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Influence of 7-nitroindazole on the anticonvulsive action of conventional antiepileptic drugs

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Abstract

7-Nitroindazole (a selective neuronal nitric oxide (NO) synthase inhibitor) at 25 and 50 mg/kg, 30 min before the test, did not influence the electroconvulsive threshold. However, at 50 mg/kg, it enhanced the anticonvulsive activity of phenobarbital against maximal electroshock and did not affect that of carbamazepine, diphenylhydantoin and valproate. L-Arginine (500 mg/kg) did not modify the protective activity of phenobarbital alone or the 7-nitroindazole-induced enhancement of its anticonvulsive potency against maximal electroshock. 7-Nitroindazole did not alter the plasma levels of antiepileptic drugs, so a pharmacokinetic interaction, in terms of total and free plasma levels, is not probable. 7-Nitroindazole combined with the antiepileptics resulted in motor disturbances, except for the combination with phenobarbital. On the other hand, the combined treatment of 7-nitroindazole with carbamazepine or phenobarbital produced effects superior to those produced by single drugs, as regards long-term memory. Our results indicate that the protective activity of carbamazepine, diphenylhydantoin, or valproate against maximal electroshock may be not dependent upon the central NO level. The enhancement of the anticonvulsive action of phenobarbital by 7-nitroindazole is probably not related to the decrease of NO in the central nervous system. © 1997 Elsevier Science B.V.

Keywords: Nitric oxide (NO); 7-Nitroindazole; Antiepileptic drug; Seizure

1. Introduction

Nitric oxide (NO) is an atypical regulatory molecule that acts both as a second messenger and as a neurotransmitter and has been implicated in diverse physiological functions (Moncada et al., 1991). The continuous release of NO from endothelium appears to play an important role in modulation of blood flow in different tissues. Apart from regulating the macrovessels, NO is also known to modulate the microvessels of the blood–brain barrier (Shukla et al., 1995). NO may be involved in neuron–glia interactions (Garthwaite, 1991), synaptic plasticity, long-term potentiation (Böhme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991), long-term depression (Shibuki and Okada, 1991) and desensitization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor (Ito and Karachot, 1991). As a retrograde messenger, NO induces presynaptically the release of several neurotransmitters (Montague et al., 1994). This indicates that NO deranges the neurotransmitter balance in the central nervous system and affects neuronal excitability. Therefore, the role of NO in seizures has been investigated by several authors, who used various NO synthase inhibitors (see Section 4). However, these experiments have yielded conflicting results. Arginine analogues, such as N^G-nitro-L-arginine (L-NAME) may be not selective for behavioral experimental studies, because they inhibit the activity of both endothelial and neuronal NO synthase and cause pronounced increases of arterial blood pressure (Dwyer et al., 1991), which may, in turn, affect the excitability of central neurons (Fevell and Johnson, 1994). 7-Nitroindazole has been described as a selective inhibitor of neuronal NO synthase in vivo (Babbedge et al., 1993; Moore et al., 1993). Another relevant fact is that L-NAME and other alkyl esters of arginine are muscarinic receptor antagonists (Buxton et al., 1993). This means that, in addition to inhibiting NO synthesis, they may affect neuronal excitability by the derangement of other neurotransmitters as well. The functional consequences of NO syn-

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thase inhibition are complicated by the fact, that NO not only serves as a messenger to raise cGMP in response to *N*-methyl-D-aspartic acid (NMDA) receptor stimulation, but also to induce feedback inhibition of the NMDA receptor via a redox modulatory site on the receptor complex (Theard et al., 1995).

Since NO may be involved in seizure phenomena, we intended to study the influence of 7-nitroindazole on the protective effects of conventional antiepileptic drugs against maximal electroshock-induced seizures in mice. In order to avoid the effect of arginine-derived NO synthase inhibitors on blood pressure and muscarinic receptors, we used 7-nitroindazole, an inhibitor of neuronal NO synthase, which does not affect endothelial NO synthase (in vivo) and blood pressure (Moore et al., 1993; Yoshida et al., 1994).

