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European Journal of Pharmacology 394 (2000) 275–288

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7-Nitroindazole potentiates the antiseizure activity of some anticonvulsants in DBA/2 mice

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Received 12 August 1999; received in revised form 20 January 2000; accepted 25 January 2000

Abstract

7-Nitroindazole, a selective neuronal nitric oxide synthase inhibitor (25–200 mg kg^{-1}, intraperitoneally (i.p.)) antagonized audiogenic seizures in DBA/2 mice in a dose-dependent manner. We investigated the effects of 7-nitroindazole at a dose of 25 mg kg^{-1} i.p., which per se did not show anticonvulsant activity against audiogenic seizures in DBA/2 mice, on the antiseizure activity of some conventional antiepileptic drugs. 7-Nitroindazole sometimes potentiated the anticonvulsant activity of carbamazepine, diazepam, lamotrigine, phenytoin, phenobarbital and valproate against audiogenic seizures in DBA/2 mice. The degree of potentiation by 7-nitroindazole was greatest for phenobarbital and diazepam, less for valproate and least for carbamazepine, lamotrigine and phenytoin. The increase in anticonvulsant activity was associated with a comparable increase in motor impairment. However, the therapeutic index of combined treatment with diazepam + 7-nitroindazole, phenobarbital + 7-nitroindazole or valproate + 7-nitroindazole was more favourable than that of the diazepam + vehicle, phenobarbital + vehicle or valproate + vehicle treatment. The results indicate that 7-nitroindazole is able to increase the protective activity of some conventional antiepileptics and this effect appears not to result only from the impaired synthesis of nitric oxide. In fact, mice receiving 7-nitroindazole (25 mg kg^{-1}, i.p.) and L-arginine (30 μg/mouse, intracerebroventricularly (i.c.v.) did not show significant changes of ED_{50} values in comparison to those of related groups of animals treated with 7-nitroindazole and anticonvulsants. 7-Nitroindazole was able to increase the brain levels of dopamine and noradrenaline and its anticonvulsant effects and changes in catecholamine content were antagonized by pretreatment with α-methyl-paratyrosine, an agent inhibiting the synthesis of catecholamines. The fact that α-methyl-paratyrosine reverses concomitantly both the increase in brain levels of dopamine and noradrenaline and the anticonvulsant properties of 7-nitroindazole strongly suggests an important role of catecholamines in the antiseizure activity of 7-nitroindazole. Since 7-nitroindazole did not significantly influence the total and free plasma levels of the anticonvulsant drugs studied, we suggest that pharmacokinetic interactions, in terms of total or free plasma levels, are not probable. 7-Nitroindazole did not significantly affect the hypothermic effects of the anticonvulsant compounds studied. 7-Nitroindazole showed an additive effect when administered in combination with some classical anticonvulsants, most notably diazepam, phenobarbital and valproate and its activity could be, in part, due to an increase of monoamine levels.

Keywords: Nitric oxide (NO); Epilepsy; 7-Nitroindazole; Carbamazepine; Diazepam; Lamotrigine; Phenobarbital; Phenytoin; Valproate; Anticonvulsant; Audiogenic seizure; DBA/2 mouse

1. Introduction

During the last decade, the gaseous chemical messenger, nitric oxide (NO), has attracted a great deal of attention since it was found to play a role in a variety of physiological processes in the brain (Moncada et al., 1989, 1991; Bredt and Snyder, 1991, 1992, 1994; Garthwaite, 1991, 1993). NO is an atypical regulatory molecule that acts both as a second messenger and as a neurotransmitter (Moncada et al., 1991). Several isoforms of NO synthase have been cloned including neuronal, endothelial and hepatic forms and one such form is present in macrophages (Dawson and Snyder, 1994). It was reported that an increase in cGMP levels follows the stimulation of glutamate receptors, mainly of the *N*-methyl-D-aspartate (NMDA)

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00086-8

type (Garthwaite et al., 1988; Bredt and Snyder, 1989), and that NO may serve as a messenger molecule mediating the physiological actions of L-glutamate and overall neuronal excitability (Bredt and Snyder, 1989; Knowles et al., 1989). It is widely accepted that enhanced NMDA receptor-mediated synaptic transmission plays a crucial role in the pathophysiology of a number of neurological diseases (Klockgether and Turski, 1990; Loschmann et al., 1991; Meldrum, 1995). NO may be involved in neuron–glia interactions (Garthwaite, 1991), synaptic plasticity, long-term potentiation (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991), long-term depression (Shibuki and Okada, 1991), and desensitization of the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptor (Ito and Karachot, 1991).

The role of NO in epilepsy has been investigated by several authors and contradictory roles for NO in the development and pathogenesis of seizures have been suggested. L-N^G-nitroarginine methyl ester (L-NAME) attenuates seizures induced by focal injection of NMDA or kainic acid into the deep prepiriform cortex (De Sarro et al., 1991, 1993) and into the hippocampus (Moncada et al., 1992). In addition, L-NAME and L-N^G-monomethyl-arginine (L-NMMA) attenuate cocaine- bicuculline- or pentylenetetrazol-induced seizures in mice (Osonoe et al., 1994; Przegalinski et al., 1994; Hara et al., 1996). In contrast, administration of L-NAME increases convulsions induced by NMDA in mice (Buisson et al., 1993). Another NO synthase inhibitor, L-N^G-nitroarginine (L-NOARG), potentiates seizures induced in rats by various convulsants, such as quinolinic acid (Haberny et al., 1992), kainic acid (Rondouin et al., 1993; Tutka et al., 1996) and bicuculline (Wang et al., 1994). According to Theard et al. (1995), acute inhibition of NO synthase activity by L-NOARG prolongs the duration of bicuculline-induced seizures. L-NOARG potentiates aminophylline-induced seizures, but remains ineffective against electroconvulsions, aminooxyacetic acid- and pentylenetetrazol-induced convulsions in mice (Urbanska et al., 1996). L-NOARG has a protective effect against clonic seizures induced by intracerebroventricular glutamate but does not significantly affect the convulsive activity of the remaining intracerebroventricular excitatory amino acids: AMPA, NMDA and *trans*-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (Tutka et al., 1996). L-NOARG is also ineffective against tonic-clonic seizures induced by systemic bicuculline, pentylenetetrazol and pilocarpine (Tutka et al., 1996).

Drawing a conclusion about the role of NO in epilepsy is quite complicated, since a wide variety of experimental epileptic models has been used and often full dose-responses were not or could not be investigated. In addition, in the earlier studies, non selective inhibitors of neuronal NO synthase were used. The discovery of the indazole series of NO synthase inhibitors has allowed better clarification of the role of NO in the Central nervous system (CNS), while 7-nitroindazole proved to have greater selectivity as an inhibitor for neuronal NO synthase than for endothelial NO in vivo (Babbedge et al., 1993). Although 7-nitroindazole is devoid of hypertensive effects in rodents (Moore et al., 1993a,b; Kelly et al., 1995; Wang et al., 1995; Smith et al., 1996), few reports have demonstrated that this compound affects endothelial synthase activity (Fabricius et al., 1996; Zagvazdin et al., 1996). In addition, 7-nitroindazole attenuates the severity of kainic acid-induced seizures (Mülsch et al., 1994) and pilocarpine-induced seizures in mice (Van Leeuwen et al., 1995).

To clarify better the different effects of NO synthase inhibitors on the susceptibility to different types of seizures, we decided to investigate the effects of treatment with 7-nitroindazole on the anticonvulsant properties of carbamazepine, diazepam, lamotrigine, phenytoin, phenobarbital and valproate against audiogenic seizures in DBA/2 mice. The effects of the combined treatment of 7-nitroindazole with the above anticonvulsant drugs on rotarod performance, body temperature and total and free plasma levels of antiepileptics were studied. In addition, an indazole derivative related to 7-nitroindazole, 3-indazolinone, which has negligible NO synthase activity (Babbedge et al., 1993), was used to characterize better the pharmacokinetics and pharmacodynamic interactions of 7-nitroindazole and anticonvulsant drugs.

Finally, since recent results have demonstrated an effect of 7-nitroindazole on monoamine oxidase activity (Desvignes et al., 1999), we investigated whether the anticonvulsant effects of 7-nitroindazole were related to a possible inhibitory action on monoamine oxidase activity.

2. Materials and methods

2.1. Animals

Male DBA/2 mice weighing 8–12 g (22–26 days old) or 20–28 g (48–56 days old) were used (Charles River, Calco, Como, Italy). The animals were housed in groups of 8–10 under a 12-h light/dark cycle (lights on at 7:00 am) with food and water available ad libitum. Procedures involving animals and their care were conducted in conformity with international and national law and policies.

2.2. Experimental design

DBA/2 mice were exposed to auditory stimulation, 15, 30 or 60 min following intraperitoneal (i.p.) administration of 7-nitroindazole, 3-indazolinone or vehicle and 45 min following i.p. injection of carbamazepine, diazepam, lamotrigine, phenytoin, phenobarbital and valproate. A group of animals was pretreated, 2 h before, with α-methyl paratyrosine 125 or 250 mg kg^{-1} and then given 7-nitroindazole i.p. L-Arginine (30 μg/mouse) was administered i.c.v. 25 min before auditory testing, as previously described (De Sarro et al., 1994, 1995). This route was

used in order to exclude possible pharmacokinetic interference between L-arginine, 7-nitroindazole and antiepileptics. Each mouse was placed under a hemispheric Perspex dome (diameter 58 cm) and 1 min was allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 1 min or until tonic extension occurred. Seizure response as previously reported (De Sarro et al., 1984) was assessed using the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus, 4 = respiratory arrest. The maximum response was recorded for each animal. Rectal temperature was recorded immediately prior to auditory testing using an Elektrolaboratoriet thermometer type T.E.3. Behavioural changes were observed and recorded during the period between drug administration and auditory testing.

