in vivo *18*: 357-366 (2004)

Review

Nitric Oxide and Brain Hyperexcitability

GIUSEPPE FERRARO and PIERANGELO SARDO

Dipartimento di Medicina sperimentale, Sezione di Fisiologia umana "G. Pagano", Università degli Studi di Palermo, Corso Tuköry, 129, 90134 Palermo, Italy

Abstract. *Nitric oxide (NO) is a gaseous messenger involved in atypical forms of intercellular communications, able to exert a strong functional modulation of several neurotransmitter systems. In particular, NO heavily influences the excitatory neurotransmitter glutamate, mainly through NMDA receptors, and the inhibitory neurotransmitter GABA, mainly through GABA A receptors. Due to the involvement of glutamate and GABA in a delicate balance conditioning the functional status of the neural cells, this interaction suggests a role for NO in regulating neuronal excitability and its transition towards hyperexcitability phenomena. This article reviews the main knowledge about the relationships existing between the activity of the NO system and the experimental aspects of epilepsy, focusing on the somewhat antithetic findings about the proconvulsant or the anticonvulsant roles exerted by nitric oxide.*

Nitric oxide

Nitric oxide (NO) is a labile, gaseous messenger first identified in the blood vessels as the endothelial-derived relaxation factor (1, 2). In the brain it is able to mediate many neuronal processes, thus influencing the cell's activity. In fact, it seems to play a crucial role in a new form of interneuronal communication, characterised by the absence of synaptic contacts and a high affinity and selectivity between the released transmitter and the affected receptors (3, 4). It also participates in the modulation of the release of classical neurotransmitters, representing a physiological linkage between synaptic and non synaptic interactions in the context of brain function. Through this modulatory action, NO strongly influences the excitability status of neurones, either in basal conditions or during paroxysmal activity.

Biosynthesis and mechanism of action. NO is synthesised directly from arginine by nitric oxide synthase (NOS), which is able to catalyse the oxidation of one of the guanidine groups of arginine, to produce NO through the formation of citrulline. Three different isoforms of NOS have been identified: neuronal NOS (nNOS), endothelial NOS (eNOS) and macrophage or inducible NOS (iNOS) (5-7). In order to exert their actions, all NOS isoforms need co-factors represented by flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and nicotinamide adenine dinucleotide phosphate (NADPH). NOS is divided into two sub-units: the catalytic site, which is able to oxidise L-arginine, and the binding site for L-arginine. Both sub-units are constituted by both a reductase and an oxygenase domain, which are able to bind calmodulin in a Ca^{2+}-dependent manner. The Ca^{2+}-calmoduline complex regulates only nNOS and eNOS; on the contrary, the iNOS shows Ca^{2+}-independent properties (3, 8). nNOS is Ca^{2+}- and NADPH-dependent, in fact the influx of Ca^{2+} activates nNOS through the phosphorylation of a protein kinase C (9). eNOS shows constitutive characteristics similar to those of nNOS and several brain areas contain, at the same time, nNOS and eNOS (10). NO can easily reach the membranes of adjacent neurones (until 300 μm), it is not characterised by polarity and it presents a half-life of a few seconds (11). NO penetrates the neuronal membrane and activates a soluble guanylyl cyclase (sGNC), a heterodimeric molecule which contains a heme group characterised by a high affinity for NO, to form cGMP. In fact, the experimental use of inhibitors for guanylyl cyclase, such as methylene blue or LY83583, blocks the modifications induced by the local application of NO donors (12). On the other hand, cGMP acts in the context

Correspondence to: Prof. Giuseppe Ferraro, Dipartimento di Medicina sperimentale, Sezione di Fisiologia umana "G. Pagano", Università degli Studi di Palermo, Corso Tuköry, 129, 90134 Palermo, Italy. Tel: ++390916555811, Fax:++390916520701, e-mail: vlgfisio@unipa.it

Key Words: Nitric oxide, glutamate, GABA, epilepsy, review.

of neurones through several mechanisms: i) modification of the activity of Ca^{2+} and Na^{+} channels, ii) activation of protein kinase and iii) modulation of phosphodiesterase (11). Furthermore, other experimental investigations have demonstrated cGMP-independent NO modality of action: i) NO can rapidly interact with superoxide anions to form peroxynitrite (ONOO-) which causes cell death (13); ii) NO is also able to S-nitrosylate ion-channels through a slow kinetic; this mechanism is involved in the control of membrane excitability and in more complex cerebral circuits (14).