2. Materials and methods

2.1. Animals and experimental conditions

The experiments were carried out on female Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food (chow pellets) and tap water. The experimental temperature was 21 ± 1°C and mice were on a natural light–dark cycle. The experimental groups consisting of 8–12 animals, were chosen by means of a randomized schedule.

2.2. Drugs

Diphenylhydantoin, carbamazepine (both drugs purchased from Sigma, St. Louis, MO, USA), valproate magnesium (Polfa, Rzeszów, Poland), phenobarbital sodium (Polfa, Cracow, Poland), L-arginine and 7-nitroindazole (both compounds from Research Biochemicals International, Natick, MA, USA) were used in this study. Diphenylhydantoin, carbamazepine and 7-nitroindazole were suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria). Valproate, phenobarbital and L-arginine were brought into solution with sterile saline. All drugs were administered i.p. in a volume of 10 ml/kg, diphenylhydantoin 120 min, phenobarbital and L-arginine 60 min, valproate, carbamazepine and 7-nitroindazole 30 min before electroconvulsions and behavioral tests. L-Arginine was administered at the dose of 500 mg/kg, 7-nitroindazole at doses of 25 and 50 mg/kg. Dose ranges of antiepileptic drugs were 225–300 mg/kg for valproate, 8–16 mg/kg for carbamazepine, 5–22 mg/kg for phenobarbital and 8–14 mg/kg for diphenylhydantoin.

2.3. Electroconvulsions

Electroconvulsions were produced according to Swinyard et al. (1952), using ear-clip electrodes and alternating current delivered by a Hugo Sachs (Type 221, Freiburg, Germany) generator, the stimulus duration being 0.2 s. Tonic hindlimb extension was taken as the endpoint. The electroconvulsive threshold was evaluated as CS_{50}, which is a current strength (in mA) necessary to produce tonic hindlimb extension in 50% of the animals tested. To estimate the electroconvulsive threshold, at least four groups of mice (8–10 animals per group) were challenged with electroshocks of various intensities (from 5 to 8 mA). Subsequently, an intensity–response curve was calculated on the basis of percentage of mice convulsing. In order to evaluate the respective ED_{50} values (in mg/kg), mice pretreated with different doses of antiepileptic drug were challenged with maximal electroshock (25 mA). Again at least four groups of mice, consisting of 8–10 animals, were used to estimate each ED_{50} value. A dose–effect curve was constructed, based on the percentage of mice protected.

2.4. Chimney test

The effects of antiepileptic drugs on motor impairment were quantified with the chimney test of Boissier et al. (1960). In this test, animals had to climb backwards up the plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated by the inability of mice to climb backwards up the tube within 60 s and the results were shown as a percentage of animals which failed to perform the test.

2.5. Passive avoidance task

The mice were placed in an illuminated box ($10 \times 13 \times 15$ cm) connected to a large dark box ($25 \times 20 \times 15$ cm) which was equipped with an electric grid floor. Entrance into the dark box was punished by an electric footshock (0.6 mA for 2 s; facilitation of acquisition). The mice that did not enter the dark compartment within 60 s were excluded from the experiment. On the next day (24 h later), the same animals were put into the illuminated box and observed up to 180 s. The mean time to enter the dark box was subsequently calculated. According to Venault et al. (1986), the step-through passive avoidance task is recognized as a measure of long-term memory.