2.3. Determination of the plasma levels of the antiepileptic compounds

DBA/2 mice, 48–56 days old, were given i.p. either vehicle + one of the antiepileptic compounds and 7-nitroindazole + one of the antiepileptic drugs, or 3-indazolinone + one of the antiepileptic drugs. The animals were lightly anaesthetized with ethyl ether and killed by decapitation at appropriate times and blood samples of approximately 1 ml were collected in Eppendorf tubes. For lamotrigine, the assay was carried out by high performance liquid chromatography (HPLC) (Rizzo et al., 1997).

Blood samples were centrifuged at 2000 rpm for 15 min for carbamazepine, diazepam, lamotrigine, phenytoin and phenobarbital determination. The plasma was put into an MPS-1 system (Amicon, Danvers, MA, USA) for the separation of free microsolutes from protein-bound ones. Plasma samples, 60 μl, were transferred to special sample cups and inserted into an Automatic Clinical Analyser (ACA II, du Pont, Wilmington D.E. USA) which uses a method based on the homogenous enzyme immunoassay technique. For magnesium valproate determination, a serum sample, 50 μl, was diluted twice with Tris-buffer and analysed by the same method. Control drug solutions were put before and after the respective antiepileptic experimental samples.

2.4. Effects on motor movements

Behavioural changes, their onset and duration were recorded after drug injection until the time of rotarod testing. Two independent observers followed gross behavioural changes consisting of locomotor activity, ataxia, squatting posture and possible piloerection. These behavioural changes were noted but not statistically analysed. Groups of 10 DBA/2 mice, weighing 6–12 g, (22–26 days old) were trained to do coordinated motor movements continuously for 2 min on a rotarod 3-cm diameter 8 rpm min^{-1} (U. Basile, Comerio, Varese, Italy). Impairment of coordinated motor movements was defined as inability of the mice to remain on the rotarod for a 2-min test period (Dunham and Miya, 1957). The ability of the mice to remain on the Rotarod was tested 25 min after the i.p. administration of conventional antiepileptics + vehicle, or after the combined treatment with antiepileptic drugs + 7-nitroindazole, or following the combined treatment with antiepileptic drugs + 3-indazolinone.

2.5. NO synthase activity in brain preparations

NO synthase activity was determined in mouse brain areas from the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline, as described by Dwyer et al. (1991). Briefly, tissue samples were homogenized in 10 vol. of Tris–HCl-buffer (20 mM; pH 7.4) containing 2 mM EDTA, and centrifuged at 10,000 × *g* for 15 min (4°C). A 25-μl portion of the supernatant was added to test tubes containing Tris–HCl-buffer (20 mM; pH 7.4), NADPH (0.5 mM), $CaCl_2$ (1 mM), and either 1.0 μM L-[^{3}H]arginine, or various concentrations of L-[^{3}H]arginine (0.5–20 μM) in a final volume of 100 μl. Following incubation (15 min at 25°C), the reaction was terminated by the addition of 3 ml HEPES buffer (pH 5.5) containing 2 mM EDTA, and then applied onto 0.5 ml columns of Dowex AG50WX-8 (Na^+ form), followed by 0.5 ml distilled water. L-[^{3}H]citrulline was quantified by scintillation spectroscopy of 1-ml aliquots of the flow-through. To calculate the Ca^{2+}-dependent NO synthase activity, the counts from samples where Ca^{2+} was omitted, were subtracted from the total counts. Protein concentration was determined by the method of Lowry et al. (1951), and enzyme activity was expressed in terms of picomole per milligram protein per minute double reciprocal (Lineweaver–Burke) plots were generated (1/V vs. 1/S) to determine the V_{max} and K_m values of L-[^{3}H]arginine. The animals were killed 28–32 min after 7-nitroindazole or 3-indazolinone administration, when the effect of a single injection of these compounds on brain NO synthase activity was investigated; the brain areas were used for subsequent assay.

2.6. Brain content of noradrenaline and dopamine following 7-nitroindazole

For neurochemical determination, DBA/2 mice were killed 28–32 min after 7-nitroindazole, α-methyl-paratyrosine + 7-nitroindazole, 3-indazolinone or vehicle administration. The brains were removed on an ice-chilled plate, dissected, weighed and stored at −80°C until the assay. Catecholamine analysis was perfomed by using the HPLC method with some modifications (De Saint Blanquet et al., 1987). Briefly, 1 ml of tissue homogenate contained in polycarbonate tubes, was treated with the internal standard, dihydroxybenzilamine hydrobromide (DHBA), and 10 mg of activated alumina. After vortexing for 10 min, the supernatant was discarded and then it was

alumina washed three times with buffer Tris–HCl pH = 8.6, 0.1 M. Catecholamines were then separated from alumina by using 200 μl of $HClO_4$ 0.1 M. After centrifugation at 10,000 × *g* for 5 min at 4°C, the supernatant was recovered, filtered and a 50-μl aliquot was injected into the HPLC apparatus. The HPLC consisted of a solvent delivery module (Model 422 Master, Kontron Instruments, Everett, USA), linked with a coulometric electrochemical detector (Coulochem Model 5100A, ESA, Bedford, USA) and the conditioning cell (Mod. 5021, ESA) The detector was connected to an automatic integrator (Model CR-3A, Shimadzu, Kyoto, Japan). The column used was an HR-80, 80 × 4.6 mm, 3-μ particle size (ESA). The mobile phase was composed of 6.9 g monobasic sodium phosphate, 250 mg heptanesulfonic acid, 80 mg of EDTA in 900 ml of pure water. The pH was adjusted with phosphoric acid to pH = 3. Before filtering under vacuum, 100 ml of methanol was added. The flow rate was 1 ml min^{-1} at room temperature. Potentials of the detector were: conditioning cell +0.3 V; detector 1 0.00 V; detector 2 − 0.25 V. Calibration chromatograms of extracted standards of noradrenaline and dopamine were also run every time for peak identification and quantitation. Noradrenaline and dopamine were expressed as nanomole of fresh tissue.

2.7. Statistical analysis

Statistical comparisons between groups of control and drug-treated animals were made, using Fisher's exact probability test (incidence of the seizure phases) or analysis of variance (ANOVA) and Dunnett's test (rectal temperatures) or the Scheffé post hoc test (NO synthase activity, noradrenaline and dopamine brain levels). The percent incidence of each phase per dose of compound administered and dose–response curves, was fitted using linear regression analysis. ED_{50} values (with 95% confidence limits) for each compound and each phase of seizure response were estimated using a computer program of the method of Litchfield and Wilcoxon (1949); the relative anticonvulsant activities were determined by comparison of respective ED_{50} values. The lines of best fit of conventional antiepileptic drugs + vehicle or in association with 7-nitroindazole or 3-indazolinone, were compared using chi-squared analysis, with results expressed for position, parallelism and heterogeneity. TD_{50} values (with 95% confidence limits) for each compound were estimated using the method of Litchfield and Wilcoxon (1949). The plasma levels of the drugs are expressed as means ± S.E.M. of at least eight determinations. Student's *t*-test was used for statistical comparisons.

2.8. Drugs

The sources of the drugs used were: carbamazepine (Novartis, Basel, Switzerland), diazepam (Hoffmann-La Roche, Basel, Switzerland), sodium phenobarbital (Bracco, Milano, Italy), sodium phenytoin (Recordati, Milan, Italy), lamotrigine (Glaxo-Wellcome, Verona, Italy) and magnesium valproate (Sigma Tau, Pomezia, Italy). 7-Nitroindazole was purchased from RBI (Natick, MA, USA) and dissolved initially in dimethylsulfoxide (DMSO) to which was added sterile saline (final solution DMSO/saline 3:7 respectively). 3-Indazolinone was purchased from Aldrich (Milan, Italy). L-Arginine and α-methyl-paratyrosine were purchased from Sigma (St. Louis, MO, USA). For i.c.v. administration, the injection volume was 5 μl of L-arginine; this compound was dissolved in 10 mM phosphate buffered (pH = 7.4) saline (tablet form) (Sigma). All HPLC reagents were purchased from Merck (Milan, Italy).

3. Results

3.1. Anticonvulsant properties of 7-nitroindazole in DBA/2 mice

To allow better comparisons among the interactions of 7-nitroindazole and the different anticonvulsants used, we attempted to choose a time of 7-nitroindazole pretreatment that would be best for evaluation of anticonvulsant activity.

7-Nitroindazole (100, 125, 150 and 175 mg kg^{-1} i.p.) produced significant protection ($P < 0.05$) against the clonic and tonic phases of the audiogenic seizure response in DBA/2 mice 15 min after administration (Table 1). The doses of 150 and 175 mg kg^{-1} i.p. significantly reduced the incidence of the wild running phase. ED_{50} values (±95% confidence limits) for 7-nitroindazole are shown in Table 1. When the auditory test was carried out 30 min following 7-nitroindazole administration, significant protection ($P < 0.01$) against the clonic and tonic phases of the audiogenic seizure response in DBA/2 mice was observed after 75, 100, 125 and 150 mg kg^{-1}, i.p. In addition, 7-nitroindazole (150 mg kg^{-1} i.p.), administered 30 min before auditory testing, significantly protected against the wild running phase of the audiogenic seizures. The ED_{50} values (±95% confidence limits) for 7-nitroindazole are shown in Table 1. When the auditory test was carried out 60 min following 7-nitroindazole administration (150, 175 and 200 mg kg^{-1} i.p.), it produced

Table 1
ED_{50} values (±95% confidence limits) of the 7-nitroindazole effect on audiogenic seizures in DBA/2 mice following various pretreatment times All data were calculated according to the method of Litchfield and Wilcoxon (1949)

Pretreatment time (min)	Seizure phase	
	Tonus (mg kg^{-1})	Clonus (mg kg^{-1})
15	73.2 (57.5–93.3)	83.3 (64.3–107.8)
30	61.5 (50.2–75.4)	71.3 (58.9–86.3)
60	97.1 (82.7–114.1)	119.2 (101.8–139.7)

significant protection ($P < 0.01$) against the clonic and tonic phases of the audiogenic seizures in DBA/2 mice (Table 1). The doses of 175 and 200 mg kg^{-1} i.p. significantly reduced the incidence of the wild running phase. The ED_{50} values (±95% confidence limits) for 7-nitroindazole after 60 min pretreatment are shown in Table 1. The largest doses of 7-nitroindazole studied impaired locomotor activity in a dose-dependent manner. Ataxia, loss of righting reflex and a fall in rectal temperatures were evident following i.p. administration of 7-nitroindazole (100, 125, 150, 175 and 200 mg kg^{-1} i.p.). Since 7-nitroindazole exerted its maximal anticonvulsant activity at 30 min, we decided to use this pretreatment time for subsequent studies. In addition, based on previous results, all the anticonvulsants were administered 45 min before auditory testing (De Sarro et al., 1992, 1996).

