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Further studies on anti- and proconvulsant effects of inhibitors of nitric oxide synthase in rodents

Corinne B. Alexander [a], Timothy M. Ellmore [b], Tushar G. Kokate [a,*], R. Duncan Kirkby [a]

[a] *Neuronal Excitability Section, Epilepsy Research Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 10 / 5N250, 9000 Rockville Pike, Bethesda, MD 20892-1408, USA*

[b] *Sensor Systems Inc., 103A Carpenter Drive, Sterling, VA 20164-4423, USA*

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Abstract

We confirmed that the effects of inhibitors of nitric oxide (NO) synthase, such as N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine, differ depending on several experimental factors. Both compounds but not their less active enantiomers delayed picrotoxin-induced clonus in mice yet increased the incidence of clonus following low-dose picrotoxin. N^{ω}-nitro-L-arginine methyl ester significantly reduced the latencies of both myoclonus and clonus in older but not younger Sprague–Dawley rats receiving pentylenetetrazol s.c. By contrast, there was no significant change in the latencies for myoclonus and clonus in Wistar rats (older and younger). However, when pentylenetetrazol was administered i.p. rather than s.c., N^{ω}-nitro-L-arginine methyl ester dramatically increased latencies of convulsive indicators, including tonus, in both Sprague–Dawley and Wistar rats. N^{ω}-nitro-L-arginine methyl ester also delayed tonus but not myoclonus or clonus in mice, regardless of the systemic route of administration of pentylenetetrazol. Both N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine increased the tonic CD_{50} of pentylenetetrazol in mice and N^{ω}-nitro-L-arginine methyl ester delayed 4-aminopyridine-induced tonus. However, N^{ω}-nitro-L-arginine methyl ester reduced the tonic CD_{50} of both picrotoxin and 4-aminopyridine in mice and failed to suppress tonus following maximal electroshock. Evidently, inhibitors of NO synthase are not universally effective antitonic drugs. © 1998 Elsevier Science B.V.

Keywords: N^{ω}-nitro-L-arginine methyl ester; N^{G}-nitro-L-arginine; $GABA_A$ receptor; NMDA (*N*-methyl-D-aspartate); Seizure; Strain difference; Ontogeny

1. Introduction

Despite a rapidly accumulating body of literature, the role of nitric oxide (NO) in the expression of seizures remains unclear. Central to the controversy, the effects of inhibitors of the enzyme NO synthase, which suppress the formation of NO, on behavioral and electrographic seizures differ profoundly from study to study, suggesting the interaction of numerous experimental and physiological factors (Kirkby et al., 1996a). A recurrent theme is that the pro- versus anticonvulsant role of NO depends on the model of seizure employed (Starr and Starr, 1993; Penix et al., 1994; Rundfeldt et al., 1995; Przegalinski et al., 1996; Kirkby et al., 1996a). Indeed, we observed that the inhibitors of NO synthase N^{ω}-nitro-L-arginine methyl ester and 7-nitroindazole dramatically hasten the expression of generalized convulsions following the systemic administration of the excitatory amino acid kainate in mice (Kirkby et al., 1996a,b; Penix et al., 1994) yet consistently delay the expression of clonic responses to the antagonist of $GABA_A$ receptors picrotoxin (Kirkby et al., 1996a). Our latter findings thus seem congruent with those of Osonoe et al. (1994), who observed a pronounced N^{ω}-nitro-L-arginine methyl ester-induced suppression of seizures elicited in rats by pentylenetetrazol, another noncompetitive antagonist of $GABA_A$ receptors. Complicating matters, however, we found that the effects of the inhibitors of NO synthase were not dependent solely on the model of seizure (i.e. convulsant drug). In particular, the influence of N^{ω}-nitro-L-arginine methyl ester also depended on the index of sensitivity to the convulsant, at least in the case of picrotoxin: N^{ω}-nitro-L-arginine methyl ester increased the latency of picrotoxin-induced convulsions, indicating a seizure-suppressing effect, but paradoxically reduced the dose of picrotoxin necessary to elicit convulsions, indicat-

* Corresponding author. Tel.: +1-301-4023501; fax: +1-301-4022871; e-mail: kokate@helix.nih.gov

ing a seizure-enhancing effect (Kirkby et al., 1996a). In related work, Osonoe et al. (1994) reported that N^{ω}-nitro-L-arginine suppressed myoclonic, clonic and tonic seizures elicited in rats by pentylenetetrazol. In an attempt to elucidate the role of NO in the expression of seizures, we evaluated inhibitors of NO synthase in the context of picrotoxin- and pentylenetetrazol-induced convulsions in mice and rats. In more specific assessments of tonic convulsive responses in mice, we also administered N^{ω}-nitro-L-arginine methyl ester prior to either the injection of 4-aminopyridine, a potent blocker of K^+ channels, or the delivery of maximal electroshock. We, thus, tested the hypothesis of Osonoe et al. (1994) that NO tightly regulates the expression of tonus.

2. Materials and methods

2.1. Animals

Adult male NIH Swiss mice (NIH Animal Program) weighing 23–33 g and male Wistar (Charles River) and Sprague–Dawley rats (Taconic) served as subjects and were maintained on a constant 12:12-h light:dark cycle with constant access to food and water. Both strains of rat were tested in either of two ranges of weight (i.e. 210–260 and 120–170 g), as specified below. All procedures rigidly complied with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the NIH Animal Care and Use Committee.

2.2. Drugs

Mice and rats were transferred from home cages (4 mice/cage; 2–3 rats/cage) to individual cages 1.5–2 h before the administration of drugs. N^{ω}-nitro-L-arginine methyl ester, N^{ω}-nitro-D-arginine methyl ester, N^{G}-nitro-L-arginine and N^{G}-nitro-D-arginine were administered i.p. 30 min before convulsive treatments. Following the administration of picrotoxin, we observed mice for a maximum of 90 min for the occurrence of convulsive indicators. An observational period of 60 min was used for mice treated with either pentylenetetrazol or 4-aminopyridine (the behavioral syndrome associated with the systemic administration of 4-aminopyridine in mice has been characterized by Yamaguchi and Rogawski (1992)). We observed mice for < 30 s following maximal electroshock (delivered via saline-wetted corneal electrodes (50 mA nominal ac, 60 Hz, 200 ms)) and rats for 30 min following the administration of pentylenetetrazol. All drugs (Sigma) were dissolved in saline (0.9%) with the exceptions of N^{G}-nitro-L-arginine and N^{G}-nitro-D-arginine (Research Biochemicals International), which were dissolved in HCl, phosphate-buffered saline (pH = 7.4; phosphates = 0.007 M; NaCl = 0.15 M) and NaOH (final pH = 6.2–6.5). Appropriate vehicle controls were employed in all experiments.