2.6. Estimation of plasma levels of antiepileptic drugs

The animals were administered with the vehicle plus one of the antiepileptics or 7-nitroindazole plus one of the antiepileptics. Mice were killed by decapitation at times scheduled for the convulsive test and samples of blood of approximately 1 ml were collected into Eppendorf tubes. The samples of blood were centrifuged at 10,000 rpm (Abbott centrifuge, Irving, TX, USA) for 3 min. Plasma samples of 70 μl were transferred into Abbott system cartridges and the rest of the plasma was put into MPS-1

system tubes (Amicon, Danvers, MA, USA) for the separation of free from protein bound microsolutes. Then the MPS-1 tubes were centrifuged at 3000 rpm (MPW-360 centrifuge; Mechanika Precyzyjna, Warsaw, Poland) for 10 min and the filtrate samples of 50 μl were put into Abbott system cartridges. The total and free plasma levels of antiepileptic drugs were estimated by immunofluorescence, using an Abbott TDx analyzer (Abbott). Plasma levels were expressed as means ± S.D of at least 8 determinations.

2.7. Statistics

CS_{50} or ED_{50} values and statistical analysis of the data, obtained in the electroconvulsive tests, were estimated by computer probit analysis, according to Litchfield and Wilcoxon (1949). The results from the chimney test were compared statistically by using Fisher's exact probability test and those from the passive avoidance task by use of the Mann–Whitney test. Plasma levels of antiepileptic drugs were evaluated with the Student's *t*-test.

3. Results

3.1. Effects of 7-nitroindazole on the electroconvulsive threshold

7-Nitroindazole did not significantly influence the threshold for electroconvulsions. CS_{50} values varied insignificantly from the control value of 6.1 (5.4–6.9) to 6.7 (6.0–7.4) and 7.0 (6.4–7.8) mA, respectively, after 7-nitroindazole in doses of 25 and 50 mg/kg.

3.2. Influence of 7-nitroindazole on the protective efficacy of antiepileptic drugs against maximal electroshock-induced seizures

7-Nitroindazole (at 50 mg/kg, but not 25 mg/kg) co-administered with phenobarbital, significantly reduced its ED_{50} from 17.7 (14.6–21.4) to 7.3 (5.5–9.9) mg/kg.

Table 1
Influence of 7-nitroindazole upon the anticonvulsive activity of carbamazepine (CBZ), diphenylhydantoin (DPH), phenobarbital (PB) and valproate (VPA) against maximal electroshock

Treatment	7-Nitroindazole (mg/kg)		
	0	25	50
CBZ	12.4 (10.5–14.8)	N.D.	10.9 (9.3–12.7)
DPH	11.2 (9.2–13.6)	11.1 (9.5–13.1)	11.5 (10.2–13.1)
PB	17.7 (14.6–21.4)	16.5 (13.5–20.4)	7.3 (5.5–9.9) *
VPA	261 (240–284)	N.D.	242 (224–260)

All drugs were administered i.p., DPH 120 min, PB 60 min, VPA and CBZ 30 min prior to the test. Table data are ED_{50} values (in mg/kg) with 95% confidence limits in parentheses. ED_{50} values and statistical analysis of the data were calculated according to Litchfield and Wilcoxon (1949).
N.D.; not determined.
* $P < 0.001$ versus respective control.

Table 2
Influence of L-arginine on the anticonvulsant effects of phenobarbital (PB) and on 7-nitroindazole (7-NI)-induced potentiation of anticonvulsive efficacy of PB against maximal electroshock

Treatment (mg/kg)	ED_{50} (mg/kg)
PB	17.7 (14.6–21.4)
PB + L-arginine (500)	17.0 (14.3–20.1)
PB + 7-NI (50)	7.3 (5.5–9.9) *
PB + 7-NI (50) + L-arginine (500)	7.5 (6.2–9.1) *

Table data are ED_{50} values (in mg/kg) with 95% confidence limits in parentheses. See also Table 1.
* $P < 0.001$ versus control.

However, it remained without effect upon the protective activity of valproate, diphenylhydantoin and carbamazepine against maximal electroshock-induced seizures in mice (Table 1).

3.3. Influence of L-arginine on the 7-nitroindazole-induced enhancement of the anticonvulsive action of phenobarbital against maximal electroshock

L-Arginine (500 mg/kg) did not affect the protective activity of phenobarbital alone or the 7-nitroindazole-induced potentiation of its anticonvulsive activity against maximal electroshock (Table 2).