3.2. Influence of 7-nitroindazole on the anticonvulsant activity of conventional antiepileptic drugs against audiogenic seizures

The influence of 7-nitroindazole on the activity of the conventional antiepileptic drugs on the audiogenic seizure response, varied according to the different classes of drugs. As shown in Tables 2 and 3, diazepam, carbamazepine, lamotrigine, phenobarbital, phenytoin and valproate (administered 45 min before testing) exhibited anticonvulsant activity in the audiogenic seizure model of DBA/2 mice. Pretreatment with conventional antiepileptic drugs (45 min before testing) and then with 7-nitroindazole (25 mg kg^{-1} i.p.) (30 min before testing) was sometimes able to produce a consistent shift to the left of the dose–response curves compared with concurrent groups; this suggests an increase in anticonvulsant activity. All dose–response curves were parallel except that of carbamazepine and valproate plus 7-nitroindazole. There was no significant heterogeneity; i.e. any residual variation was consistent with binomial sampling. The degree of potentiation by 7-nitroindazole varied among the anticonvulsant drugs, being greatest for diazepam and phenobarbital, less for valproate and least for phenytoin, lamotrigine and carbamazepine. In addition, 7-nitroindazole (25 mg kg^{-1} i.p.) was able to potentiate significantly the anticonvulsant activity of phenytoin against tonus but did not significantly affect the activity of the same drugs against clonus.

Table 2
ED_{50} values (±95% confidence limits) of vehicle + lamotrigine or vehicle + phenytoin and 7-nitroindazole (25 mg kg^{-1} i.p.) + lamotrigine or 7-nitroindazole (25 mg kg^{-1} i.p.) + phenytoin effect against the audiogenic seizures in DBA/2 mice
All data above reported are expressed in mg kg^{-1}.

Seizure phase	Test drug	Drug + vehicle (mg kg^{-1})	Drug + 7-nitroindazole (mg kg^{-1})
Wild running	lamotrigine	6.1 (4.6–8.1)	5.8 (5.5–8.4)
	phenytoin	4.3 (3.1–6)	3.4 (2.7–4.3)
Clonus	lamotrigine	3.5 (2.4–5.1)	2.8 (2.4–3.3)
	phenytoin	2.5 (1.8–3.5)	2.1 (1.8–2.4)
Tonus	lamotrigine	1.1 (0.7–1.8)	0.8 (0.6–1.1)
	phenytoin	2.0 (1.6–2.5)	1.0 (0.6–1.7)[a]

[a] Significant differences in the ED_{50} values between concurrent groups of vehicle + antiepileptic drug and 7-nitroindazole + antiepileptic groups are denoted by [a]$P < 0.01$ according to the method of Litchfield and Wilcoxon (1949).

3.3. Influence of L-arginine on the 7-nitroindazole-induced enhancement of the antiseizure activity of carbamazepine, phenobarbital, diazepam and valproate on audiogenic seizures

Treatment (25 min before testing) with L-arginine (30 μg/mouse, i.c.v.) did not affect either the anticonvulsant properties of carbamazepine, phenobarbital, diazepam and valproate or the 7-nitroindazole-induced increase in anticonvulsant activity of the same compounds against audiogenic seizures (Table 3).

3.4. Influence of 7-nitroindazole upon the motor impairment induced by antiepileptic drugs

When applied at doses equal to their ED_{50} values against the clonic phase of the audiogenic seizures, carbamazepine (4.4 mg kg^{-1}), diazepam (0.28 mg kg^{-1}), lamotrigine (3.5 mg kg^{-1}), phenytoin (2.5 mg kg^{-1}), phenobarbital (3.4 mg kg^{-1}) and valproate (43 mg kg^{-1}) did not influence the motor performance of DBA/2 mice. Higher doses were necessary to produce motor impairment. 7-Nitroindazole at dose levels up to 50 mg kg^{-1}, did not significantly affect locomotor performance. Concomitant treatment with valproate and 7-nitroindazole resulted in marked motor impairment. Considerable impairment of locomotor performance was also observed when 7-nitroindazole was administered with carbamazepine, diazepam, phenytoin, phenobarbital and lamotrigine. However, the therapeutic index of combined treatment with diazepam + 7-nitroindazole, lamotrigine + 7-nitroindazole, valproate + 7-nitroindazole or phenobarbital + 7-nitroindazole was more favourable than that with diazepam + vehicle, lamotrigine + vehicle, phenobarbital + vehicle or valproate + vehicle (Fig. 1).

3.5. Effects of combined treatment with 7-nitroindazole and antiepileptic compounds on body temperature

Body temperature was recorded in animals given anticonvulsant drugs + vehicle and anticonvulsant drugs + 7-nitroindazole. We observed hypothermic effects after administration of the highest doses of carbamazepine, diazepam and valproate. No significant differences among groups treated concomitantly with carbamazepine, diazepam, lamotrigine, phenytoin, phenobarbital or valproate

Table 3
Influence of vehicle, L-arginine (30 μg/mouse) administered i.c.v., 7-nitroindazole (25 mg kg^{-1} i.p.) and 7-nitroindazole + L-arginine on ED_{50} values (±95% confidence limits) of some antiepileptic drugs against the audiogenic seizures in DBA/2 mice
All data above reported are expressed in mg kg^{-1}.

Seizure phase and test drug	Drug + vehicle (mg kg^{-1})	Drug + L-arginine (mg kg^{-1})	Drug + 7-nitroindazole (mg kg^{-1})	Drug + 7-nitroindazole + L-arginine (mg kg^{-1})
Wild running				
Carbamazepine	10.6 (8.1–13.8)	9.8 (7.9–12.2)	8.8 (7.5–10.3)	9.3 (7.6–11.4)
Diazepam	0.49 (0.34–0.71)	0.44 (0.28–0.69)	0.24 (0.16–0.36)[a]	0.32 (0.21–0.49)
Phenobarbital	7.1 (5.6–9)	6.1 (5.2–7.2)	3.5 (2.3–5.3)[a]	3.7 (2.5–5.5)
Valproate	84 (63–114)	88 (61–127)	54 (39.1–74.6)	66 (37–117.7)
Clonus				
Carbamazepine	4.4 (3.6–5.4)	4.1 (3.2–5.2)	3.8 (2.4–6.0)	3.9 (2.6–5.85)
Diazepam	0.28 (0.2–0.39)	0.22 (0.16–0.3)	0.15 (0.13–0.18)[b]	0.18 (0.14–0.23)
Phenobarbital	3.4 (2.3–5)	3.1 (1.9–5.1)	2.0 (1.7–2.3)[b]	2.5 (1.8–3.47)
Valproate	43 (33–56)	43 (33–56)	25.1 (18.5–33.7)[b]	29.2 (18.9–45.1)
Tonus				
Carbamazepine	3.0 (2.6–3.8)	3.4 (2.6–4.4)	2.0 (1.3–3.1)	2.1 (1.4–3.1)
Diazepam	0.24 (0.15–0.39)	0.22 (0.16–0.30)	0.11 (0.09–0.13)[a]	0.15 (0.11–0.2)
Phenobarbital	2.4 (1.7–3.4)	2.6 (1.7–3.98)	1.1 (0.7–1.7)[a]	1.4 (0.8–2.45)
Valproate	31 (22–43)	30 (21–42.9)	15.4 (12.7–18.7)[a]	18.2 (13.1–25.3)

[a] Significant differences in the ED_{50} values among concurrent groups of antiepileptic drug + vehicle and antiepileptic drug + 7-nitroindazole groups are denoted by $P < 0.01$, using the method of Litchfield and Wilcoxon (1949).
[b] Significant differences in the ED_{50} values among concurrent groups of antiepileptic drug + vehicle and antiepileptic drug + 7-nitroindazole groups are denoted by $P < 0.05$, using the method of Litchfield and Wilcoxon (1949).

and vehicle or concurrent group treated with 7-nitroindazole were evident (data not shown).

3.6. Influence of 7-nitroindazole on the total and free plasma levels of antiepileptic drugs

The blood concentrations of carbamazepine, diazepam, lamotrigine, phenytoin, phenobarbital and valproate are presented in Table 4. The dose of 7-nitroindazole (25 mg kg^{-1} i.p.) studied did not significantly modify the plasma level of carbamazepine (15 mg kg^{-1} i.p.), lamotrigine (10 mg kg^{-1} i.p.), phenytoin (10 mg kg^{-1}, i.p.), phenobarbital (20 mg kg^{-1} i.p.), valproate (200 mg kg^{-1} i.p.) or diazepam (5 mg kg^{-1} i.p.).

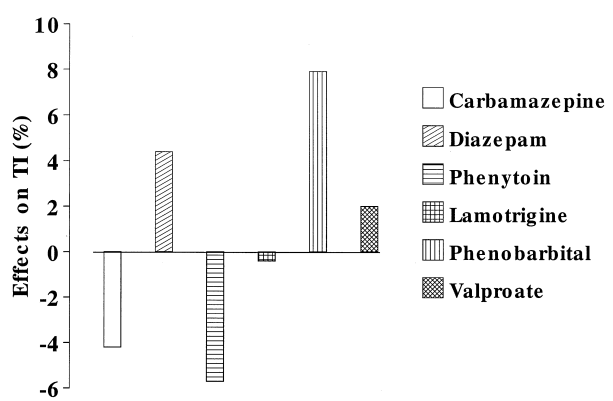

Fig. 1. Effects of a single administration of 7-nitroindazole (25 mg kg^{-1}, i.p.) in combination with some antiepileptics on changes in percentage incidence of therapeutic index (TI). Note that the combined treatments with 7-nitroindazole (25 mg kg^{-1}, i.p.) plus diazepam, phenobarbital or valproate resulted in a favourable therapeutic index, whereas a combination of 7-nitroindazole with carbamazepine, lamotrigine or phenytoin caused an increase of motor impairment.