2.3. Statistical analyses

We analyzed latencies of convulsive events with one-tailed independent *t*-tests, except where noted. We used permutation tests for ranks for ordinal data and either Fisher's exact tests or probit analysis (Pharmacological Calculation System) to assess frequency data.

3. Results

3.1. Effects of inhibitors of NO synthase on picrotoxin-induced clonus in mice

As revealed in Table 1, we confirmed our previous observations that N^{ω}-nitro-L-arginine methyl ester can either inhibit or potentiate convulsive responses, depending on the dose of picrotoxin. N^{ω}-nitro-L-arginine methyl ester delayed the expression of clonus following the administration of a moderate dose of picrotoxin yet increased the proportion of mice expressing clonus following an otherwise minimally effective dose of picrotoxin. N^{G}-nitro-L-arginine exerted qualitatively similar anti- and proconvulsant effects, suggesting that both actions of N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine reflect inhibition of NO synthase, a conclusion further substantiated by the ineffectiveness of the less active enantiomers N^{ω}-nitro-D-arginine methyl ester and N^{G}-nitro-D-arginine (Table 1).

3.2. Effects of N^{ω}-nitro-L-arginine methyl ester on pentylenetetrazol-induced convulsions in rats

As shown in Table 2, N^{ω}-nitro-L-arginine methyl ester significantly reduced the latencies of both myoclonus and clonus in Sprague–Dawley but not Wistar rats that weighed 210–260 g and were thus 7–8 weeks of age during testing. A slightly different pattern of results was obtained using lighter (120–170 g) and hence younger rats (5–6 weeks) and is suggestive of important development-dependent differences in the expression of seizures and the effects of N^{ω}-nitro-L-arginine methyl ester. First, lighter (younger) control rats (both strains) tended to demonstrate shorter latencies of myoclonus and clonus than did heavier (older) rats (Table 2; note that we have not addressed the differences statistically, because weight (age) was not employed as a variable in the context of a single experiment, rather, two separate experiments were conducted, one employing heavier (older) rats and the other employing lighter (younger) rats). Second, lighter (younger) Sprague–Daw-

Table 1
Stereospecific anti- and proconvulsant effects of NO synthase-inhibiting analogues of L-arginine on picrotoxin-induced clonus in mice

Dose of picrotoxin (mg/kg)	Drug (dose, mg/kg)	*n*	Latency of clonus (s ± S.E.M.)	Proportion of group exhibiting clonus
7	vehicle	24	672 (20)	1.0
	L-NAME (100)	12	751 (44)[a]	1.0
	D-NAME (100)[e]	12	660 (36)	1.0
7	vehicle	12	673 (20)	1.0
	L-NNA (5)	10	662 (36)	1.0
	L-NNA (50)	11	749 (26)[a]	1.0
	L-NNA (100)	11	774 (22)[b]	1.0
	D-NNA (100)[e]	11	699 (22)	1.0
1.25	vehicle	17	—	0.12
	L-NAME (0.5)	18	—	0.50[c]
	L-NAME (5)	18	—	0.56[c]
	L-NAME (50)	18	—	0.72[d]
	L-NAME (100)	18	—	0.61[d]
	D-NAME (0.5)[f]	17	—	0.12
1.25	vehicle	18	—	0.11
	L-NNA (0.5)	18	—	0.44[c]
	L-NNA (5)	18	—	0.56[c]
	L-NNA (50)	18	—	0.67[d]
	L-NNA (100)	17	—	0.94[d]
	D-NNA (0.5)[f]	18	—	0.00

N^{ω}-nitro-L-arginine methyl ester (L-NAME) and N^{G}-nitro-L-arginine (L-NNA) but not their less active enantiomers (i.e. N^{ω}-nitro-D-arginine methyl ester (D-NAME) and N^{G}-nitro-D-arginine (D-NNA)) delayed clonus provoked by a moderate dose of picrotoxin (7 mg/kg; s.c.).

[a] and [b] indicate significant difference from vehicle control at $P < 0.05$ and $P < 0.005$, respectively. In contrast to their anticonvulsant effects, N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine but not their less active enantiomers (i.e. N^{ω}-nitro-D-arginine methyl ester and N^{G}-nitro-D-arginine) increased the probability that mice receiving a low dose of picrotoxin (1.25 mg/kg; s.c.) would express clonus.

[c] and [d] indicate significant difference from vehicle control at $P < 0.05$ and $P < 0.005$, respectively, according to Fisher's exact tests. Doses of N^{ω}-nitro-D-arginine methyl ester and N^{G}-nitro-D-arginine differed depending on whether we assessed the [e] latency (i.e. 100 mg/kg) versus the [f] expression of clonus (i.e. 0.5 mg/kg), which reflects our unpublished observations that relatively high doses of either N^{ω}-nitro-L-arginine methyl ester or N^{G}-nitro-L-arginine are generally required to delay picrotoxin-induced seizures, whereas the inhibitors of NO synthase can increase the incidence of seizures across a wide range of doses.

ley rats failed to demonstrate the N^{ω}-nitro-L-arginine methyl ester-induced shortening of latencies of myoclonus and clonus exhibited by their heavier (older) strain-mates. It thus appears that younger Sprague–Dawley rats are indeed *less* vulnerable to the proconvulsant effects of N^{ω}-nitro-L-arginine methyl ester than are older Sprague–Dawley rats. In these experiments, tonus occurred in relatively few rats and was thus not clearly related to the administration of N^{ω}-nitro-L-arginine methyl ester. Incidences of tonus for the groups were (N^{ω}-nitro-L-arginine methyl ester at doses of 0, 30 and 100 mg/kg, respectively): Heavier (older) Wistar, 3/8, 1/6, 3/6; heavier (older) Sprague–Dawley, 0/9, 1/6, 0/7; lighter (younger) Wistar, 3/8, 4/8, 0/7; lighter (younger) Sprague–Dawley, 1/8, 2/7, 1/7.