Distribution of NO synthesising system. The NO synthesising system is distributed throughout the CNS but the co-localisation between the NOS system and sGNC has been only occasionally described. This experimental observation, together with the spatial proximity between NOS-positive and sGNC-positive structures, demonstrates that NO can function as an intercellular neuronal messenger in the range of 20-100 μm (11, 15). A lot of *in vivo* and *in vitro* techniques have been used to reveal the presence of NOS and sGNC throughout the brain (15). NOS and cGMP activities were found in the context of frontal, parietal, cingulate and piriform cortices. Similarly, the caudate-putamen complex and the globus pallidus showed abundance of NOS activity. In the context of the diencephalon, lateral and medial habenular nuclei revealed the presence of NOS and cGNC activities, but in thalamic reticular formation, in the subthalamic nucleus, hypothalamus, suprachiasmatic nucleus, nucleus arcuate, median eminence and mammillary region no co-localisation between the two activities was highlighted. In the context of the mesencephalon, an abundant presence of NOS activity was revealed in the substantia nigra and pretectal nucleus. In the cerebellum, and in particular in the context of Purkinjie cells, it is possible to evidence the existence of a co-localisation between NOS and cGNC activities. Also pons and medulla oblongata show rich NOS and cGNC activities. An alternative modality to detect the NOS activity was performed through the evaluation of the distribution of NOS mRNA using an antisense-strand probe. Higher levels of nNOS mRNA were evidenced in the context of the olfactory bulb and in the cerebellum, in the caudate/ putamen complex associated with the entire ventral striatum structures. Furthermore, in the hippocampus a diffuse presence of neurones exhibiting a moderate but significant presence of nNOS mRNA was highlighted, associated with small areas with a strong presence of nNOS mRNA (16).

Interaction with glutamate and GABA

Much research has demonstrated that NO or NO donors are capable of significantly increasing the release of glutamate from excitatory synapses (17,18). Subsequently, other experimental observations have demonstrated that the modulation of glutamate release is strictly related to the level of NO in the context of cerebral tissue. In fact, low concentrations of cerebral NO or treatment with low doses of NO donors caused the reduction of neuronal glutamate release (19). On the contrary, in the context of the same experimental observations, elevated intracerebral NO levels and/or NO donor treatment at high concentrations caused a significant increase of glutamate outflow (19). The biphasic effect seems to be strictly linked to the intracellular level of cGMP, which is involved in mediating the glutamatergic response. Several experimental lines have demonstrated that the functional interaction between NO and glutamate could take place at different levels of the synaptic terminal. In particular, NO may act at the level of the pre-synaptic membrane by inhibiting glutamate release through the functional inactivation of the thiol group of the redox site of the NMDA receptor complex (20-22). Furthermore, the reduction of glutamate availability induced by NO through the removal of extracellular glutamate by activation of glial cells has also been demonstrated (23). Finally, a post-synaptic functional interaction has been reported by a down-regulation of NMDA receptors in strict relation to the extracellular levels of NO or free radicals including superoxide (21, 24). All the reported experimental observations demonstrate that the interaction between NO and glutamate in the context of a putative neuroprotective or neurotoxic effect still remains controversial. In fact, the reduction of cerebral NO levels, obtained through systemic or intracerebral administration of NOS inhibitors, causes a significant increase of extracellular glutamate and ensuing ischemic/neurotoxic damage (25, 26). Research has also demonstrated that NO is produced in response to NMDA receptor activation. In fact, during sustained depolarization of NMDA-type glutamatergic post-synaptic receptors, the following calcium influx causes the activation of nNOS (17).

Although NO is widely considered to be functionally linked to glutamate activity, a relationship between NO activity and the inhibitory GABA has been highlighted (27). In particular, it has been demonstrated that NO or NO donors, such as 3-morpholinosidnonimine (SIN1) or S-Nitroso-N-acetylpenicillamine (SNAP), or NO precursors, such as L-arginine, increase inhibitory post-synaptic potentials (IPSP) frequency as the result of local activation of GABAergic neurones (28, 29). On the other hand, many experimental observations have demonstrated a co-localisation between NOS and GABA (*e.g.* in the context of cuneate nucleus, periaqueductal grey, ventral geniculate nucleus and the I and II superficial laminae of the spinal dorsal horn) (30-33). More recently, it has been shown that the different isoforms of NOS activity are able to