3.7. Effects of 7-nitroindazole on brain NO synthase activity

Brain NO synthase activity was determined in the absence and presence (in vitro) of 7-nitroindazole and 30 min after a single i.p. administration of the drug (25 mg kg^{-1}). Initially, the effects of several concentrations of 7-nitroindazole (0.1–10 μM) were studied (data not shown). The IC_{50} value of 7-nitroindazole for the inhibition of the conversion of L-[^{3}H]arginine (1.0 μM) to L-[^{3}H]citrulline was calculated to be 1.25 ± 0.19 μM, in agreement with the IC_{50} value previously reported by Babbedge et al. (1993). We also found that all antiepileptics studied, at the highest dose level now used, did not significantly affect brain NO synthase activity (data not shown). The competitive type of NO synthase inhibition by 7-nitroindazole seems to be demonstrated by the finding that in presence of exogenously added 7-nitroindazole (1.0 μM), the K_i values for the enzymatic reaction were increased 2.3–2.5 times, with no apparent changes in V_{max} values (Table 5). The vehicle used to dissolve 7-nitroindazole had no significant effects on NO synthase kinetics. A single i.p. administration of 7-nitroindazole (25 mg kg^{-1}) induced a 2.5–2.9

Table 4
Influence of 7-nitroindazole (25 mg kg^{-1} i.p.) on total and free plasma levels of some antiepileptic compounds in DBA/2 mice
Antiepileptic compounds (carbamazepine, diazepam, lamotrigine, phenytoin, phenobarbital and valproate) were administered i.p., lamotrigine 45 min, carbamazepine and diazepam 45 and 60 min, phenobarbital 45, 60 and 120 min, phenytoin 45 and 120 min and valproate 30, 45 and 60 min before the test in association with vehicle or with 7-nitroindazole. Values are means (μg ml^{-1}) of at least eight determinations ± S.E.M. Student's *t*-test was used for statistical analysis of the data.

Treatment (time, min) (dose, mg kg^{-1})	Vehicle + compound		7-Nitroindazole + compound	
	Total (μg ml^{-1})	Free (μg ml^{-1})	Total (μg ml^{-1})	Free (μg ml^{-1})
Carbamazepine (45) (15)	6.5 ± 0.9	0.77 ± 0.2	6.6 ± 0.7	0.79 ± 0.2
Carbamazepine (60) (15)	5.2 ± 0.7	0.62 ± 0.2	5.3 ± 0.6	0.63 ± 0.2
Diazepam (45) (5)	2.8 ± 0.3	0.19 ± 0.07	2.8 ± 0.4	0.19 ± 0.07
Diazepam (60) (5)	2.1 ± 0.2	0.15 ± 0.05	2.1 ± 0.3	0.14 ± 0.05
Phenytoin (45) (10)	2.7 ± 0.6	0.3 ± 0.03	2.8 ± 0.6	0.3 ± 0.03
Phenytoin (120) (10)	8.8 ± 1.8	0.9 ± 0.1	8.9 ± 2.1	0.9 ± 0.1
Phenobarbital (45) (20)	39.2 ± 3.5	4.9 ± 0.3	38.5 ± 3.8	4.8 ± 0.4
Phenobarbital (60) (20)	35.3 ± 3.1	4.4 ± 0.3	34.7 ± 3.4	4.3 ± 0.4
Phenobarbital (120) (20)	22.4 ± 2.5	3.1 ± 0.3	22.1 ± 2.7	2.9 ± 0.3
Valproate (30) (200)	251 ± 22	40.2 ± 3.9	253 ± 27	43.7 ± 4.1
Valproate (45) (200)	276 ± 24	44.2 ± 4.2	278 ± 29	48.1 ± 4.5
Valproate (60) (200)	309 ± 29	49.4 ± 4.1	311 ± 36	49.6 ± 4.0
Lamotrigine (45) (10)	1.8 ± 0.2	0.67 ± 0.07	1.8 ± 0.3	0.71 ± 0.08

times increase in the K_i values with no apparent change in the V_{max} values (Table 5). The degree of inhibition, expressed as a percentage of the control, differed in all brain areas. Cerebellar NO synthase activity was significantly more inhibited than the same enzymatic activity in the diencephalon, cortex and brainstem (Fig. 2).

Table 5
Effects of 7-nitroindazole on the kinetic parameter of brain NO synthase
The conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline was determined in cortical, diencephalon, brainstem and cerebellar preparation as described in Materials and methods. The V_{max} values were derived from double reciprocal (Lineweaver–Burke) plots and represent the means ± S.E.M. of five to six determinations. ''Vehicle'' indicates the solvent for the preparation of 7-nitroindazole solution: DMSO/saline 3:7 , respectively.

Brain area	Treatment	V_{max} (pmol/mg protein min)
Cortex	control	80 ± 8
	+ vehicle	82 ± 8
	+ 7-nitroindazole (1.0 μM; in vitro)	77 ± 8
	+ 7-nitroindazole (25 mg kg^{-1}; in vivo)	89 ± 10
Diencephalon	control	78 ± 7
	+ vehicle	81 ± 9
	+ 7-nitroindazole (1.0 μM; in vitro)	78 ± 8
	+ 7-nitroindazole (25 mg kg^{-1}; in vivo)	84 ± 8
Brainstem	control	77 ± 8
	+ vehicle	80 ± 9
	+ 7-nitroindazole (1.0 μM; in vitro)	77 ± 8
	+ 7-nitroindazole (25 mg kg^{-1}; in vivo)	83 ± 8
Cerebellum	control	79 ± 6
	+ vehicle	82 ± 8
	+ 7-nitroindazole (1.0 μM; in vitro)	76 ± 6
	+ 7-nitroindazole (25 mg kg^{-1}; in vivo)	84 ± 7

3.8. Effects of 3-indazolinone in DBA/2 mice

3-Indazolinone (25, 50, 100, 125, 150, 175 and 200 mg kg^{-1} i.p.), did not produce significant protection against all the phases of the audiogenic seizure response in DBA/2 mice 30 min after i.p. administration. The largest doses of 3-indazolinone studied impaired locomotor activity in a dose-dependent manner. Ataxia, loss of righting reflex and a fall in rectal temperature were evident following i.p. administration of 3-indazolinone (150, 175 and 200 mg

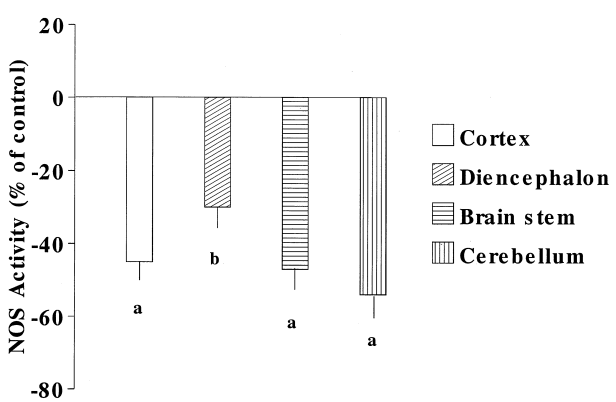

Fig. 2. Effects of a single administration of 7-nitroindazole (25 mg kg^{-1}, i.p.) on percent inhibition of mouse brain nitric oxide synthase activity. Results are means ± S.E.M., $n = 5–6$. Statistical significance is denoted by $^aP < 0.01$ and $^bP < 0.05$.

kg^{-1} i.p.). Since 3-indazolinone failed to inhibit significantly, mouse brain NO synthase activity at a concentration of 25 mg kg^{-1} after 30 min, we decided to use this dose and a 30 min pretreatment time for subsequent studies. In addition, as in previous studies, all the anticonvulsants were administered 45 min before auditory testing (De Sarro et al., 1992, 1996).

3.9. Influence of 3-indazolinone upon the anticonvulsant activity of conventional antiepileptic drugs against audiogenic seizures

3-Indazolinone did not significantly affect the activity of the conventional antiepileptic drugs on the audiogenic seizures. Pretreatment with some conventional antiepileptic (administered 45 min before testing) and then with 3-indazolinone (25 mg kg^{-1} i.p.) (30 min before testing) was unable to produce significant changes in ED_{50} values of groups receiving antiepileptics + 3-indazolinone when compared with the related groups.

3.10. Effects of 7-nitroindazole on noradrenaline and dopamine brain levels

Acute administration of 3-indazolinone 25 or 50 mg kg^{-1} i.p. did not produce any change in the content of noradrenaline or dopamine in any brain area studied (data not shown). Pretreatment (30 min before) with 7-nitroindazole (12.5, 25 or 50 mg kg^{-1}) was able to increase the level of noradrenaline and dopamine in comparison to those in vehicle and 3-indazolinone groups (Table 6). Examination of brain samples obtained from diencephalon revealed that 7-nitroindazole 25 and 50 mg kg^{-1} when acutely administered, caused a significant increase of both noradrenaline and dopamine in this region. This effect was more evident following 7-nitroindazole, 100 mg kg^{-1} (data not shown). In the brainstem and cortex, 7-nitroindazole produced a non-significant and non-dose-dependent increase in noradrenaline and dopamine content (Table 6). Pretreatment, 2 h before, with α-methyl-paratyrosine (125 or 250 mg kg^{-1}) was able to

Table 6
Effects of acute administration of 7-nitroindazole, α-methyl-paratyrosine + 7-nitroindazole, 3-indazolinone or vehicle on the content of noradrenaline (NA) and dopamine (DA) in cortex, diencephalon and brainstem of the DBA/2 mice
Data analysis was performed using one-way ANOVA with the Scheffé post hoc test for multiple comparisons. Each group was composed of six animals. Data are expressed as the means ± S.E.M.