3.4. Chimney test

When applied at doses corresponding to their ED_{50} values, phenobarbital (17.7 mg/kg), carbamazepine (12.4 mg/kg) and diphenylhydantoin (11.2 mg/kg) did not influence the performance of mice in the chimney test. 7-Nitroindazole (50 mg/kg) also did not cause any significant motor impairment. On the contrary, valproate (261 mg/kg) produced strong motor disturbances in 38.9% of mice. The combined treatment of 7-nitroindazole (50 mg/kg) with valproate (242 mg/kg), carbamazepine (10.9 mg/kg) or diphenylhydantoin (11.5 mg/kg), providing a 50% protection against maximal electroshock, produced a significant motor impairment in 44.4, 27.7 and 27.7% of the animals, respectively. The combined treatment of 7-nitroindazole (50 mg/kg) with phenobarbital (7.3 mg/kg) did not affect the motor performance of mice (Table 3).

Table 3
Motor impairment after administration of 7-nitroindazole (7-NI) and phenobarbital (PB) alone or in combination

Treatment (mg/kg)	Mice impaired (%)
Vehicle	0
PB (17.7)	5.5
PB (7.3)	0
PB (7.3) + 7-NI (50)	16.6
7-NI (50)	11.1

The results show the percentage of animals failing to perform the chimney test. PB at the higher dose or the combined treatment provide a 50% protection against seizures. Experimental groups consisted of 18 mice. See also legend to Table 1.

Table 4
Effects of phenobarbital (PB) and 7-nitroindazole (7-NI) administered alone or in combination on the retention of a passive avoidance task in mice

Treatment (mg/kg)	Time (s) for entering the dark box
Vehicle	> 180
PB (17.7)	138.2 ± 17.5 [a]
PB (7.3)	169.9 ± 14.6
PB (17.3) + 7-NI (50)	167.0 ± 11.9
7-NI (50)	164.2 ± 7.2

Data are the means of 12 determinations ± S.E. The retention was quantified as a time period the animals avoided the dark compartment for. See also legends to Tables 1 and 2.
[a] $P < 0.05$ (Mann–Whitney test).

3.5. Dark-avoidance acquisition and retention testing

Phenobarbital, valproate, diphenylhydantoin and carbamazepine, when given at doses corresponding to their ED_{50}s against maximal electroshock, caused a moderate impairment of long-term memory ($P < 0.05$). The combined treatment of 7-nitroindazole (50 mg/kg) with doses of valproate (242 mg/kg) or diphenylhydantoin (11.5 mg/kg), providing a 50% protection against maximal electroshock, also resulted in a worsened retention in this test ($P < 0.05$ and $P < 0.01$, respectively). On the contrary, the combined treatment of 7-nitroindazole (50 mg/kg) with carbamazepine (10.9 mg/kg) or phenobarbital (7.3 mg/kg) did not affect the acquisition of mice. The detailed results concerning the effects of phenobarbital alone or combined with 7-nitroindazole are listed in Table 4.

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

7-Nitroindazole (50 mg/kg) did not alter either the total or free plasma levels of diphenylhydantoin (9.0 mg/kg), valproate (242 mg/kg) and carbamazepine (10.9 mg/kg). The total plasma levels of phenobarbital (7.3 mg/kg) were not affected, either (Table 5).