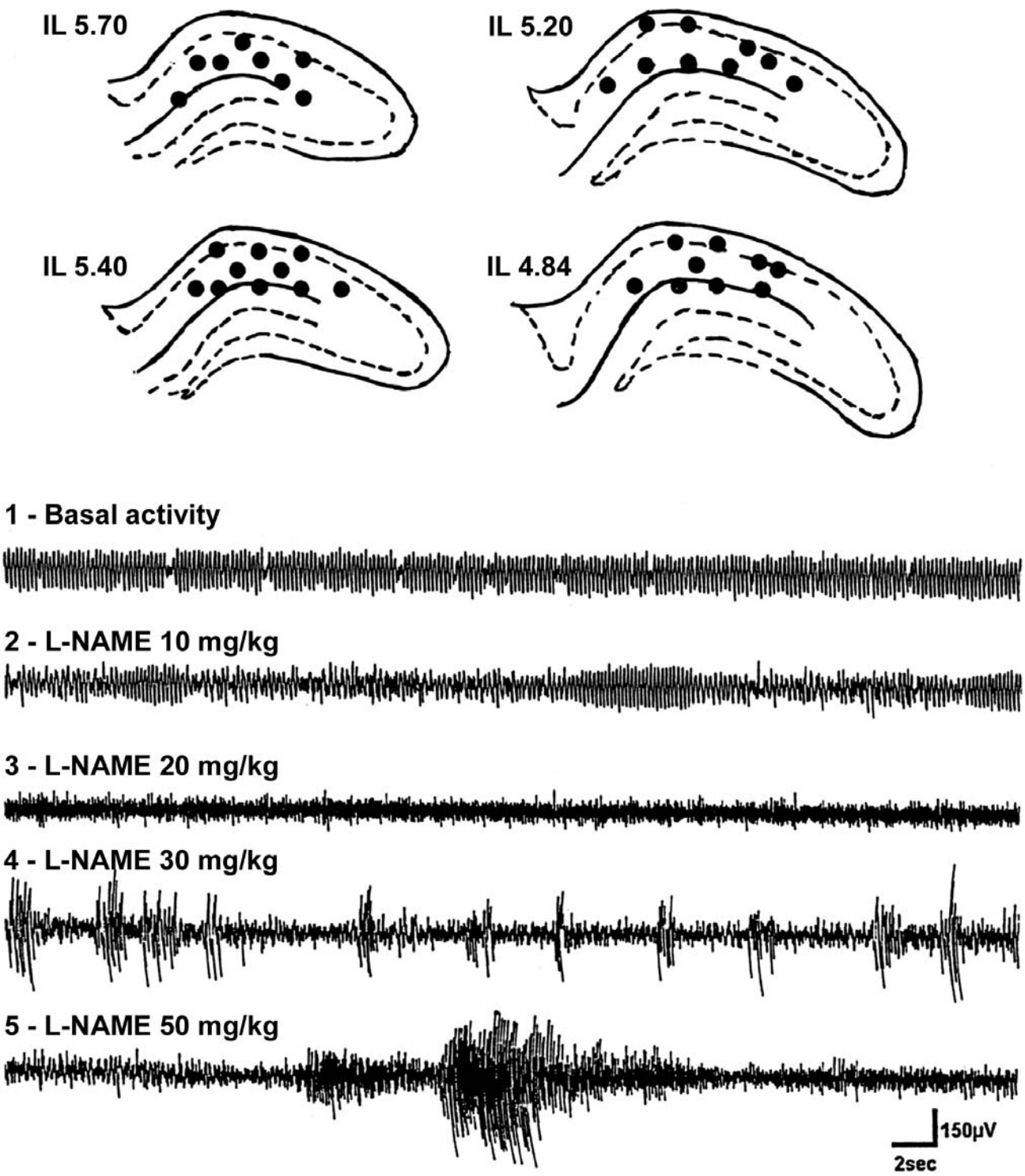

Figure 1. *Depth EEG recordings in the hippocampus of male urethane-anaesthetised Wistar rats (220 - 280 g) treated with increasing doses of L-NAME (0.5 - 50 mg/kg body weight, intraperitoneal). Top : locations of the coaxial bipolar recording electrodes in the sterotaxic planes explored (IL : interaural line). Bottom: representative EEG traces showing the effects of different doses of L-NAME on the bioelectric hippocampal activity. Trace 1 : spontaneous basal activity. Traces 2, 3, 4, 5 : activity recorded after L-NAME administration. Note the interictal epileptic activity in trace 4 and the afterdischarge in trace 5. All effects evoked by L-NAME treatment had an onset latency of 5-10 min and a duration of 30-39 min (modified, from Ferraro et al., Epilepsia 40: 830-836, 1999).*

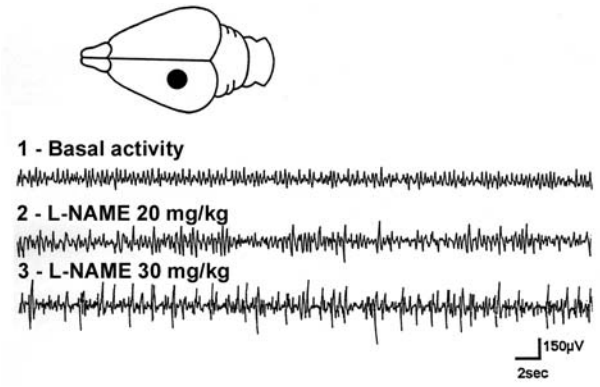

Figure 2. *Surface EEG recordings in the somatosensory cortex of male Wistar rats (same experimental conditions as in Fig. 1) treated with L-NAME (0.5 – 50 mg/kg body weight, intraperitoneal). Top : location of the cortical silver chloride ball electrodes. Bottom : representative EEG traces showing the effects of different doses of L-NAME on the bioelectric activity of the somatosensory cortex. Trace 1 : spontaneous basal activity. Traces 2 and 3 : cortical interictal activity after L-NAME administration. All effects evoked by L-NAME treatment had an onset latency of 7-10 min and a duration of 30-34 min (modified, from Ferraro et al., Epilepsia 40: 830-836, 1999).*