Brain area	Treatment (mg kg^{-1})	NA (nmol g^{-1})	DA (nmol g^{-1})
Cortex	vehicle	10.13 ± 2.1	106.60 ± 6.0
	7-nitroindazole (12.5)	10.39 ± 1.6	110.63 ± 5.7
	7-nitroindazole (25)	11.12 ± 1.8	109.41 ± 6.1
	7-nitroindazole (50)	11.45 ± 1.8	110.62 ± 6.6
	3-indazolinone (25)	10.64 ± 1.4	110.41 ± 5.3
	3-indazolinone (50)	10.91 ± 1.6	111.73 ± 5.8
	α-methyl-paratyrosine (125)	8.39 ± 1.6	91.17 ± 9.1
	α-methyl-paratyrosine (125) + 7-nitroindazole (50)	9.16 ± 1.8	101.14 ± 9.5
	α-methyl-paratyrosine (250)	6.18 ± 2.0[a]	71.47 ± 8.3[a]
	α-methyl-paratyrosine (250) + 7-nitroindazole (50)	8.34 ± 1.9[b]	81.55 ± 8.5[b]
Diencephalon	vehicle	22.42 ± 1.1	11.20 ± 0.89
	7-nitroindazole (12.5)	23.64 ± 1.0	11.72 ± 0.74
	7-nitroindazole (25)	27.91 ± 1.1[b]	15.32 ± 0.81[b]
	7-nitroindazole (50)	31.15 ± 0.9[a]	18.88 ± 0.80[a]
	3-indazolinone (25)	22.43 ± 15	11.00 ± 0.62
	3-indazolinone (50)	23.85 ± 1.2	11.09 ± 0.70
	α-methyl-paratyrosine (125)	11.2 ± 3.4[a]	9.3 ± 1.61
	α-methyl-paratyrosine (125) + 7-nitroindazole (50)	13.6 ± 3.8[a]	10.45 ± 1.35
	α-methyl-paratyrosine (250)	9.18 ± 2.9[a]	5.8 ± 0.99[a]
	α-methyl-paratyrosine (250) + 7-nitroindazole (50)	11.28 ± 4.0[a]	8.12 ± 1.16[b]
Brainstem	vehicle	18.78 ± 1.1	2.96 ± 0.26
	7-nitroindazole (12.5)	18.45 ± 0.7	2.91 ± 0.24
	7-nitroindazole (25)	19.20 ± 0.8	2.95 ± 0.27
	7-nitroindazole (50)	19.77 ± 0.6	2.97 ± 0.20
	3-indazolinone (25)	18.31 ± 0.6	2.88 ± 0.18
	3-indazolinone (50)	18.94 ± 0.6	2.91 ± 0.12
	α-methyl-paratyrosine (125)	12.92 ± 1.02[a]	1.67 ± 0.24[a]
	α-methyl-paratyrosine (125) + 7-nitroindazole (50)	13.5 ± 3.2[b]	2.34 ± 0.26
	α-methyl-paratyrosine (250)	11.4 ± 2.9[a]	1.41 ± 0.22[a]
	α-methylparatyrosine (250) + 7-nitroindazole (50)	12.8 ± 3.4[b]	1.77 ± 0.27[b]

[a] Statistical significance was set at $P < 0.01$ vs. vehicle.
[b] Statistical significance was set at $P < 0.05$ vs. vehicle.

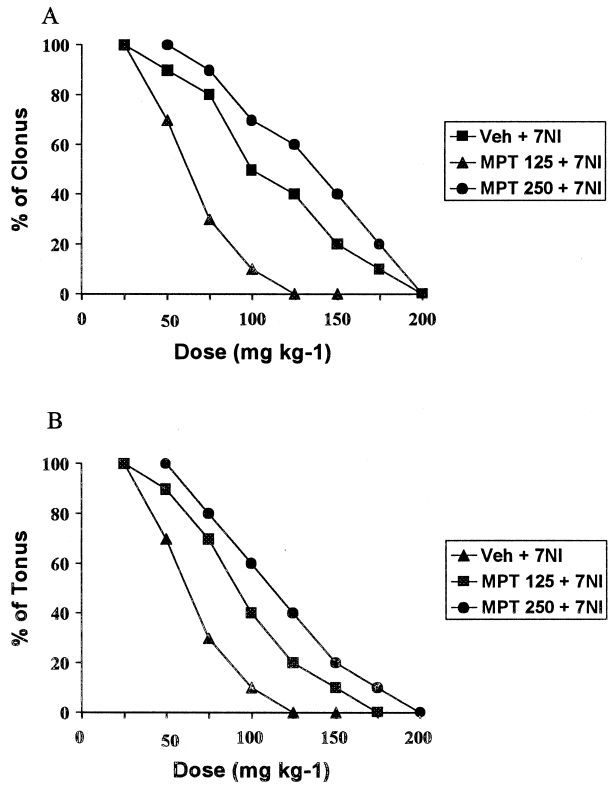

Fig. 3. Effects of pretreatment with α-methyl-paratyrosine (MPT, 125 or 250 mg kg^{-1} i.p.) on dose–response curve for 7-nitroindazole administered 30 min before auditory testing in DBA/2 mice. A% clonus; B% tonus. Pretreatment with MPT (125 mg kg^{-1} i.p.) increased the ED_{50} values for clonus to 97.21 (79.21–119.29) and for tonus to 84.67 (68.65–104.41). Pretreatment with MPT (250 mg kg^{-1} i.p.) increased the ED_{50} values for clonus to 125.75 (104.19–146.17) an for tonus to 108.27 (91.19–128.54).

antagonize both anticonvulsant properties of 7-nitroindazole (Fig. 3) and the increase in noradrenaline and dopamine brain content (Table 6).

4. Discussion

The present results clearly demonstrate that 7-nitroindazole, at doses which do not significantly affect or slightly influence the audiogenic seizures in DBA/2 mice, enhances the anticonvulsant properties of diazepam, phenobarbital, valproate and those of phenytoin against tonus in this strain of mice. The present data are in part, agreement with those of Borowicz et al. (1997) who found that 7-nitroindazole strongly potentiated the anticonvulsant activity of phenobarbital, while they did not find a significant enhancement of anticonvulsant effects of carbamazepine, phenytoin and valproate when these antiepileptics were administered in conjunction with 7-nitroindazole. The partial discrepancy in these results could be accounted for by the different mechanisms for inducing seizures, i.e. electroshock instead of a genetic model of reflex epilepsy.

According to several reports, NO may exert either anticonvulsant or proconvulsant activity in various animal models of epilepsy. Results of earlier studies suggested an involvement of NO in seizures induced by focal microinjection of NMDA into the deep prepiriform cortex. In fact, seizures were prevented by the local pretreatment with NO synthase inhibitors (De Sarro et al., 1991, 1993). Mollace et al. (1991) observed a proconvulsive effect of i.c.v. administered L-arginine, the precursor of NO, on NMDA-induced seizures; this effect was reversed by L-NAME. Subsequently, Smith et al. (1996) demonstrated that L-arginine, at a dose of 300 μg i.c.v., was anticonvulsant, while at a dose of 1000 μg i.c.v. it became convulsant. In seizures induced by other chemoconvulsants in rodents, NO synthase inhibitors have both anticonvulsant and proconvulsant actions (Osonoe et al., 1994; Penix et al., 1994). Apparent anticonvulsant activity of 7-nitroindazole was described against pilocarpine-induced seizures (Van Leeuwen et al., 1995) in DBA/2 mice and genetically epilepsy-prone rats (Smith et al., 1996) and kainic acid-induced seizures in rats (Mülsch et al., 1994). 7-Nitroindazole and L-NAME appeared to have only a weak anticonvulsant action against seizures induced by i.c.v. injection of NMDA in mice. Both compounds had a tendency to delay the onset of seizures (Eblen et al., 1996) and the occurrence of tonic extension following injection of pentylenetetrazole in rats (Kirkby et al., 1996). Wiley et al. (1997) showed that 7-nitroindazole inhibited acoustic startle responses, but the dose of 7-nitroindazole (25 mg kg^{-1}), used in the present study, was found unable to induce a reduction of auditory thresholds.

It was reported that the treatment of cultured neuronal cells with L-NOARG significantly augmented the NMDA- and kainate-induced increase in intracellular Ca^{2+} (Tanaka et al., 1993). Consequently, the endogenous NO would inhibit NMDA- and kainate-induced increase in intracellular Ca^{2+} as a negative feedback system independent of guanylate cyclase activation. It is interesting to note that the NO synthase inhibitors decreased the release of NO and the levels of cGMP without affecting the release of excitatory amino acids (Lizasoain et al., 1995). On the other hand, the stimulation of NMDA receptors results in an influx of Ca^{2+} followed by activation of Ca^{2+}/calmodulin-dependent NO synthase enzyme. It was postulated that NO alone is responsible for the increase of cGMP level as a result of the NMDA receptor stimulation (Lizasoain et al., 1995). Several studies showed that NO released upon NMDA receptor stimulation might subsequently modulate NMDA receptor function through the redox site (Theard et al., 1995). NO oxidises some redox site within the NMDA receptor complex and inhibits an influx of Ca^{2+} as a negative feedback system (Kutsuwada et al., 1992; Lipton et al., 1993). This supports the hypothesis that central NO might play an anticonvulsant role in CNS. Many results, however, supported a convulsant role for NO in epilepsy: kindling produces a long-lasting in-

crease in NO synthase activity (Al-Ghoul et al., 1995). Direct administration of NO (330–800 μmol) into the rat brain has been attempted, resulting in brief tonic convulsive episodes (Smith et al., 1991), while 3-morpholinosydnonimine (SIN-1), a NO donor, is convulsant in DBA/2 mice and genetically epilepsy-prone rats (Smith et al., 1996).

In addition, L-NOARG induces an enhancement of the kindling processes (Rondouin et al., 1992, 1993) and of the severity of kindled seizures (Herberg et al., 1995) or increases the severity of ferric chloride, kainate or bicuculline-induced seizures or quinolinate-induced neurotoxicity in rats (Haberny et al., 1992; Mülsch et al., 1994; Penix et al., 1994; Przegalinski et al., 1994; Wang et al., 1994; Maggio et al., 1995; Kabuto et al., 1996).