Table 5
Influence of 7-nitroindazole (7-NI) upon the total and free plasma levels of carbamazepine (CBZ), diphenylhydantoin (DPH), phenobarbital (PB) and valproate (VPA)

Treatment (mg/kg)	Plasma levels	
	Total	Free
CBZ (10.9)	5.26 ± 1.00	1.02 ± 0.08
CBZ (10.9) + 7-NI (50)	6.07 ± 1.07	1.03 ± 0.14
DPH (9.0)	5.62 ± 1.17	0.56 ± 0.08
DPH (9.0) + 7-NI (50)	5.23 ± 0.59	0.62 ± 0.05
PB (7.3)	9.24 ± 1.18	N.D.
PB (7.3) + 7-NI (50)	9.47 ± 0.96	N.D.
VPA (242)	251.65 ± 33.08	208.65 ± 29.67
VPA (242) + 7-NI (50)	267.44 ± 38.73	215.24 ± 27.49

Presented values are the means (μg/ml of plasma) of 8 determinations ± S.D. Unpaired Student's *t*-test was used for statistical evaluation of the data. For treatment times see also legend to Table 1.
N.D.; not determined.

4. Discussion

The present study has revealed that 7-nitroindazole (25 and 50 mg/kg, i.p.) did not influence the electroconvulsive threshold in mice. Moreover, 7-nitroindazole (50 mg/kg) significantly enhanced the protective activity of phenobarbital, but not that of valproate, diphenylhydantoin or carbamazepine against maximal electroshock-induced convulsions. L-Arginine (500 mg/kg), used as a donor of NO, did not reverse the 7-nitroindazole-induced enhancement of the anticonvulsive activity of phenobarbital against maximal electroshock. This specific inhibitor of neuronal NO synthase, used at the similar dose-range, was active in other tests. In mice, the harmaline-induced increase in cerebellar cGMP was dose-dependently reversed by pretreatment with 7-nitroindazole (12.5–50 mg/kg). It is well established that the action of harmaline on cerebellar cGMP levels is mediated by neuronal NO synthase present in the cerebellar cortex (Wood et al., 1990). Consequently, 7-nitroindazole, in the dose-range used in the present study, actually inhibited neuronal NO synthase. Also, 7-nitroindazole (25–100 mg/kg) attenuated the severity of pilocarpine-induced seizures in mice (Van Leeuwen et al., 1995).

As mentioned earlier, 7-nitroindazole selectively inhibits neuronal NO synthase without affecting endothelial NO synthase. The results of in vitro studies, however, call the specificity of 7-nitroindazole for neuronal NO synthase into question. As demonstrated by Wolff and Gribin (1994), 7-nitroindazole may also be an inhibitor of constitutive NO synthase in bovine endothelial cells. The reason for the discrepancy between the in vivo and in vitro studies, with respect to the action of 7-nitroindazole on endothelial NO synthase, is unclear. Possibly, 7-nitroindazole is metabolized in vivo to a compound with a more specific action on NO synthase (Medhurst et al., 1994).

The inhibitors of NO synthase may produce diverse effects upon seizure susceptibility. 7-Nitroindazole and L-NAME had only weak actions against seizures induced by intracerebroventricular injection of NMDA in mice. At the highest dose tested, both compounds conferred partial protection against NMDA-induced seizures and had only a tendency to delay the onset of seizures (Eblen et al., 1996). De Sarro et al. (1991) reported that local pretreatment with the NO synthase inhibitor, N^G-monomethyl-L-arginine prevented seizures induced by local injection of NMDA into the deep prepiriform cortex of rats. Mollace et al. (1991) observed a proconvulsant effect of intracerebroventricularly administered L-arginine, the precursor of NO, on seizures induced by intracerebroventricular injection of NMDA. This effect was reversed by the co-administration

of L-NAME. In seizures induced by other chemoconvulsants, NO synthase inhibitors possessed both anticonvulsant and proconvulsant actions (Bagetta et al., 1992; Osonoe et al., 1994; Penix et al., 1994; Przegaliński et al., 1994; Tutka et al., 1996; Urbańska et al., 1996).