simultaneously modulate the excitatory *versus* inhibitory neurotransmission, probably in relation to their specific neuronal localisation (34). All the data suggest that NO seems to be a linkage between excitatory and inhibitory neurotransmission in physiological and pathological conditions and it functions as an efficacious regulator of neuronal excitability.

Interaction with other neurotransmitters

A functional relationship between NO activity and cholinergic neurotransmission has been demonstrated. In particular, NO could have an anterograde and/or retrograde influence on different cholinergic neurones and the majority of cholinergic neurones, particularly in the context of the cerebral cortex, basal ganglia and hippocampus, is coupled to NO-mediated cGMP synthesis (35, 36). An indirect NO-mediated increase of acetylcholine (ACh) release has been demonstrated in the hippocampal formation (37). Furthermore, endogenous NO is able to influence neighbouring excitatory neurones which, in turn, increase the cholinergic input (38).

In various regions of the CNS a functional relationship between NO and dopamine (DA) has been demonstrated (39). In particular, the experimental treatment with L-arginine, a precursor of NO, or NO donors, such as hydroxylamine, sodium nitroprusside (SNP) and Roussin's black salt (RBS), induces an augmentation of the DA release, suggesting that endogenous NO stimulates DA activity (40). More recently, in the context of the frontal cortex and raphe nucleus a biphasic effect of NO on NMDA-evoked release of DA was demonstrated, suggesting a crucial role exerted by the redox state of the NMDA receptor and the ability of NO to influence the various subtypes of GLU receptors in a different manner (41).

Studies performed on hippocampal slices demonstrate a direct relationship between NO or NO donors and the increase of the release of norepinephrine (42). Furthermore, NO is capable of increasing the release of noradrenaline induced by NMDA receptor activation in the context of both the hippocampus and the cerebral cortex (43, 44).

The relationship between NO and serotonin has been investigated in the context of the striatum, locus coeruleus and hypothalamus. In particular, it has been demonstrated that NO donors increase the serotonin production in the striatum (45). On the contrary, the NO-induced hypothalamic influence shows a biphasic effect: NO donors, administered at low or high doses, cause a decrease or increase of serotonin production, respectively, and all these effects are mediated by cGMP functional involvement (46). Finally, in the locus coeruleus, NO donors or L-arginine treatment causes an increase of serotonin production (47). All these experimental results show a significant relationship between cerebral levels of NO and the augmentation of serotonergic neurotransmission, but a controversy still remains about a functional involvement of glutamate neurotransmission in this interaction.

Nitric oxide and experimental epilepsy

Nitric oxide is supposed to have an important role in the genesis and/or the maintenance of several diseases of the CNS. In particular, in the last 10 years a strong influence of NO in various experimental models of epilepsy has been documented. However, definitive conclusions are still not available about an anti-convulsant and/or pro-convulsant role exerted by NO.

Evidence for an anticonvulsant role. The first hypotheses about an anticonvulsant/neuroprotective role exerted by NO were reported ten years ago and the term "endogenous" anticonvulsant substance was introduced in the literature in relation to the functional action of NO in physiology and pathology (48). In particular, in an experimental model of generalised epilepsy, induced by the intraventricular injection of NMDA, the pharmacological blockade of nNOS caused an increase of both the duration and the severity of seizures (48). Subsequently, a direct relationship between the decrease of cerebral NO levels and a facilitatory effect on the genesis and/or the course of both focal and generalised seizures was demonstrated (49-51). Moreover, the use of a precursor such

Table I. *Nitric oxide as anticonvulsant.*

Model	Animal	Structure	Reference
NMDA	Mice	Gen. seizures	Buisson *et al.* (1993) (48)
LTP	Rat	CA1	Williams *et al.* (1993) (88)
Kindling	Rat	Gen. seizures	Herberg *et al.* (1995) (52)
Iron ion	Rat	Cerebral cortex	Kabuto *et al.* (1996) (50)
Aminopyridine	Rat	Neocortex	Boda *et al.* (1996) (49)
PTZ	Mice	Gen. seizures	Przegalinski *et al.* (1996) (51)
DMCM	Mouse	Gen. seizures	Tsuda *et al.* (1997) (58)
Kainate	Rat	Hippocampus	Alabadı *et al.* (1999) (89)
Basal conditions	Rat	Hippocampus/cortex	Ferraro *et al.* (1999) (59)

