NO synthase irreversibly (Xu et al., 1993). For instance, after systemic application of 20 mg/kg N^G-nitro-L-arginine methyl ester in rats, NO synthase activity in the brain was attenuated by 33% at 0.5 h, 52% at 2 h and 55% at 24 h, respectively (Xu et al., 1993). For comparison, N^G-nitro-L-arginine inhibits brain NO synthase activity by more than 50% within 2 h after application of 2 mg/kg (Carreau et al., 1994), indicating that N^G-nitro-L-arginine is about 10 times more potent than N^G-nitro-L-arginine methyl ester after systemic administration. The irreversible inhibition of NO synthase by N^G-nitro-L-arginine could explain the present observation that a low dose of N^G-nitro-L-arginine, i.e. 1 mg/kg, exhibited a delayed effect on seizure threshold, while a higher dose, i.e. 10 mg/kg, had a more rapid onset of anticonvulsant activity.

Carreau et al. (1994) recently demonstrated that the neuroprotective effect of N^G-nitro-L-arginine followed a biphasic pattern in that it increased dose dependently over doses of 0.1–1 mg/kg, but the effect was lost at higher doses, viz. 3 and 10 mg/kg, although these higher doses inhibited NO synthase activity more effectively. This biphasic dose-response for N^G-nitro-L-arginine-induced neuroprotection may be another important factor explaining the discrepancies previously noted in the literature concerning the neuroprotective effects of this drug in models of focal ischaemia

(Hara et al., 1994). Similar observations were made in the present experiments in that the anticonvulsant effect of N^G-nitro-L-arginine observed at doses of 1–10 mg/kg was lost at a higher dose. The lack of anticonvulsant efficacy of high doses of N^G-nitro-L-arginine could be related to the blockade of a NO-mediated negative feedback exerted on the NMDA receptor. Alternatively, the proconvulsant effect of high doses of N^G-nitro-L-arginine observed in the present and previous studies (Penix et al., 1994) could be due to toxic effects of the drug, such as an adverse vascular effect. Penix et al. (1994) have recently suggested that high doses of N^G-nitro-L-arginine may exert proconvulsant activity through suppression of NO synthase activity in the vascular endothelium, thereby impairing cerebrovascular autoregulation. N^G-Nitro-L-arginine methyl ester also tended to decrease seizure threshold at 40 mg/kg in the present experiments, but this effect was not significant. The differences between N^G-nitro-L-arginine and N^G-nitro-L-arginine methyl ester found in this study cannot be fully explained in terms of differential potency to inhibit NO synthase, although this would be the most plausible explanation.

In conclusion, we have demonstrated that NO synthase inhibitors can exert both anticonvulsant and proconvulsant effects in the same model, depending on the dose used. Similarly, NMDA receptor antagonists and clinically used antiepileptic drugs such as phenytoin and carbamazepine can produce proconvulsant effects at doses which are only 3–5 times higher than the anticonvulsant doses of such drugs (e.g., Klockgether et al., 1988; Löscher and Hönack, 1990; Löscher and Nolting, 1991; Starr and Starr, 1993; Rundfeldt et al., 1994). The present results on the anticonvulsant effects produced by NO synthase inhibition suggest that the recent proposal of Buisson et al. (1993), that NO may play the role of an endogenous anticonvulsant substance, may be warranted for NMDA-induced seizures but not as a general principle. Similarly, proposals to develop NO precursors such as nitroglycerin for treatment of epilepsy (Lipton, 1993) should be considered with caution. Studies are under way to evaluate NO precursors such as nitroglycerin and sodium nitroprusside in the present model of cortical seizure threshold.

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