Kainic acid-induced convulsions were inhibited by 7-nitroindazole (40 mg/kg) in rats (Mülsch et al., 1994). 7-Nitroindazole can prevent pilocarpine-induced seizures and lethality in mice (Van Leeuwen et al., 1995). However, the anticonvulsant effect of 7-nitroindazole and the decrease in lethality were less pronounced after high doses of this drug. This may indicate that higher doses of 7-nitroindazole (above 50 mg/kg) possess some additional non-specific effect, unrelated to brain NO synthase inhibition (Van Leeuwen et al., 1995). The anticonvulsant effect of 7-nitroindazole contrasts with the effect of the other NO synthase inhibitor, L-NAME, that was found to be proconvulsant when injected in conjunction with a subconvulsant dose of pilocarpine (Starr and Starr, 1993). Interestingly, in our previous study (unpublished data) we provided evidence that L-NAME reduced the protective activity of phenobarbital against maximal electroshock. 7-Nitroindazole exerted an opposite effect, it enhanced the anticonvulsive efficacy of phenobarbital. The mechanism of these contradictory effects of L-NAME and 7-nitroindazole upon pilocarpine-induced seizures and the protective activity of phenobarbital against electrically evoked convulsions is not clear. These discrepancies may result from the actions of 7-nitroindazole or L-NAME that could be unrelated to brain NO synthase inhibition. Furthermore, concomitant administration of L-arginine (500 mg/kg) prevented the proconvulsive effect of L-NAME at the dose of 5 mg/kg, but was ineffective against L-NAME at the dose of 25 mg/kg (Starr and Starr, 1993). According to Shibata et al. (1995), the neuroprotective effect of L-NAME was also significantly attenuated by co-treatment with L-arginine. Although the most of available studies demonstrated that 7-nitroindazole did not affect peripheral functions, there is a recent report demonstrating that the pretreatment with L-arginine (300 mg/kg) inhibited the effects of 7-nitroindazole (50 mg/kg) on mean arterial blood pressure and acetylcholine-induced hypotension, suggesting an involvement of the L-arginine pathway in the effects of this selective NO synthase inhibitor (Zagvazdin et al., 1996). Generally, however, we observed a failure of L-arginine to antagonize the opposite effects of L-NAME (unpublished data) and 7-nitroindazole upon the anticonvulsive activity of phenobarbital.

The most evident finding of this study was the interaction between 7-nitroindazole and phenobarbital. Phenobarbital exerts its effects through the GABA-related receptor complex (Haefely, 1980). 7-Nitroindazole decreases NO level in the brain and this effect may be responsible for its anticonvulsant action in some models of epilepsy and for the potentiation of the protective activity of phenobarbital against maximal electroshock. This may support the idea of a proconvulsant role of central NO. Nevertheless, the influence of 7-nitroindazole upon phenobarbital was not reversed by L-arginine so the participance of NO in this particular effect of 7-nitroindazole may be questionable.

It should be stressed that the potentiation of the anticonvulsive activity of phenobarbital induced by 7-nitroindazole was not accompanied by increased adverse effects. On the other hand, the combination of 7-nitroindazole with carbamazepine or diphenylhydantoin resulted in the disturbed performance in the chimney test, whilst the same antiepileptic drugs when administered alone did not affect the motor coordination. Interestingly, the combination of 7-nitroindazole with carbamazepine did not affect the retention time as carbamazepine alone did. These findings seem to indicate that the manipulations of the central NO level may actually affect the expression of some antiepileptic drug-induced adverse effects.

In conclusion, our data show that 7-nitroindazole, a selective inhibitor of neuronal NO synthase, increases the protective activity of phenobarbital against maximal electroshock-induced seizures in mice. It may not be excluded that this effect of 7-nitroindazole is unspecific (i.e. non-dependent on NO level in the central nervous system). Finally, central NO does not seem involved in the expression of the anticonvulsive action of carbamazepine, diphenylhydantoin and valproate.

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References

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, 225–229.

Bagetta, G., Iannone, M., Scorsa, A.M., Nistico, G., 1992. Tacrine-induced seizures and brain damage in LiCl-treated rats can be prevented by *N*-omega-nitro-L-arginine methyl ester. Eur. J. Pharmacol. 213, 301–304.

Böhme, 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. 199, 379–381.