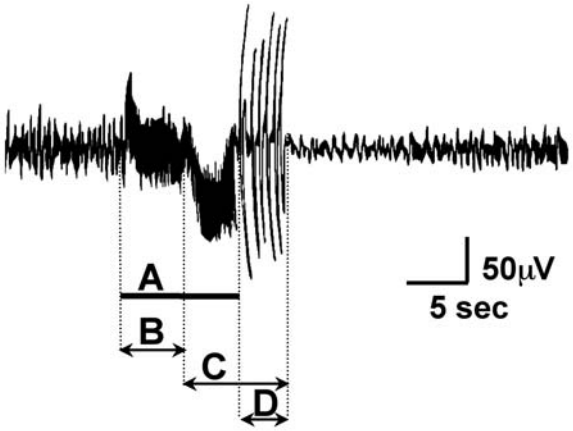

Figure 3. *Studied parameters of Maximal Dentate gyrus Activation (MDA) obtained in male urethane-anaesthetised Wistar rats (220 – 280 g) through the electrical stimulation of the Angular Bundle for 10 sec (train of 0.3 ms biphasic pulses, intensity 100 – 800 μA, 20 Hz). A: Angular Bundle stimulating train duration. B: MDA onset latency. C: MDA duration. D: afterdischarge duration. Spike amplitude, number and frequency were also measured.*

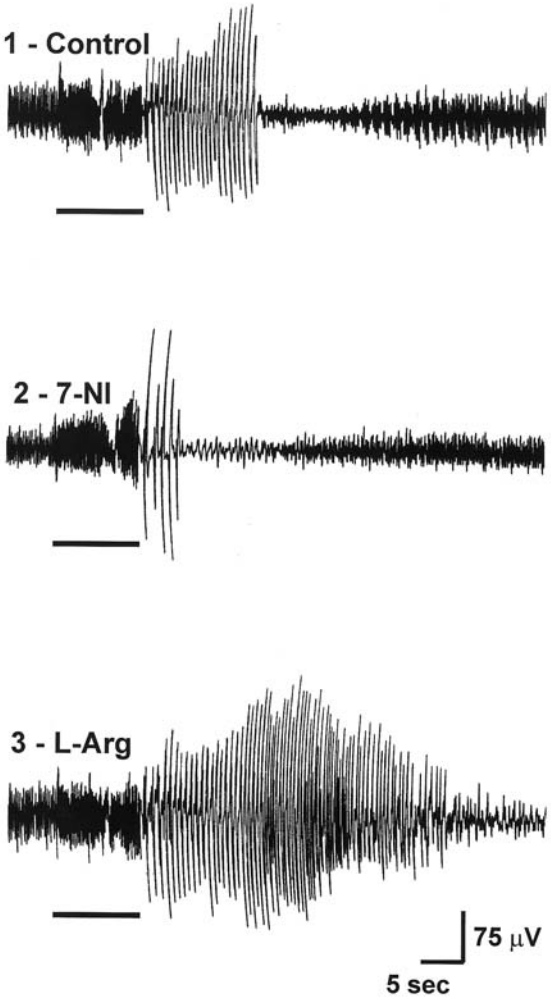

Figure 4. *Effects of the pharmacological modifications of NO levels on Maximal Dentate gyrus Activation (MDA). Trace 1 - representative depth EEG trace of MDA in control animals. Trace 2 - effect of 7-Nitro-indazole (7-NI, nNOS inhibitor, dose of 50 mg/kg body weight) on MDA parameters: increased onset latency, decreased MDA duration, decreased afterdischarge duration and population spike's frequency. Trace 3 - effect of L-arginine (L-Arg, NO donor, dose of 1 mg/kg body weight) on MDA parameters: decreased onset latency, increased MDA duration, increased afterdischarge duration and population spike's amplitude.*

as L-arginine showed an efficacious action in decreasing the susceptibility to seizure, comparable to the common antiepileptic drugs, suggesting a potential involvement of NO as an anticonvulsant (51, 52). Further data revealed a significantly increased susceptibility to convulsions in animals treated with inhibitors of NOS (53). Recent experimental observations have demonstrated a relative natural protection against pentylenetrazol-induced seizures in immature rats which show a high availability of L-arginine, a precursor of NO (54). Different hypotheses have been suggested to explain the putative anticonvulsant action exerted by NO: 1) it could act *via* a negative feedback on the NMDA receptor through a competitive blockade of the recognition site; 2) it could functionally interact with the redox modulatory site, either in basal conditions or during all the pathological events characterised by the "overactivity" of the NMDA receptor complex (20, 55). On the other hand, other experimental

Table II. Nitric oxide as a proconvulsant.