Arbitrarily using rats of the lighter range of weight (i.e. 120–170 g), we obtained radically different results by varying the route of administration of pentylenetetrazol (Table 2). With respect to control responses to i.p. pentylenetetrazol, myoclonus, which occurred with average latencies of less than 1 min, preceded clonus by only several seconds in both strains of rat and tonus subsequently occurred with average latencies of approximately 3 min. This time, latencies of all convulsive events, including tonus, increased dramatically and significantly as a function of the administration of N^{ω}-nitro-L-arginine methyl ester. With respect to clonus and tonus, increased latencies at least partially reflected a failure of some N^{ω}-nitro-L-arginine methyl ester-treated rats to exhibit the behaviors (Table 2): Using the method of Osonoe et al. (1994), we arbitrarily assigned latencies of 1800 s (the limit of the observational period) when convulsive components of pentylenetetrazol failed to occur. Incidences of clonus for the groups were (N^{ω}-nitro-L-arginine methyl ester at doses of 0, 30 and 100 mg/kg, respectively): Wistar, 8/8, 7/8 and 7/8; Sprague–Dawley, 10/10, 9/9 and 7/9. Incidences of tonus for the groups were: Wistar, 8/8, 5/8 and 4/8 and Sprague–Dawley, 9/9, 8/9 and 7/9. Note that Wistar (but not Sprague–Dawley) rats treated with N^{ω}-nitro-L-arginine methyl ester (100 mg/kg) were significantly less likely than controls to express tonus ($P < 0.05$, Fisher's exact test). Although Wistar rats treated with N^{ω}-nitro-L-arginine methyl ester tended to exhibit longer latencies of convulsive events than did identically

Table 2
Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME) on seizures in rats vary with strain and weight of rat and systemic route of administration of the convulsant pentylenetetrazol (PTZ; 80 mg/kg)

Dose of L-NAME (mg/kg)	*n*	Strain of rat	Weight (age) of rat	Route of delivery of PTZ	Latency of myoclonus (s ± S.E.M.)	Latency of clonus (s ± S.E.M.)	Latency of tonus (s ± S.E.M.)
0	8	Wistar	heavier (older)	s.c.	291 (22)	426 (36)	—
30	6				240 (23)	407 (105)	—
100	6				261 (29)	380 (64)	—
0	9	Sprague–Dawley	heavier (older)	s.c.	338 (39)	430 (47)	—
30	6				223 (16)[a]	294 (26)[b]	—
100	7				270 (31)	328 (47)[a]	—
0	8	Wistar	lighter (younger)	s.c.	215 (19)	371 (62)	—
30	8				238 (26)	306 (43)	—
100	7				234 (27)	314 (33)	—
0	8	Sprague–Dawley	lighter (younger)	s.c.	263 (35)	334 (66)	—
30	7				232 (61)	294 (93)	—
100	7				252 (26)	365 (62)	—
0	8	Wistar	lighter (younger)	i.p.	55 (8)	62 (9)	192(73)
30	8				194 (103)[c]	354 (213)[c]	613 (234)
100	8				189 (109)[c]	777 (304)[d]	1022 (302)[c]
0	10	Sprague–Dawley	lighter (younger)	i.p.	53 (4)	57 (4)	172 (59)
30	9				79 (11)[c]	84 (11)[c]	304 (189)
100	9				88 (22)[c]	455 (254)[d]	552 (240)[c]

N^{ω}-nitro-L-arginine methyl ester accelerated the expression of myoclonus and clonus in heavier (210–260 g; older) but not lighter (120–170 g; younger) Sprague–Dawley rats receiving pentylenetetrazol s.c. [a] and [b] indicate $P < 0.05$ and $P < 0.005$, respectively. However, when we administered pentylenetetrazol i.p rather than s.c., as we did in our previous experiments, N^{ω}-nitro-L-arginine methyl ester dramatically increased latencies of convulsive indicators, including tonus, which was expressed by all control rats.
[c] and [d] indicate $P < 0.05$ and $P < 0.005$, respectively, according to nonparametric permutation tests for ranks, which we used because not all rats treated with N^{ω}-nitro-L-arginine methyl ester prior to pentylenetetrazol (i.p.) expressed clonic and tonic components (see text).

Table 3
Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME) on seizures in mice provoked by pentylenetetrazol (PTZ; 100 mg/kg) or picrotoxin (30 mg/kg) delivered either s.c. or i.p.

Dose of L-NAME (mg/kg)	*n*	Convulsant	Route of delivery of convulsant	Latency of myoclonus (s ± S.E.M.)	Latency of clonus (s ± S.E.M.)	Latency of tonus (s ± S.E.M.)
0	10	PTZ	s.c.	185 (36)	229 (38)	417 (63)
5	12			159 (23)	205 (30)	605 (91)
50	10			136 (16)	182 (18)	633 (82)[a]
100	12			169 (19)	241 (30)	590 (69)[a]
0	12	PTZ	i.p.	57 (6)	71 (8)	110 (13)
5	11			61 (5)	71 (9)	122 (27)
50	12			71 (8)	75 (8)	309 (28)[b]
100	12			67 (4)	75 (4)	269 (39)[b]
0	18	Picrotoxin	s.c.	226 (11)	276 (11)	460 (14)
5	12			244 (16)	317 (12)[a]	505 (16)[a]
50	12			240 (16)	319 (10)[a]	491 (26)
100	18			217 (10)	281 (13)	499 (245)
0	12	Picrotoxin	i.p.	184 (11)	226 (15)	341 (17)
5	11			215 (9)[a]	254 (7)	360 (16)
50	12			210 (10)[a]	279 (11)[b]	442 (34)[a]
100	12			207 (8)	260 (10)[a]	422 (32)[a]

High doses of N^{ω}-nitro-L-arginine methyl ester significantly increased the latency of tonus induced by pentylenetetrazol administered either s.c. or i.p. Whereas N^{ω}-nitro-L-arginine methyl ester exerted only minimal clonus- and tonus-delaying effects in mice treated with picrotoxin (s.c.), the inhibitor of NO synthase significantly delayed all convulsive events assessed following the i.p. administration of picrotoxin (i.e. myoclonus, clonus and tonus, [a]$P < 0.05$; [b]$P < 0.005$).

treated Sprague–Dawley rats, strain-related differences were not statistically significant.