Boissier, J.R., Tardy, J., Diverres, J.C., 1960. Une nouvelle methode simple pour explorer l'action'tranquilisante: Le test de la cheminee. Med. Exp. Basel 3, 81–84.

Buxton, I.L.O., Cheek, D.J., Eckman, D., Westfall, D.P., Sanders, K.M., Keef, K.D., 1993. N^G-Nitro-L-arginine methyl ester and other alkyl esters of arginine are muscarinic receptor antagonists. Circ. Res. 72, 387–391.

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.

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

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

Fevell, J.E., Johnson, P., 1994. Acute increases of blood pressure cause arousal from sleep in lambs. Brain. Res. 311, 258–262.

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

Haefely, W.E., 1980. GABA and the anticonvulsant action of benzodiazepines and barbiturates. Brain Res. Bull. 5 (Suppl. 2), 873–877.

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

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

Medhurst, A.D., Greenless, C., Parsons, A.A., Smith, S.J., 1994. Nitric oxide synthase inhibitors 7- and 6-nitroindazole relax smooth muscle in vitro. Eur. J. Pharmacol. 256, R5.

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

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

Montague, P.R., Gancayco, C.D., Winn, M.J., Marchase, R.B., Friedlander, M.J., 1994. Role of NO production in NMDA receptor mediated neurotransmitter release in cerebral cortex. Science 263, 973–977.

Moore, P.K., Babbedge, R.C., Wallace, P., Gaffen, Z.A., Hart, S.L., 1993. 7-Nitroindazole, an inhibitor of nitric oxide synthase, exhibits anti-nociceptive activity in the mouse without increasing blood pressure. Br. J. Pharmacol. 110, 296–297.

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. USA 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.

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

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

Shibata, S., Yamamoto, Y., Tanaka, T., Watanabe, S., 1995. N^G-Nitro-L-arginine protects against hypoxia/hypoglycemia-induced decrease in CA1 presynaptic spikes in rat hippocampal slices. Eur. J. Pharmacol. 273, 223–228.

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

Shukla, A., Dikshit, M., Srimal, R.C., 1995. Nitric oxide modulates blood–brain barrier permeability during infections with an inactivated bacterium. NeuroReport 6, 1629–1632.

Starr, M.S., Starr, B.S., 1993. Paradoxal facilitation of pilocarpine-induced seizures in the mouse by MK-801 and the nitric oxide synthesis inhibitor L-NAME. Pharmacol. Biochem. Behav. 45, 321–325.

Swinyard, E.A., Brown, W.C., Goodman, L.S., 1952. Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. Exp. Ther. 106, 319–330.

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

Urbańska, E., Drelewska, E., Borowicz, K.K., Błaszczak, 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 neuronal nitric oxide synthase, attenuates pilocarpine-induced seizures. Eur. J. Pharmacol. 287, 211–213.

Venault, P., Chapouthier, G., De Carvalho, L.P., Simiand, J., Morre, M., Dodd, R.H., Rossier, J., 1986. Benzodiazepines impair and beta-carbolines enhance performance in learning and memory task. Nature 321, 864–866.

Wolff, D.J., Gribin, B.J., 1994. The inhibition of the constitutive and inducible nitric oxide synthase isoforms by indazole agents. Arch. Biochem. Biophys. 311, 300–304.

Wood, P.L., Emmett, M.R., Rao, T.S., Cler, J., Mick, S., Iyengar, S., 1990. Inhibition of nitric oxide synthase blocks *N*-methyl-D-aspartate-, kainate-, quisqualate-, harmaline-, and pentylenetetrazole-dependent increases in cerebellar cyclic GMP in vivo. J. Neurochem. 55, 346–351.

Yoshida, T., Limmroth, V., Irikura, K., Moskowitz, M.A., 1994. The NOS inhibitor, 7-nitroindazole, decrease focal infarct volume but not the response to topical acetylcholine in pial vessels. J. Cereb. Blood Flow Metab. 14, 924–929.

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.