Model	Animal	Structure	Reference
Reverberatory epilepsy	Rat	Hippocampus	Stringer and Erden (1995) (71)
Bicuculline	Rat	Frontal cortex	Pereira de Vasconcelos *et al.* (1995) (90)
Hereditary convulsion	Chick	Forebrain	Sandirasegarane *et al.* (1996) (91)
Pentylenetrazole (PTZ)	Mice	Gen. seizures	Urbanska *et al.* (1996) (53)
Bicuculline	Rat	Pyriform cortex	Proctor *et al.* (1997) (92)
Penicillin	Rat	Hippocampus	Lu *et al.* (1998) (57)
EL mice	Mice	Hippocampus	Murashima *et al.* (2000) (93)
Kainate	Rat	Cortex	Huh *et al.* (2000) (94)
PTZ-induced kindling	Rat	Hippocampus	Han *et al.* (2000) (95)
Amygdala kindling	Rat	Gen. Seizures	Borowicz *et al.* (2000) (96)
Kainate	Rat	Hippocampus	Yasuda *et al.* (2001) (69)
MDA	Rat	Hippocampus	Ferraro *et al.* (2004) (73)

observations, together with the analogies between the role of NO and GABA in the generalised decrease of neuronal excitability (56), have suggested the possibility that NO and GABA can act synergically in the neuroprotective and/or antiepileptic action (32). An interesting link between NMDA receptor activity, increase of NO activity and potentiation of GABAergic neurotransmission has been proposed to explain the aggravation of seizures induced by NOS inhibitors either in a generalised model of experimental epilepsy, due to the blockade of $GABA_A$ receptors or in focal hippocampal penicillin epilepsy (57, 58). Our experimental results have demonstrated that a severe reduction of cerebral NO causes an increase of neuronal synchronisation in both the hippocampal formation (Figure 1) and the cerebral cortex (Figure 2) in non epileptic rats. In particular, we have shown that the inhibition of NOS, obtained through non-selective or brain-selective NOS inhibitors, causes a marked modification of the bioelectric activity of both these neural structures, until it evokes an epileptiform activity as evidenced by the presence of spikes, polyspikes, or spikes and waves, collectively called interictal discharge activity. Furthermore, we have recognised the appearance of afterdischarges (ADs) in the hippocampal formation (Figure 1) following a severe reduction in NO cerebral availability (L-NAME *i.p.* administration at high doses). All these effects were completely abolished by pre-treatment with NMDA receptor antagonists such as 2-AP5 and MK-801, and partially reduced by pre-treatment with CNQX, a non-NMDA receptor antagonist (59). These data demonstrate a strong link between NO and the glutamate system in the genesis of epileptic hyperexcitability, without any kind of interference due to type and aetiology of seizures, genetic factors or, in general, methodological differences that have been re-proposed recently (27, 60, 61). Significantly, all the NOS inhibitor-mediated excitatory effects were abolished by the pre-treatment with different types of NMDA and non-NMDA receptor antagonists. All the results, in agreement with the data existing in the literature reported in Table I, constitute further support for a natural neuroprotective/anticonvulsant role exerted by NO. In this context, a co-operative effect between NGF and NO has been investigated, which demonstrates that NO regulates NGF gene expression through the cGMP pathway (62). It has also been hypothesised that chronic delivery of NGF causes a stimulatory effect of neuronal NOS synthesis, reducing, through a regulatory feedback loop, the effects of the inhibition of neuronal NOS activity (63). Finally, NO production may be up-regulated by NGF, suggesting a powerful and rapid co-ordinated action (64).

Evidence for a pro-convulsant role. It has been hypothesised that NO could also act as a pro-convulsant agent, inducing seizures through a complex mechanism involving a functional alteration of the control of vascular motility. In fact, the functional involvement of NO has been hypothesised in the neurotoxicity phenomenon induced by the activation of NMDA receptor during the epileptic disorder related to the modification of cerebral blood flow (CBF) (65-67). Furthermore, several conditions associated with the appearance of seizures related to the vasodilation seem to be prevented or delayed by preliminary treatment with NOS inhibitors (68). More recently, in the context of a model of experimental epilepsy, obtained through intrahippocampal injection of kainic acid, an increase of NOS activity was demonstrated. This observation suggests a direct relationship between the cerebral level of NOS and the severity of the epileptiform phenomena (69). All these experimental observations highlight a functional involvement of NO in excytotoxic/proconvulsant mechanisms in the CNS suggesting a potential implication in a new therapeutic approach (70). In this regard, we have tested the role of NO in another model of experimental epilepsy: the "maximal dentate activation" (MDA, Figure 3), considered an example of reverberatory seizure activity in the context of the

hippocampal-parahippocampal circuit (71). It was demonstrated that repeated seizures are strictly linked to the modifications of NADPH diaphorase activity, which is considered a marker of NO synthesis (72). Then, using this model of experimental epilepsy, we modified the level of endogenous NO through the administration of 7-nitroindazole and L-arginine, a precursor of the synthesis of NO, in order to verify the modifications induced to both the onset time and the duration of the ictal events (Figure 4). The inhibition of nNOS caused an increase of the MDA onset time and a decrease of MDA and AD duration. On the contrary, the administration of L-arginine caused opposite effects: a decrease of the MDA onset time and an increase of MDA and AD duration (73). Studies evidencing the proconvulsant role of NO are reported in Table II.