3.3. Effects of N^{ω}-nitro-L-arginine methyl ester in mice treated with pentylenetetrazol or picrotoxin (s.c. or i.p.)

To characterize further the anticonvulsant effects of N^{ω}-nitro-L-arginine methyl ester in rodents, we assessed convulsions in mice while varying the route of systemic administration of GABA-inhibiting drugs, an important factor in the suppression of pentylenetetrazol-induced convulsions in rats by N^{ω}-nitro-L-arginine methyl ester. As in rats, convulsive indicators in mice occurred sooner following i.p. administration than following s.c. administration (Table 3). Although N^{ω}-nitro-L-arginine methyl ester failed to affect either myoclonus or clonus after the s.c. or i.p. administration of pentylenetetrazol, the inhibitor of NO synthase exerted clear anticonvulsant effects, significantly delaying the expression of tonus (hindlimb) in mice receiving pentylenetetrazol via either route. Contrasting these results, N^{ω}-nitro-L-arginine methyl ester minimally affected convulsions elicited by the s.c. administration of picrotoxin yet moderately delayed all convulsive consequences of picrotoxin administered i.p.

3.4. Effects of N^{ω}-nitro-L-arginine methyl ester on tonus in mice

Consistent with our observation that N^{ω}-nitro-L-arginine methyl ester increased the latency of pentylenetetrazol-induced tonus in mice, both N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine significantly raised the tonic CD_{50} (convulsive $dose_{50}$, dose of the convulsant (i.e. pentylenetetrazol) required to elicit tonus in 50% of the mice; Fig. 1A and B). Moreover, N^{ω}-nitro-L-arginine methyl ester significantly delayed the expression of tonus following the administration of 4-aminopyridine (15.2 mg/kg; s.c.): 4-aminopyridine-induced tonus occurred in controls ($n = 12$) and N^{ω}-nitro-L-arginine methyl ester-treated mice ($n = 12$) with mean latencies of 760.5 ± 35.4

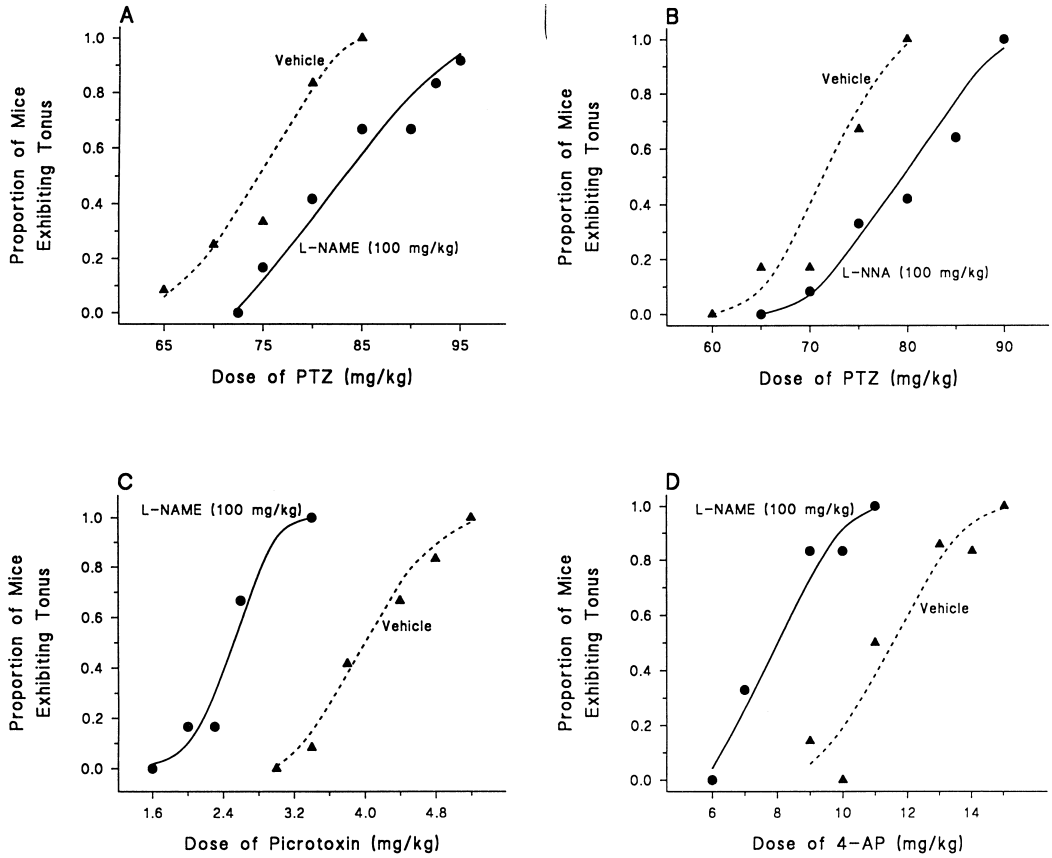

Fig. 1. Effects of inhibitors of NO synthase on tonus in mice. Both N^{ω}-nitro-L-arginine methyl ester (L-NAME; 100 mg/kg) and N^{G}-nitro-L-arginine (L-NNA; 100 mg/kg) substantially increased the dose of pentylenetetrazol (PTZ) required to elicit tonus in mice. (A) We estimated the tonic CD_{50} of pentylenetetrazol to be 74.1 mg/kg in vehicle-treated mice ($n = 72$) as compared to 83.5 mg/kg in mice treated with N^{ω}-nitro-L-arginine methyl ester ($n = 84$). (B) N^{G}-nitro-L-arginine similarly increased the tonic CD_{50} of pentylenetetrazol from 72.3 mg/kg (vehicle-treated mice; $n = 60$) to 79.4 mg/kg (N^{ω}-nitro-L-arginine methyl ester-treated mice; $n = 71$). In marked contrast, N^{ω}-nitro-L-arginine methyl ester reduced the tonic CD_{50} of (C) picrotoxin from 4.0 mg/kg (vehicle-treated mice, $n = 72$) to 2.5 mg/kg (N^{ω}-nitro-L-arginine methyl ester-treated mice; $n = 30$) and of (D) 4-aminopyridine (4-AP) from 11.3 mg/kg (vehicle-treated mice; $n = 40$) to 7.8 mg/kg (N^{ω}-nitro-L-arginine methyl ester-treated mice; $n = 30$). All differences were statistically significant, as determined with probit analysis (95% confidence intervals).