The problem with the use of non-selective inhibitors of NO in the CNS experiments was demonstrated in the study of Rundfeldt et al. (1995); the threshold for seizures after cortical electrical stimulation in rats was increased by L-NOARG (1–10 mg kg^{-1} i.p.), while L-NOARG (40 mg kg^{-1}, i.p.) decreased the threshold for seizures. These discrepancies might result from the different actions of 7-nitroindazole, L-NAME and other NO synthase inhibitors that could be unrelated to brain NO synthase inhibition. Furthermore, although most of the reports demonstrated that 7-nitroindazole did not affect peripheral functions, a few demonstrated that 7-nitroindazole can also affect endothelial NO synthase (Fabricius et al., 1996) and that pretreatment with L-arginine inhibits the peripheral effects of 7-nitroindazole (50 mg kg^{-1} i.p.), suggesting an involvement of the L-arginine pathway in the effects of this selective NO synthase inhibitor (Zagvazdin et al., 1996). Several reports by Moore and colleagues seem to indicate that the dose of 7-nitroindazole used in the present study affects principally neuronal NO synthase (Moore and Handy, 1996; Moore et al., 1993a,b; Handy and Moore, 1998).

These apparently divergent data do not, however, exclude either that NO might play a different role in various models of epileptic disorders or that it may act as an anticonvulsant or as a proconvulsant agent, depending on the experimental procedures and/or particular brain structures, as recently suggested by Libri et al. (1997). The lack of effects of 3-indazolinone on antiseizure activity of conventional antiepileptics suggests that the increase of anticonvulsant activity is related to the pharmacological activity of 7-nitroindazole. The impairment of NO synthase induced by 7-nitroindazole may not be solely responsible for the increase of antiseizure activity observed with some anticonvulsant drugs because its effects were not reversed by L-arginine. Therefore, the effects of 7-nitroindazole on antiseizure activity of conventional antiepileptics could be the result of various pharmacological actions. There is a great body of evidence showing that excitatory amino acid antagonists themselves possess anticonvulsant efficacy in various models of epilepsy (Meldrum, 1984, 1995; Dingledine et al., 1991; Rogawski, 1992; MacDonald and Meldrum, 1995) and that NO is able to modulate the presynaptic release of excitatory amino acids (Theard et al., 1995). In this context, the lowering of the NO level by 7-nitroindazole would impair the negative feedback system within the NMDA receptor complex which could, in turn, lead to the lack of significant additive anticonvulsant activity of some antiepileptic drugs (carbamazepine, lamotrigine and sometimes phenytoin).

The additive effects of 7-nitroindazole on anticonvulsant activity and the effects of lamotrigine on locomotor activity could be expected. In fact, in a previous report lamotrigine seemed to be able to inhibit the level of GMP without affecting the NO synthase activity but did reduce the release of NO (Lizasoain et al., 1995). On the other hand, the anticonvulsant properties of diazepam, phenobarbital and valproate which act by enhancing γ-aminobutyric acid GABAergic transmission (Meldrum, 1996) were potentiated by the concomitant administration of 7-nitroindazole. This is in agreement with previous results which showed that 7-nitroindazole was able to potentiate the GABA brain accumulation induced by aminoxyacetic acid, a compound which acts by enhancing GABAergic neurotransmission (Löscher et al., 1991). Another recent study by Volke et al. (1997) showed that 7-nitroindazole had potent anxiolitic effects which might affect both anticonvulsant activity and locomotor impairment caused by diazepam and phenobarbital. In the present study, 7-nitroindazole appeared more active to enhance anticonvulsant properties than the locomotor impairment (Fig. 1).

A pharmacokinetic interaction does not seem to be responsible for the potentiation by 7-nitroindazole of the antiseizure effects of the anticonvulsant drugs studied. In fact, we found no effect of 7-nitroindazole on the total and free plasma levels of carbamazepine, diazepam, lamotrigine, phenytoin, phenobarbital and valproate. However, the present data do not exclude the possibility that (1) 7-nitroindazole modifies the time course of the anticonvulsants which penetrate the brain or (2) the clearance of some antiepileptics. In addition, we have demonstrated, following a single administration of 7-nitroindazole, that this compound has different effects on the antiseizure activity of the anticonvulsants.

The first hypothesis appears unlikely to hold, since 7-nitroindazole (25 mg kg^{-1} i.p.) did not significantly modify the changes in body temperature induced by the antiepileptic drugs studied.

Furthermore, 7-nitroindazole 25 mg kg^{-1}, in combination with phenobarbital and diazepam, caused some motor impairment but still showed a favourable therapeutic index (Fig. 1), whereas a combination of 7-nitroindazole and phenytoin or carbamazepine caused an increase in motor impairment. It was also shown that 7-nitroindazole per se caused significant motor impairment (Smith et al., 1996) and we observed, as previously reported (Borowicz et al., 1997), that 7-nitroindazole potentiated the motor distur-

bances induced by antiepileptic drugs in mice. These results support the hypothesis that continued activity of constitutive NO synthase is necessary for normal body movements (Starr and Starr, 1995).

7-Nitroindazole shows a new and different mechanism of action in comparison to those of the conventional antiepileptic drugs used in the present study. In particular, it appears to act via specific mechanisms (i.e. inhibition of NO brain synthase and increase of cerebral level of L-arginine) and/or as recently suggested, it may alter seizures NO-independently, suppressing the stereochiometric formation of L-citrulline and promoting the accumulation of L-arginine (Smith et al., 1996). Nevertheless, the influence of 7-nitroindazole upon carbamazepine, phenobarbital, diazepam and valproate was not reversed by L-arginine so the participation of NO in this particular effect of 7-nitroindazole might be questionable.

7-Nitroindazole was able to increase both dopamine and noradrenaline contents and this effect was particularly significant in the diencephalon. Such an effect on catecholamines appears quite important in our study. In fact, the audiogenic seizures in DBA/2 mice seem to be particularly sensitive to monoamine pharmacological manipulations. Previous data showed that increasing dopamine and/or noradrenaline levels exerted anticonvulsant activity (Dailey and Jobe, 1984; Chapman and Meldrum, 1987), while reserpine, which depletes stores of monoamines (Lehmann, 1977) and α-methyl-paratyrosine, which inhibits synthesis of catecholamines, facilitated audiogenic seizures in DBA/2 mice (Schlesinger et al., 1970). In addition, a body of data suggests a greater role for dopamine than for noradrenaline as anticonvulsant in such an epileptic model (Chapman and Meldrum, 1987; Jobe et al., 1991; Buchhalter, 1993). The dose of 7-nitroindazole now used was able to increase the dopamine and the noradrenaline levels with a more evident effect in diencephalon. The fact that α-methyl-paratyrosine reversed the anticonvulsant effects of 7-nitroindazole further confirms our hypothesis. The effects of 7-nitroindazole on dopamine and noradrenaline brain concentrations are smaller than those obtained with monoamine oxidase inhibitors (see Dailey and Jobe, 1984; Chapman and Meldrum, 1987), which could be due to various actions of 7-nitroindazole. The latter compound could induce a dopamine and a noradrenaline increase by its monoamine oxidase inhibitor action. On the other hand, 7-nitroindazole could decrease dopaminergic transmission in the striatum by its inhibitory action on NO synthase because NO has been reported to facilitate dopamine release in in vitro experiments (Zhu and Luo, 1992; Black et al., 1994) and in vivo microdialysis studies (Strasser et al., 1994). However, more recent studies have shown that NO synthase inhibitors also facilitate the striatal release of dopamine (Shibata et al., 1996; West and Galloway, 1997). Indeed, other studies have clearly demonstrated that both NO synthase and monoamine oxidase-B inhibitory effects of 7-nitroindazole are likely to contribute to the pharmacological changes induced by 7-nitroindazole and some other NO synthase inhibitors (Castagnoli et al., 1997; Di Monte et al., 1997; Desvignes et al., 1999). We observed in vivo, in the diencephalon, an increase of dopaminergic and perhaps noradrenergic contents similar to those already described (Silva et al., 1995; Castagnoli et al., 1997; Di Monte et al., 1997; Desvignes et al., 1999). The effects obtained with 7-nitroindazole are of special importance for studies on the role of NO on catecholaminergic transmission, using 7-nitroindazole as a specific neuronal NO synthase inhibitor. The inhibitory activity of 7-nitroindazole on monoamine oxidase-B seems to be an important target for the pharmacological development of new indazole derivatives similar to 7-nitroindazole. It could be suggested that the observed increase in antiseizure activity of some antiepileptics, administered concomitantly with 7-nitroindazole, might be related to the additional or synergic effects elicited by drugs acting with different mechanism of actions.

Finally, the present experimental data, showing a potentiation of effects of some conventional antiepileptic agents induced by 7-nitroindazole in DBA/2 mice, suggest further investigation, in other models of epilepsy.

Acknowledgements

This research was supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST 60%). Our thanks to Dr. Rodolfo Testa (Recordati Labs) and Sigma Tau Labs for generous supply of sodium phenytoin and magnesium valproate.

References

Al-Ghoul, W.M., Meeker, R.B., Greenwood, R.S., 1995. Kindling induces a long-lasting increase in brain nitric oxide synthase activity. NeuroReport 6, 457–460.

Babbedge, R.C., Bland-Ward, P.A., Hart, S.L., Moore, P.K., 1993. Inhibition of rat cerebellar nitric oxide synthase by 7-nitroindazole and related substituted indazoles. Br. J. Pharmacol. 110, 225–228.

Black, M.D., Matthews, E.K., Humphrey, P.P.A., 1994. The effects of a photosensitive nitric oxide donor on basal and electrically-stimulated dopamine afflux from the rat striatum in vitro. Neuropharmacology 33, 157–1365.

Bohme, G.A., Bon, C., Stutzman, J.M., Doble, A., Blanchard, J.C., 1991. Possible involvement of nitric oxide in long-term potentiation. Eur. J. Pharmacol. 19, 379–381.

Borowicz, K.K., Kleinrok, Z., Czuczwar, S.J., 1997. Influence of 7-nitroindazole on the anticonvulsive action of conventional antiepileptic drugs. Eur. J. Pharmacol. 331, 127–132.