Taking into consideration the large number of experimental studies showing a proconvulsant *versus* an anticonvulsant effect of NO, it has been hypothesised that such a variability could depend on either the model of seizure employed, or genetic factors or methodological differences. In spite of the significant influences exerted by all these variables, all the experimental observations, showing both pro-convulsant and anticonvulsant NO- induced effects, recognise the crucial role of the redox site of the NMDA receptor complex whose pharmacological manipulation is able modify the course of experimental epilepsy (74).

Nitric oxide and other neurological disorders

The role of NO has been widely investigated in several experimental models of other common neurological disease such as the ischemic stroke (75-77). In particular, it has been shown that activation of the endothelial form of NOS exerts a protective action against the neurotoxic effects caused by the cerebral ischemia. On the contrary, the increased activity of nNOS and/or iNOS induced an aggravation of the excytotoxic phenomena (75, 78). Study of the involvement of nitric oxide in the context of ischemic damage in humans showed that the increase of NO levels due to the activation of inducible NOS activity is not related to the release of glutamate and calcium influx (79). On the other hand, the increased levels of cerebral NO can promote the apoptotic events following a severe brain ischemia (80).

A severe neurodegenerative disorder such as Alzheimer's disease has been related to the activity of the NO system. In fact, several investigations have revealed that the neurotoxic role exerted by NO in the context of the CNS could be responsible for this neurodegenerative disorder; the event could be based on the excessive calcium influx which is the key of the oxidative stress (81).

Further, in schizophrenia the involvement of nitric oxide has been hypothesised. In particular, it has been shown that the inhibition of nNOS could be responsible for the low levels of nitrates and cyclic GMP evidenced in patients affected by schizophrenic disease (82).

Another neuropsychiatric disorder, namely catalepsy, was investigated in mice and the results have highlighted a relationship with NO activity: in fact, high levels of NG-nitro-L-arginine, an efficacious inhibitor of neuronal NOS, caused motor effects in mice quite similar to those shown by cataleptic subjects (83). Furthermore, an elevated NOS activity was demonstrated in pyramidal neurones and its neuroprotective effects were evidenced against the reactive oxygen damage induced by pathological ageing (84).

Finally, it has been demonstrated that the inhibition of nNOS could be responsible for an increase of aggressive behaviour, showing a putative functional involvement of NO in adverse behaviour (85).

Concluding remarks

Although the role of NO as an unconventional transmitter in the CNS was discovered only a few years ago, its functional involvement in brain physiology and pathophysiology has been widely demonstrated. In this regard, the influence of the NO system on normal and paroxysmal neuronal excitability constitutes an interesting field of research, aiming to explore possible and innovative approaches to the pharmacological control of epileptic phenomena (86, 87). According to the literature reviewed in this article, our study has highlighted opposing effects (proconvulsant *vs* anticonvulsant) induced by using several drugs which modify the cerebral availability of NO. Furthermore, in this review we have reported and discussed evidence supporting either the pro-convulsant/zneurotoxic or the anticonvulsant/neuroprotective effects, or both. Although no definitive conclusions are possible yet, one can observe that the NO system, characterised by a surprising functional adaptability, is able to induce totally differing effects on neuronal hyperexcitability, in relation to the modifications induced on neighbouring neurotransmitters by the different epileptic models.

Acknowledgements

This work was supported by grants from the Ministero dell'Istruzione, dell'Università e della Ricerca Scientifica e Tecnologica, Rome, Italy.

References

1 Snyder SH : Nitric oxide and neurones. Curr Op Neurobiol *2*: 323-327, 1992.
2 Palmer RMJ, Ferrige AG and Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxation factor. Nature *327*: 524-526, 1987.
3 Vizi ES: Non Synaptic Interactions Between Neurones: Modulation of Neurochemical Transmission. John Wiley and Sons, 1984.