and 1100.9 ± 148.6 s, respectively, $t(22) = 2.23$, $P < 0.05$ (two-tailed). In striking contrast, N^{ω}-nitro-L-arginine methyl ester dramatically *reduced* the tonic CD_{50} of both picrotoxin (Fig. 1C) and 4-aminopyridine (Fig. 1D). Finally, N^{ω}-nitro-L-arginine methyl ester did not exert obvious antitonic actions against maximal electroshock, with 100% of controls ($n = 10$) and N^{ω}-nitro-L-arginine methyl ester-treated mice ($n = 9$) exhibiting tonic extension of the hindlimbs immediately following the delivery of current.

4. Discussion

4.1. Anti- and proconvulsant effects of inhibitors of NO synthase in picrotoxin-treated mice

Confirming our previous results (Kirkby et al., 1996a), N^{ω}-nitro-L-arginine methyl ester exerted either anti- or proconvulsant effects against systemically administered picrotoxin, a blocker of $GABA_A$ receptors, depending on the measure of seizure-sensitivity/susceptibility in mice. First, N^{ω}-nitro-L-arginine methyl ester modestly increased the latency of clonus following the administration of a moderate dose of picrotoxin (i.e. 7 mg/kg; s.c.). Second, N^{ω}-nitro-L-arginine methyl ester paradoxically increased the probability that mice would express clonus after an otherwise minimally effective dose of picrotoxin (i.e. 1.25 mg/kg; s.c.). Implicating the suppressed formation of NO in both the anti- and proconvulsant effects of N^{ω}-nitro-L-arginine methyl ester, which inhibits NO synthase but also blocks muscarinic receptors (Buxton et al., 1993), we obtained qualitatively similar anti- and proconvulsant effects with N^{G}-nitro-L-arginine, a nonesterified NO synthase-inhibiting analogue of L-arginine that unlike N^{ω}-nitro-L-arginine methyl ester lacks anticholinergic actions. Moreover, the less active enantiomers of N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine (i.e. N^{ω}-nitro-D-arginine methyl ester and N^{G}-nitro-D-arginine) failed to suppress or enhance picrotoxin-induced clonus. We previously observed that 7-nitroindazole, the NO synthase-inhibiting actions of which are reputedly restricted to the central nervous system in vivo (Moore et al., 1991, 1993; but see Kelly et al., 1994; Medhurst et al., 1994; Bland-Ward and Moore, 1995), delays clonus without increasing the probability of its occurrence in mice treated with picrotoxin (Kirkby et al., 1996a). We therefore proposed that the parallel suppression and enhancement of picrotoxin-induced clonus by N^{ω}-nitro-L-arginine methyl ester reflects inhibition of central and peripheral (i.e. vascular) NO synthase, respectively (Kirkby et al., 1996a). Our finding that N^{G}-nitro-L-arginine, also commonly viewed as a nonselective inhibitor of NO synthase, closely mimics both the anti- and proconvulsant effects of N^{ω}-nitro-L-arginine methyl ester in mice treated with picrotoxin is consistent with the hypothesis. We caution, however, that we have not related regional levels of NO to specific behavioral effects of inhibitors of NO synthase. Moreover, the relative regional selectivity of nitroindazole-derived compounds, including 7-nitroindazole, is not unequivocal (Kelly et al., 1994; Medhurst et al., 1994; Bland-Ward and Moore, 1995).

4.2. N^{ω}-nitro-L-arginine methyl ester in pentylenetetrazol-treated rats

Further assessing the role of NO in the regulation of seizures, we attempted to replicate the findings of Osonoe et al. (1994), who reported dramatic anticonvulsant effects of high doses of N^{ω}-nitro-L-arginine methyl ester in Wistar rats receiving pentylenetetrazol, as revealed by significantly delayed expression of myoclonus, clonus and tonus. Despite using the same dose of pentylenetetrazol as Osonoe et al. (1994) and a dose of N^{ω}-nitro-L-arginine methyl ester reportedly in the behaviorally effective range (Osonoe et al., 1994), we failed to observe such anticonvulsant effects in either Wistar or Sprague–Dawley rats. In fact, Sprague–Dawley rats treated with N^{ω}-nitro-L-arginine methyl ester actually exhibited shortened latencies of myoclonus and clonus, suggesting strain-dependent proconvulsant effects of N^{ω}-nitro-L-arginine methyl ester, a conclusion consistent with our previous observation that N^{ω}-nitro-L-arginine methyl ester significantly shortens the latency of kainate-induced clonus in Sprague–Dawley but not Wistar rats (Kirkby et al., 1996a). In this context, it is noteworthy that investigators claiming unambiguous anticonvulsant effects of inhibitors of NO synthase have tended to employ Wistar rats (De Sarro et al., 1991, 1993; Mollace et al., 1991; Bagetta et al., 1992; Mulsch et al., 1994; Osonoe et al., 1994; Becker et al., 1995), with the notable exceptions of Przewlocka et al. (1994), Van Leeuwen et al. (1995), Hara et al. (1996) and Yamamoto (1995, 1996), who employed mice and Smith et al. (1996) who employed genetically seizure-susceptible rats and mice; previous studies unambiguously demonstrating proconvulsant effects of inhibitors of NO synthase used either mice (Buisson et al., 1993; Starr and Starr, 1993; Penix et al., 1994; Przegalinski et al., 1994, 1996; Kirkby et al., 1996a,b), Sprague–Dawley rats (Haberny et al., 1992; Rondouin et al., 1993; Penix et al., 1994; Maggio et al., 1995; Kirkby et al., 1996a), or hooded rats (Herberg et al., 1995), with notable exceptions being the studies of Rigaud-Monnet et al. (1994) and Wang et al. (1994), which involved Wistar rats. In addition, Grooms and Jones (1994) reported enhanced stimulus train-induced bursting following the application of inhibitors of NO synthase to hippocampal slices taken from Sprague–Dawley rats. The only other direct test of the strain-related hypothesis proved inconclusive, however, as the focal administration of N^{ω}-nitro-L-arginine methyl ester failed to alter seizures provoked in either Wistar or Sprague–Dawley rats by the infusion of kainate into the deep prepiriform cortex (Proctor et al., 1997).