Bredt, D.S., Snyder, S.H., 1989. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. Proc. Natl. Acad. Sci. U.S.A. 86, 9030–9033.

Bredt, D.S., Snyder, S.H., 1991. Nitric oxide as a neuronal messenger. Trends Pharmacol. Sci. 12, 125–128.

Bredt, D.S., Snyder, S.H., 1992. Nitric oxide, a novel neuronal messenger. Neuron 8, 3–11.

Bredt, D.S., Snyder, S.H., 1994. Nitric oxide: a physiologic messenger molecule. Annu. Rev. Biochem. 63, 175–195.

Buchhalter, J.R., 1993. Animal models of inherited epilepsy. Epilepsia 34, S31–S41.

Buisson, A., Lackhmeche, N., Verrecia, C., Plotkine, M., Bolou, R.G., 1993. Nitric oxide: an endogenous anticonvulsant substance. NeuroReport 4, 444–446.

Castagnoli, K., Palmer, S., Anderson, A., Bueters, T., Castagnoli, N. Jr., 1997. The neuronal nitric oxide synthase inhibitor 7-nitroindazole also inhibits the monoamine oxidase-B-catalyzed oxidation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Chem. Res. Toxicol. 10, 364–368.

Chapman, A.G., Meldrum, B.S., 1987. Epilepsy-prone mice: genetically determined sound-induced seizures. In: Jobe, P.C., Laird, H.E. III (Eds.), Neurotransmitters and Epilepsy. Humana Press, Clifton, NJ, pp. 9–40.

Dailey, J.W., Jobe, P., 1984. Effect of increments in the concentration of dopamine in the central nervous system on audiogenic seizures in DBA/2J mice. Neuropharmacology 23, 1019–1024.

Dawson, T.M., Snyder, S.H., 1994. Gases as biological messengers: nitric oxide and carbon monoxide in the brain. J. Neurosci. 14, 5147–5159.

De Saint Blanquet, G., Lambouef, Y., Fritsch, P., 1987. Determination of catecholamines in biological tissues by liquid chromatography with coulometric detection. J. Chromatog. 415, 388–392.

De Sarro, G.B., Ammendola, D., Nava, F., De Sarro, A., 1995. Effects of some excitatory amino acid antagonists on imipenem-induced seizures in DBA/2 mice. Brain Res. 671, 131–140.

De Sarro, G.B., Croucher, M.J., Meldrum, B.S., 1984. Anticonvulsant actions of DS 103-282: pharmacological studies in rodents and the baboon, *Papio papio*. Neuropharmacology 23, 526–530.

De Sarro, G.B., De Sarro, A., Trimarchi, G.R., Nisticò, G., 1992. Effects of some calcium antagonists upon the activity of common antiepileptic compounds on sound-induced seizures in DBA/2 mice. Gen. Pharmacol. 23, 75–82.

De Sarro, G.B., Donato Di Paola, E., De Sarro, A., Vidal, M.J., 1991. Role of nitric oxide in the genesis of excitatory amino acid-induced seizures from the deep prepiriform cortex. Fundam. Clin. Pharmacol. 5, 503–511.

De Sarro, G.B., Donato Di Paola, E., De Sarro, A., Vidal, M.J., 1993. L-Arginine potentiates excitatory amino acid-induced seizures elicited in the deep prepiriform cortex. Eur. J. Pharmacol. 230, 151–158.

De Sarro, G.B., Nava, F., Aguglia, U., De Sarro, A., 1996. Lamotrigine potentiates the antiseizure activity of some anticonvulsants in DBA/2 mice. Neuropharmacology 35, 153–158.

De Sarro, G.B., Ongini, E., Bertorelli, R., Aguglia, U., De Sarro, A., 1994. Excitatory amino acid neurotransmission through both NMDA and non-NMDA receptors is involved in the anticonvulsant activity of felbamate in DBA/2 mice. Eur. J. Pharmacol. 262, 11–19.

Desvignes, C., Bert, L., Vinet, L., Denory, L., Renaud, B., Lambas-Senas, L., 1999. Evidence that neuronal oxide synthase inhibitor 7-nitroindazole inhibits monoamine oxidase in rat: in vivo effects on extracellular striatal dopamine and 3,4-dihydrophenilacetic acid. Neurosci. Lett. 264, 5–8.

Di Monte, D.A., Royland, J.E., Anderson, A., Castagnoli, K., Castagnoli, N., Langston, W., 1997. Inhibition of monoamine oxidase contributes to the protective effects of 7-nitroindazole against MPTP neurotoxicity. J. Neurochem. 69, 1771–1773.

Dingledine, R., Mc Bain, C.J., Mc Namara, J.O., 1991. Excitatory amino acid receptors in epilepsy. Trends Pharmacol. Sci. 11, 49–53.

Dunham, N.W., Miya, T.S., 1957. A note on a simple apparatus for detecting neurological deficit in rats and mice. J. Am. Pharm. Assoc. 46, 208–209.

Dwyer, M.A., Bredt, D.S., Snyder, S.H., 1991. Nitric oxide synthase: irreversible by the L-N^g-nitroarginine in brain in vitro and in vivo. Biochem. Biophys. Res. Commun. 176, 1136–1141.

Eblen, F., Loschmann, P.A., Wulner, U., Turski, L., Klockgether, T., 1996. Effects of 7-nitroindazole, N^g-L-arginine and CPPene on harmaline-induced postural tremor, *N*-methyl-D-aspartate seizures and lisuride-induced rotations in rat with nigral 6-hydroxydopamine lesions. Eur. J. Pharmacol. 299, 9–16.

Fabricius, M., Rubin, I., Bundgaard, M., Lauritzen, M., 1996. NOS activity in brain and endothelium: reaction to hypercapnic rise of cerebral blood flow in rats. Am. J. Physiol. 271, H2035–H2044.

Garthwaite, J., 1991. Glutamate, nitric oxide and cell–cell signalling in the nervous system. Trends Neurosci. 14, 60–67.

Garthwaite, J., 1993. Nitric oxide signalling in the nervous system. Semin. Neurosci. 5, 171–180.

Garthwaite, J., Charles, S.L., Chess-Williams, R., 1988. Endothelium-derived relaxing factor release on activation of NMDA receptor suggests a role as intercellular messenger in the brain. Nature 336, 385–388.

Haberny, K.A., Pou, S., Eccles, C.U., 1992. Potentiation of quinolinate-induced hippocampal lesions by inhibition of NO synthesis. Neurosci. Lett. 146, 187–190.

Handy, R.L.C., Moore, P.K., 1998. Handy and Moore reply. Trends Pharmacol. Sci. 19, 350.

Hara, S., Kuriiwa, F., Iwata, N., Mukai, T., Kano, S., Endo, T., 1996. Distinct effects of N^{ω}-nitro-L-arginine on seizures induced by several drugs in mice. Pharmacol. Biochem. Behav. 53, 673–677.

Herberg, L.J., Grottick, A., Rose, I.C., 1995. Nitric oxide synthesis, epileptic seizures and kindling. Psychopharmacology 119, 115–123.

Ito, M., Karachot, L., 1991. Messengers mediating long-term desensitization in cerebellar Purkinje cells. NeuroReport 1, 129–132.

Jobe, P., Mishra, P., Ludvig, N., Dailey, J.W., 1991. Scope and contribution of genetic models to an understanding of the epilepsies. Crit. Rev. Neurobiol. 6, 183–220.

Kabuto, H., Yokoi, I., Habu, H., Willmore, L.J., Mori, A., Ogawa, N., 1996. Reduction in nitric oxide synthase activity with development of an epileptogenic focus induced by ferric chloride in the rat brain. Epilepsy Res. 25, 65–68.

Kelly, P.A., Ritchie, I.M., Arbuthnott, G.W., 1995. Inhibition of neuronal nitric oxide synthase by 7-nitroindazole: effects upon local cerebral blood flow and glucose use in rat. J. Cereb. Blood Flow Metab. 15, 766–773.

Kirkby, R.D., Carroll, D.M., Grossman, A.B., Subramaniam, S., 1996. Factors determining proconvulsant and anticonvulsant effects of inhibitors of nitric oxide synthase in rodents. Epilepsy Res. 24, 91–100.

Klockgether, T., Turski, L., 1990. NMDA antagonists potentiate antiparkinsonian actions of L-dopa in monoamine-depleted rats. Ann. Neurol. 28, 539–544.

Knowles, R.G., Palacios, M., Palmer, R.M.J., Moncada, S., 1989. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. Proc. Natl. Acad. U.S.A. 89, 5159–5162.

Kutsuwada, T., Kashiwabuchi, M., Mori, H., Sakimura, K.E., Kushiya, E., Araki, K., Mehuro, H., Masaki, H., Kumanishi, T., Arakawa, M., Mishina, M., 1992. Molecular diversity of the NMDA receptor channel. Nature 358, 36–41.

Lehmann, A., 1977. Mechanisms underlying modifications in the severity of audiogenic convulsions. Life Sci. 20, 2047–2059.

Libri, V., Santarelli, R., Nisticò, S., Azzena, G., 1997. Inhibition of nitric oxide synthase prevents magnesium-free-induced epileptiform activity in guinea-pig piriform cortex neurones in vitro. Naunyn–Schmiedeberg's, Arch. Pharmacol. 355, 452–456.

Lipton, S.A., Choi, Y.B., Pan, Z.H., Lei, S.Z., Chen, H.S., Sucher, N.J., Loscalzo, J., Singel, D.J., Stamler, J.S., 1993. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364, 626–632.

Litchfield, J.T., Wilcoxon, F., 1949). A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Lizasoain, I., Knowles, R.G., Moncada, S., 1995. Inhibition by lamotrigine of the generation of nitric oxide in rat forebrain slices. J. Neurochem. 64, 636–642.

Löscher, W., Honack, D., Taylor, C.P., 1991. 7-Nitroindazole increases aminoxyacetic acid-induced GABA accumulation in several regions of rat brain. Neurosci Lett. 128, 150–154.