The rats employed by Osonoe et al. (1994) were considerably lighter and presumably younger than those initially used in our study. Because development-dependent changes in both seizure-susceptibility and the impact of particular drugs on seizures have been reported (e.g. Mareš et al., 1983), it is possible that our failure to confirm the previously published results reflects age-related factors. We thus repeated our experiments employing rats similar in weight and hence age to those used in the study of Osonoe et al. (1994). As expected, convulsive responses to pentylenetetrazol occurred somewhat sooner in the lighter (younger) control rats as compared to the heavier (older) control rats used in our previous experiment, indicating age-related decreases in seizure-susceptibility. However, N^{ω}-nitro-L-arginine methyl ester once again failed to delay convulsions. It thus appears that developmental variables cannot independently account for our initial inability to suppress pentylenetetrazol-induced convulsions in rats with N^{ω}-nitro-L-arginine methyl ester.

An important distinction between our results and those of Osonoe et al. (1994) pertains to basal responses to pentylenetetrazol. To elaborate, Osonoe et al. (1994) reported that all behavioral components of the convulsive syndrome (i.e. myoclonus, clonus and whole-body tonus) reliably occurred at very short latencies in control rats, with mean latencies of myoclonus and clonus being less than one quarter the length of those recorded in our study, even in lighter (younger) rats. Moreover, despite using a dose of pentylenetetrazol identical to that used by Osonoe et al. (1994) (i.e. 80 mg/kg), tonus occurred only unreliably in control rats of either strain or of either range of weight and hence age (i.e. Wistar and Sprague–Dawley; 210–260 and 120–170 g). Because Osonoe et al. (1994) injected pentylenetetrazol i.p., whereas we injected pentylenetetrazol s.c., we speculated that the anticonvulsant effects of N^{ω}-nitro-L-arginine methyl ester in rats may reflect the route of systemic administration of the convulsant drug. To test the hypothesis, we repeated the experiment yet again, this time administering pentylenetetrazol i.p. and our results were highly similar to those of Osonoe et al. (1994). First, myoclonus, clonus and tonus occurred at very short latencies in control rats of either strain. Second, N^{ω}-nitro-L-arginine methyl ester significantly increased the latencies of all convulsive components and, in some rats, prevented clonus and/or tonus.

4.3. Effects of N^{ω}-nitro-L-arginine methyl ester in pentylenetetrazol- and picrotoxin-treated mice

The dependence of the anticonvulsant effects of N^{ω}-nitro-L-arginine methyl ester on the route of systemic administration of inhibitors of $GABA_A$ receptors was somewhat less obvious in mice than in rats. First, N^{ω}-nitro-L-arginine methyl ester did not influence myoclonus or clonus but significantly delayed tonus in mice, regardless of the route of administration of pentylenetetrazol. On the other hand, whereas N^{ω}-nitro-L-arginine methyl ester was only minimally effective against a very high dose of picrotoxin administered s.c. (i.e. 30 mg/kg), the inhibitor of NO synthase significantly delayed myoclonus, clonus and tonus induced by picrotoxin administered i.p. It is possible that our failure to demonstrate anticonvulsant effects of the highest dose of N^{ω}-nitro-L-arginine methyl ester (i.e. 100 mg/kg) in mice treated with picrotoxin (30 mg/kg; s.c.) merely reflects chance events, because we have invariably obtained seizure-delaying effects of this dose of N^{ω}-nitro-L-arginine methyl ester with doses of picrotoxin $\geq 3.5–4.0$ mg/kg (Kirkby et al., 1996a; but see Hara et al., 1996). To the contrary, this constitutes our first attempt to assess the effects of N^{ω}-nitro-L-arginine methyl ester in mice treated with a dose of picrotoxin > 20 mg/kg, which may indicate an upper limit of the experimentally useful dose of s.c. picrotoxin (dictating our use of the higher dose of picrotoxin (i.e. 30 mg/kg) in the present series of experiments was the desire to equate baseline responses of control mice receiving picrotoxin with those of control mice receiving pentylenetetrazol (limits of solubility of picrotoxin precluded our achievement of this end, with solutions being injected in volumes of 10 ml/kg). To summarize, although the results of the experiments involving mice are suggestive, they do not unequivocally demonstrate that N^{ω}-nitro-L-arginine methyl ester preferentially exerts anticonvulsant effects in mice treated i.p. (as opposed to s.c.) with inhibitors of $GABA_A$ receptors.

4.4. Inhibitors of NO synthase as antitonic agents

Based on a striking N^{ω}-nitro-L-arginine methyl ester-induced increase in latency of tonus observed in rats treated with pentylenetetrazol, Osonoe et al. (1994) postulated pronounced antitonic capacities of N^{ω}-nitro-L-arginine methyl ester, a view consistent with several aspects of our data. First, we replicated the findings of Osonoe et al. (1994) regarding the arguably disproportionate latency-increasing effects of high-dose N^{ω}-nitro-L-arginine methyl ester on pentylenetetrazol-induced tonus (relative to myoclonus and clonus) in rats, provided that the pentylenetetrazol was injected i.p. (see Table 2). Second, we found in mice that N^{ω}-nitro-L-arginine methyl ester significantly delayed the expression of tonus but not myoclonus or clonus, regardless of the systemic route of administration of pentylenetetrazol. Third, both N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine significantly *increased* the tonic CD_{50} of pentylenetetrazol, but N^{ω}-nitro-L-arginine methyl ester failed to affect the clonic CD_{50} (unpublished data), further suggesting a relatively selective antitonic influence. Finally, N^{ω}-nitro-L-arginine methyl ester significantly increased the latency of 4-aminopyridine-induced tonus. In marked contrast to these observations, however, N^{ω}-nitro-L-arginine methyl ester dramatically decreased the tonic CD_{50} of both picrotoxin and 4-amino-

pyridine in mice (note that the convulsants were delivered s.c. rather than i.p.) and invariably failed to block the expression of tonus following the delivery of maximal electroshock. It therefore appears that the substantial antitonic effects of high-dose N^{ω}-nitro-L-arginine methyl ester are restricted to rodents treated with pentylenetetrazol (i.p. in rats, it is noteworthy, however, that we did not assess anticonvulsant effects of N^{ω}-nitro-L-arginine methyl ester in rats treated with a s.c. dose of pentylenetetrazol sufficient to elicit tonus reliably, as was done in mice). It is also possible that nonspecific actions of the inhibitor of NOS are involved (Przegalinski et al., 1996). Hence, it is unlikely that N^{ω}-nitro-L-arginine methyl ester acts as a general antitonic agent.

The opposing influences of N^{ω}-nitro-L-arginine methyl ester on the tonic CD_{50} of pentylenetetrazol and of picrotoxin are particularly striking given that both convulsants act at the picrotoxinin site (within the GABA-sensitive chloride ionophore; Woodbury, 1980; Klunk et al., 1983; Ramanjaneyulu and Ticku, 1984; Levine et al., 1985). Differential effects of specific treatments on pentylenetetrazol- and picrotoxin-induced convulsions are not unprecedented, however. Parallelling our findings, for example, the central administration of the peptide endothelin-3, which like N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine promotes vasoconstriction (Yanagisawa and Mamasaki, 1989), decreased the proportion of mice expressing pentylenetetrazol-induced tonus, suggesting an anticonvulsant effect, while exacerbating picrotoxin-induced convulsions (Getova et al., 1994). In addition, Eigyo et al. (1994) reported that a potent antihypertensive blocker of L-channels, S-312-d, decreased the incidence of pentylenetetrazol- but not picrotoxin-induced clonus. Developmental studies may also be relevant in this light, as early postnatal exposure to caffeine chronically increased the myoclonic threshold of i.v. pentylenetetrazol, as determined in 4-week-, 6-week- and 10-week-old rats; elevated thresholds of picrotoxin-induced myoclonus were delayed and transient, by contrast, evident only in 6-week-old rats (Guillet, 1995). Complementing these observations, the convulsive thresholds of pentylenetetrazol and picrotoxin were unchanged and reduced, respectively, as determined in adult rats exposed to diazepam in utero (Bitran et al., 1991). Mechanisms influencing and mediating pentylenetetrazol- and picrotoxin-induced convulsions are clearly not unitary, therefore, being pharmacologically and developmentally dissociable, perhaps involving either an incomplete overlap of binding of pentylenetetrazol and picrotoxin to the picrotoxinin site (Holland et al., 1992) or nonspecific effects of pentylenetetrazol on membranes (Velisek et al., 1995).

4.5. Conclusions

The behavioral interactions between inhibitors of NO synthase (like N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine) and the route of administration of convulsant drugs (systemic versus central (Kirkby et al., 1996a) and one systemic route (s.c.) versus another (i.p.), as demonstrated in rats in the present study) suggest at least some indirect effects on seizures and hence neuronal excitability (Proctor et al., 1997): As noted above, we have provided preliminary behavioral evidence that N^{ω}-nitro-L-arginine methyl ester and N^{G}-nitro-L-arginine promote picrotoxin-induced seizures by inhibiting vascular NO synthase (Kirkby et al., 1996a; but see Kelly et al., 1994; Medhurst et al., 1994; Bland-Ward and Moore, 1995). Further complicating matters, Smith et al. (1996) recently proposed that inhibitors of NO synthase may alter seizures NO-independently, suppressing the stereochiometric formation of L-citrulline and promoting the accumulation of L-arginine. In addition, the impact of inhibitors of NO synthase on the kinetics of chemoconvulsants has not to our knowledge been assessed. By virtue of highly varied experimental outcomes and interpretations within the literature, the contributions of endogenous NO to the expression of seizures and their cellular/molecular sequelae (Bagetta et al., 1992, 1993; Haberny et al., 1992; Stringer and Erden, 1995) thus remain mysterious. Coupling the uncertainty with the general tendency of studies uncompromised by such pharmacokinetic considerations (i.e. those involving electrically induced seizures, in vivo or in vitro) to reveal either mixed, null, or seizure-promoting effects of inhibitors of NO synthase but not uniform seizure-suppressing effects (Rondouin et al., 1992; Grooms and Jones, 1994; Rundfeldt et al., 1995; Stringer and Erden, 1995), it seems improbable that such compounds will become clinically useful anticonvulsants (Herberg et al., 1995).

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References

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

Bagetta, G., Corasaniti, M.T., Melino, G., Paoletti, A.M., Finazzi-Agrò, A., Nisticò, G., 1993. Lithium and tacrine increase the expression of nitric oxide synthase mRNA in the hippocampus of rat. Biochem. Biophys. Res. Commun. 197, 1132–1139.

Becker, A., Grecksch, G., Schröder, H., 1995. N^{ω}-nitro-L-arginine methyl ester interferes with pentylenetetrazol-induced kindling and has no effect on changes in glutamate binding. Brain Res. 688, 230–232.

Bitran, D., Primus, R.J., Kellogg, C.K., 1991. Gestational exposure to diazepam increases sensitivity to convulsants that act at the GABA/benzodiazepine receptor complex. Eur. J. Pharmacol. 196, 223–231.

Bland-Ward, P.A., Moore, P.K., 1995. 7-Nitro indazole derivatives are potent inhibitors of brain, endothelium and inducible isoforms of nitric oxide synthase. Life Sci. 57, PL131–PL135.

Buisson, A., Lakhmeche, N., Verrecchia, C., Plotkine, M., Boulu, R.G., 1993. Nitric oxide: An endogenous anticonvulsant. NeuroReport 4, 444–446.

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, 387–395.

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. Fund. 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.