Loschmann, P.A., Lange, K.W., Kunow, M., Rettig, K.J., Jahnig, P., Honoré, T., Turski, L., Wachtel, H., Jenner, P., Marsden, C.D., 1991.

Synergism of the AMPA-antagonist NBQX and the NMDA-antagonist CPP with L-dopa in models of Parkinsonian's disease. J. Neural Transm.: Parkinson's Dis. Dementia Sect. 3, 203–213.

Lowry, O.H., Rosebrrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Macdonald, R.L., Meldrum, B.S., 1995. Principles of antiepileptic drug action. In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.), Antiepileptic Drugs. 4th edn. Raven Press, New York, pp. 61–77.

Maggio, R., Fumagalli, F., Donati, E., Barbier, P., Racagni, G., Corsini, G.U., Riva, M., 1995. Inhibition of nitric oxide synthase dramatically potentiates seizures induced by kainic acid and pilocarpine in rats. Brain Res. 679, 184–187.

Meldrum, B.S., 1984. Amino acid neurotransmitters and new approaches to anticonvulsant drug action. Epilepsia 25, S140–S149.

Meldrum, B.S., 1995. Neurotrasmission in epilepsy. Epilepsia 36, S30–S35.

Meldrum, B.S., 1996. Update on the mechanism of action of antiepileptic drugs. Epilepsia 37, S4–S11.

Mollace, V., Bagetta, G., Nisticò, G., 1991. Evidence that L-arginine possesses proconvulsant effects mediated through nitric oxide. NeuroReport 2, 269–272.

Moncada, C., Lekieffre, D., Arvin, B., Meldrum, B., 1992. Effect of NO synthase inhibition of NMDA- and ischaemia-induced hippocampal lesions. NeuroReport 3, 530–532.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1989. Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. Biochem. Pharmacol. 38, 1709–1715.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Moore, P.K., Babbedge, R.C., Wallace, P., Gaffen, Z.A., Hart, S.L., 1993a. 7-Nitroindazole, an inhibitor of nitric oxide synthase, exhibits anti-nociceptive activity in the mouse without increasing blood pressure. Br. J. Pharmacol. 108, 296–297.

Moore, P.K., Handy, R.L.C., 1996. Selective inhibitors of neuronal nitric oxide synthase — is no really good NOS for the nervous system?. Trends Pharmacol. Sci. 18, 204–211.

Moore, P.K., Wallace, P., Gaffen, Z., Hart, S.L., Babbedge, R.C., 1993b. Characterization of the novel nitric oxide synthase inhibitor 7-nitroindazole and related indazoles: antinociceptive and cardiovascular effects. Br. J. Pharmacol. 110, 219–224.

Mülsch, A., Busse, R., Mordvintcev, P.I., Vanin, A.F., Nielsen, E.O., Scheel-Krüger, J., Olsen, S.P., 1994. Nitric oxide promotes seizure activity in kainate-treated rats. NeuroReport 5, 2325–2328.

O'Dell, T.J., Hawkins, R.D., Kandel, E.R., Arancio, O., 1991. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. Proc. Natl. Acad. Sci U.S.A. 88, 11285–11289.

Osonoe, K., Mori, N., Suzuki, K., Osonoe, M., 1994. Antiepileptic effects of inhibitors of nitric oxide synthase examined in pentylenetetrazol-induced seizures in rats. Brain Res. 663, 338–340.

Penix, L.P., Davis, W., Subramaniam, S., 1994. Inhibition of NO synthase increases the severity of kainic acid-induced seizures in rodents. Epilepsy Res. 18, 177–184.

Przegalinski, E., Baran, L., Siwanowicz, J., 1994. The role of nitric oxide in kainate-induced seizure in mice. Neurosci. Lett. 170, 74–76.

Rizzo, M., Morrone, L., Longo, P., Sinopoli, V.A., Spagnolo, C., Lo Pilato, R., Pelaggi, T., David, E., Rotiroti, D., De Sarro, G.B., 1997. Simultaneous determination of lamotrigine, felbamate and some conventional antiepileptic drugs using highperformance liquid chromatography. Pharmacol. Res. 35, 105–109.

Rogawski, M.A., 1992. The NMDA receptor, NMDA antagonists and epilepsy therapy. Drugs 44, 279–292.

Rondouin, G., Bockaert, J., Lerner-Natoli, M., 1993. L-Nitroarginine, an inhibitor of NO synthase, dramatically worsens limbic epilepsy in rats. NeuroReport 4, 1187–1190.

Rondouin, G., Lerner-Natoli, M., Manzoni, O., Lafon-Cazal, M., Bockaert, J., 1992. A nitric oxide (NO) synthase inhibitor accelerates amygdala kindling. NeuroReport 3, 805–808.

Rundfeldt, C., Koch, R., Richter, A., Mevissen, M., Gerecke, U., Löscher, W., 1995. Dose-dependent anticonvulsant and proconvulsant effects of nitric oxide synthase inhibitors on seizure threshold in a cortical stimulation model in rats. Eur. J. Pharmacol. 274, 73–81.

Schlesinger, K., Boggan, W.O., Freedman, D.X, 1970. Genetics of audiogenic seizures.3. Time response relationships between drug administration and seizure susceptibility. Life Sci. 9, 721–729.

Schuman, E.M., Madison, D.V., 1991. A requirement for the intercellular messenger nitric oxide in long-term potentiation. Science 254, 1503–1506.

Shibata, M., Araki, N., Ohta, K., Hamada, J., Shimazu, K., Fukuuchi, Y., 1996. Nitric oxide regulates NMDA-induced dopamine release in rat striatum. NeuroReport 7, 605–608.

Shibuki, K., Okada, D., 1991. Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. Nature 349, 326–328.

Silva, M.T., Rose, S., Hindmarsh, J.G., Aislaitner, G., Gorrod, J.W., Moore, P.K., Jenner, P., Marsden, C.D., 1995. Increased striatal dopamine efflux in vivo following inhibition of cerebral nitric oxide synthase by the novel monosodium salt of 7-nitroindazole. Br. J. Pharmacol. 114, 257–258.

Smith, R.P., Louis, C.A., Kruszyna, R., Kruszyna, H., 1991. Acute neurotoxicity of sodium azide and nitric oxide. Fundam. Appl. Toxicol. 17, 120–127.

Smith, S.E., Man, C.M., Yip, P.K., Tang, E., Chapman, A.G., Meldrum, B.S., 1996. The nitric oxide synthase product, L-citrulline and the nitric oxide donor, SIN-1 are convulsant, while L-arginine is anticonvulsant in rodents with inbred reflex epilepsy. Br. J. Pharmacol. 119, 165–173.

Starr, M.S., Starr, B.S., 1995. Do NMDA receptor-mediated changes in motor behaviour involve nitric oxide. Eur. J. Pharmacol. 272, 211–217.

Strasser, A., McCarron, R.M., Ishil, H., Stanimirovic, D., Spatz, M., 1994. L-Arginine induces dopamine release from the striatum in vivo. NeuroReport 5, 2298–2300.

Tanaka, T., Saito, H., Matsuki, N., 1993. Endogenous nitric oxide inhibits NMDA- and kainate-responses by a negative feedback system in rat hippocampal neurons. Brain Res. 631, 72–76.

Theard, M.A., Baughman, V.L., Wang, Q., Pellegrino, D.A., Albrecht, R.F., 1995. The role of nitric oxide in modulating brain activity and blood flow during seizure. NeuroReport 6, 921–924.

Tutka, P., Klonowski, P., Dzieciuch, J., Kleinrock, Z., Czuczwar, S.J., 1996. N^G-Nitro-L-arginine differentially affects glutamate- or kainate-induced seizures. NeuroReport 7, 1605–1608.

Urbanska, E.M., Drelewska, E., Borowicz, K.K., Blaszczak, P., Kleinrok, Z., Czuczwar, S.J., 1996. N^G-Nitro-L-arginine and seizure susceptibility in four seizure models in mice. J. Neural Transm. 103, 1145–1152.

Van Leeuwen, R., De Vries, R., Dzoljic, M.R., 1995. 7-Nitroindazole, an inhibitor of neural nitric oxide synthase, attenuates pilocarpine-induced seizures. Eur. J. Pharmacol. 287, 211–213.

Volke, V., Soosaar, A., Koks, S., Bourin, M., Mannisto, P.T., Vasar, E., 1997. 7-Nitroindazole, a nitric oxide synthase inhibitor, has anxiolitic-like properties in exploratory models of anxiety. Psychopharmacology 131, 399–405.

Wang, Q., Pellegrino, D.A., Baughman, V.L., Koenig, H.M., Albrecht, R.F., 1995. The role of neuronal nitric oxide synthase in regulation of cerebral blood flow in normocapnia and hypercapnia in rats. J. Cereb. Blood Flow Metab. 15, 774–778.

Wang, Q., Theard, M.A., Pellegrino, D.A., Baughman, V.L., Hoffman, W.E., Albrecht, R.F., Cwick, M., Paulson, O.B., Lassen, N.A., 1994. Nitric oxide (NO) is an endogenous anticonvulsant but not a mediator of the increase in cerebral blood flow accompanying bicuculline-induced seizures in rats. Brain Res. 658, 192–198.

West, A.R., Galloway, M.P., 1997. Endogenous nitric oxide facilitates striatal dopamine and glutamate efflux in vivo: role of ionotropic

glutamate receptor-dependent mechanism. Neuropharmacology 36, 1571–1581.

Wiley, J.L., Golden, K.M., Bowen, S.E., 1997. Effects of modulation of nitric oxide on acoustic startle responding and prepulse inhibition in rats. Eur. J. Pharmacol. 328, 125–130.

Zagvazdin, Y., Sancesario, G., Wang, Y.X., Share, L., Fitzgerald, M.E.C., Reiner, A., 1996. Evidence from its cardiovascular effects that 7-nitroindazole may inhibit endothelial nitric oxide synthase in vivo. Eur. J. Pharmacol. 303, 61–69.

Zhu, X.Z., Luo, L.G., 1992. Effect of nitroprusside (nitric oxide) on endogenous dopamine release from striatal slices. J. Neurochem. 59, 932–935.