Eigyo, M., Shiomi, T., Inoue, Y., Sakaguchi, I., Ishibashi, C., Murata, S., Koyabu, K., Matsushita, A., Adachi, I., Ueda, M., 1994. Pharmacological studies on a new dihydrothienopyridine calcium antagonist, S-312-d. 5th Communication: Anticonvulsant effects in mice. Jpn. J. Pharmacol. 65, 175–177.

Getova, D.P., Maslarova, J.E., Nikolov, R.K., Semkova, I.S., 1994. Effects of endothelin-1 (ET-1) and endothelin-3 (ET-3) on convulsive seizures. Methods Find. Clin. Pharmacol. 16, 173–178.

Grooms, S.Y., Jones, L.S., 1994. Hippocampal in vitro kindling is not blocked by nitric oxide synthase inhibitors. NeuroReport 5, 1102–1104.

Guillet, R., 1995. Neonatal caffeine exposure alters seizure susceptibility in rats in an age-related manner. Dev. Brain Res. 89, 124–128.

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.

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

Holland, K.D., McKeon, A.C., Canney, D.J., Covey, D.F., Ferrendelli, J.A., 1992. Relative anticonvulsant effects of GABAmimetics and GABA modulatory agents. Epilepsia 33, 981–986.

Kelly, P.A.T., Ritchie, I.M., Arbuthnitt, G.W., 1994. 7-Nitro indazole uncouples cerebral blood flow from metabolic demand in the conscious rat. Br. J. Pharmacol. 112, 348P.

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

Kirkby, R.D., Forbes, R.A., Subramaniam, S., 1996b. Modification of kainate-induced behavioral and electrographic seizures following inhibition of nitric oxide synthase in mice. Epilepsy Res. 24, 79–90.

Klunk, W.E., Kalman, B.L., Ferrendelli, J.A., Covey, D.A., 1983. Computer-assisted modeling of the picrotoxinin and γ-butyrolactone receptor site. Mol. Pharmacol. 23, 511–518.

Levine, J.A., Ferrendelli, J.A., Covey, D.F., 1985. Convulsant and anticonvulsant butyrolactones bind at the picrotoxinin/*t*-butylbicyclophosphorothionate (TBPS) receptor. Biochem. Pharmacol. 23, 4187–4190.

Maggio, R., Fumagalli, F., Donati, E., Barbier, P., Racagni, G., Corsini, 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.

Mareš, P., Hlavatá, J., Lišková, K., Mudrochová, M., 1983. Effects of carbamazepine and diphenylhydantoin on metrazol seizures during ontogenesis in rats. Physiol. Bohemoslov. 32, 92–96.

Medhurst, A.D., Greenlees, 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–R6.

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

Moore, P.K., Babbedge, R.C., Wallace, P., Gaffen, Z.A., Hart, S.L., 1991. 7-Nitro indazole, 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., Wallace, P., Gaffen, Z., Hart, S.L., Babbedge, R.C., 1993. Characterization of the novel nitric oxide synthase inhibitor 7-nitro indazole and related indazoles: Antinociceptive and cardiovascular effects. Br. J. Pharmacol. 110, 219–224.

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

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.

Proctor, M.R., Fornai. F., Afshar, J.K.B., Gale, K., 1997. The role of nitric oxide in focally-evoked limbic seizures. Neuroscience. In press.

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

Przegalinski, E., Baran, L., Siwanowicz, J., 1996. The role of nitric oxide in chemically- and electrically-induced seizures in mice. Neurosci. Lett. 217, 145–148.

Przewlocka, B., Lasoń, W., Macheleska, H., Przewlocki, R., 1994. The effects of cocaine-induced seizures on the proenkephalin mRNA level in the mouse hippocampus: A possible involvement of the nitric oxide pathway. Neurosci. Lett. 168, 81–84.

Ramanjaneyulu, R., Ticku, M.K., 1984. Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor–ionophore complex. Eur. J. Pharmacol. 98, 337–345.

Rigaud-Monnet, A.-S., Pinard, E., Borredon, J., Seylaz, J., 1994. Blockade of nitric oxide synthesis inhibits hippocampal hyperemia in kainic acid-induced seizures. J. Cereb. Blood Flow Metab. 14, 581–590.

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.

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.

Rundfeldt, L., Koch, R., Richter, A., Meuissen, M., Gerecke, V., Loscher, 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.

Smith, S.E., Man, C.M., Yip, P.K., Tang, E., Chapman, A.G., Meldrum, B.S., 1996. Anticonvulsant effects of 7-nitroindazole in rodents with reflex epilepsy may results from L-arginine accumulation or a reduction in nitric oxide or L-citrulline formation. Br. J. Pharmacol. 119, 165–173.

Starr, M.S., Starr, B.S., 1993. Paradoxical 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.

Stringer, J.L., Erden, F., 1995. In the hippocampus in vivo, nitric oxide does not appear to function as an endogenous antiepileptic agent. Exp. Brain Res. 10, 391–401.

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

Velisek, L., Veliskova, J., Moshe, S.L., 1995. Developmental seizure models. Ital. J. Neurol. Sci. 16, 127–133.

Wang, Q., Theard, M.A., Pellegrino, D.A., Baughman, V.L., Hoffman, W.E., Albrecht, R.F., Cwik, 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.
Woodbury, D.M., 1980. Convulsant drugs: Mechanisms of action. In: Glaser, G.H., Penry, J.K., Woodbury, D.M. (Eds.), Antiepileptic Drugs: Mechanisms of Action. Raven Press, New York, pp. 249–303.
Yamaguchi, S., Rogawski, M.A., 1992. Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. Epilepsy Res. 11, 9–16.
Yamamoto, H.-A., 1995. A hypothesis for cyanide-induced tonic seizures with supporting evidence. Toxicology 95, 19–26.
Yamamoto, H.-A., 1996. Preventive effect of N^G-nitro-L-arginine against L-cysteine-induced seizures in mice. Toxicol. Lett. 84, 1–5.
Yanagisawa, M., Mamasaki, T., 1989. Endothelin, a novel endothelium-derived peptide: Pharmacological activities, regulation and possible roles in cardiac control. Biochem. Pharmacol. 38, 1877–1883.