4 Vizi ES: Role of high-affinity receptors and membrane transporters in non synaptic communication and drug action in the central nervous system. Pharmacol Rev *52*: 63-90, 2000.
5 Bredt DS, Glatt CE, Hwang PM, Fotuhi M, Dawson TM and Snyder SH: Nitric oxide synthase protein and mRNA are discretely localised in neuronal populations of mammalian central nervous system together with NADPH diaphorase. Neuron *7*: 615-624, 1991.
6 Lowenstein CJ, Glatt CS, Bredt DS and Snyder SH: Cloned and expressed macrophage nitric oxide synthase contrasts with the brain enzyme. Proc Natl Acad Sci USA *89*: 6711-6715, 1992.
7 Sessa WC, Harrison JK, Barber CM, Zeng D, Durieux MS, D'Angelo DD, Lynch KR and Peach MJ: Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. J Biol Chem *267*: 15274-15276, 1992.
8 Bredt DS and Snyder SH: Nitric oxide: a physiological messenger molecule. Ann Rev Biochem *63*: 175-179, 1994.
9 Schmidt HHHW, Pollock JS, Nakane M, Forstermann U and Murad F: Ca^{2+}/calmodulin-regulated nitric oxide synthase. Cell Calcium *13*: 427-434, 1992.
10 Dinerman JL, Dawson TM, Schell MJ, Snowman A and Snyder SH: Endothelial nitric oxide synthase localized to hippocampal pyramidal cells: implications for synaptic plasticity. Proc Natl Acad Sci USA *91*: 4214-4218, 1994.
11 Garthwaite J and Boulton CL: Nitric oxide signalling in the central nervous system. Ann Rev Physiol *57*: 683-706, 1995.
12 Luo D, Das S and Vincent SR: Effects of methylene blue and LY83583 on neuronal nitric oxide synthase and NADPH-diaphorase. Eur J Pharmacol *290*: 247-251, 1995.
13 Dawson VL and Dawson TM: Nitric oxide in neurodegeneration. Progr Brain Res *118*: 215-229, 1998.
14 Ahern GP, Klyachko VA and Jackson MB: cGMP and S-nitrosylation: two routes for modulation of neuronal excitability by NO. Trends Neurosci *25*: 510-517, 2002.
15 de Vente J, Hopkins DA, Markerink-Van Ittersum M, Emson PC, Schmidt HHHW and Steinbusch HWM: Distribution of nitric oxide synthase and nitric oxide-receptive, cyclic GMP-producing structures in the rat brain. Neuroscience *87*: 207-241, 1998.
16 Iwase K, Iyama K, Akagi K, Yano S, Fukunaga K, Miyamoto E, Mori M and Takiguchi M: Precise distribution of neuronal nitric oxide synthase mRNA in the rat brain revealed by non-radioisotopic *in situ* hybridization. Mol Brain Res *53*: 1-12, 1998.
17 Garthwaite J: Glutamate, nitric oxide and cell-cell signalling in the nervous system. TiNS *14*: 60-67, 1991.
18 McNaught KSP and Brown GC: Nitric oxide causes glutamate release from rat synaptosomes. J Neurochem *70*: 1541-1546, 1998.
19 Sequeira SM, Ambrosio AF, Malva JO, Carvalho AP and Carvalho CM: Modulation of glutamate release from rat hippocampal synaptosomes. Nitric Oxide *1*: 315-329, 1997.
20 Lei SZ, Pan Z, Aggarwal SK, Chen HV, Hartman J, Sucher NJ and Lipton SA: Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. Neuron *8*: 1087-1099, 1992.
21 Lipton SA, Choi Y, Pan Z, Lei SZ, Chen HV, Sucher NJ, Loscalzo J, Singel DJ and Stamler JS: A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature *364*: 626-632, 1993.
22 Manzoni O and Bockaert J: Nitric oxide synthase activity endogenously modulates NMDA receptors. J Neurochem *61*: 368-370, 1993.
23 Nanri K, Takizawa S, Fujita H, Ogawa S and Shinoara Y: Modulation of extracellular glutamate concentration by nitric oxide synthase inhibitor in rat transient forebrain ischemia. Brain Res *738*: 243-248, 1996.
24 Beckman JS: The double-edged role of nitric oxide in brain function and superoxide-mediated injury. J Dev Physiol *15*: 53-59, 1991.
25 Zhang F and Iadecola C: Nitroprusside improves blood flow and reduces brain damage after focal ischemia. Neuroreport *4*: 559-562, 1993.
26 Zhang F, Benveniste H, Klitzman B and Piantadosi CL: Nitric oxide synthase inhibition and extracellular glutamate concentration after cerebral ischemia/reperfusion. Brain Res *610*: 57-61, 1993.
27 Bains JS and Ferguson AV: Nitric oxide regulates NMDA-driven GABAergic inputs to type I neurones of the rat paraventricular nucleus. J Physiol *499*: 733-746,1997.
28 Sequeira SM, Duarte CB, Carvalho AP and Cawalho CM: Nitric oxide differentially affects the exocytotic and the carrier-mediated release of [^{3}H]γ-aminobutyric acid in rat hippocampal synaptosomes. Mol Brain Res *55*: 337-340, 1998.
29 Tasker JG and Dudek FE: Local inhibitor synaptic inputs to neurones of the paraventricular nucleus in slices of rat hypothalamus. J Physiol *469*: 179-192, 1993.
30 Bernardi PS, Valtschanoff JG, Weinberg RJ, Schmidt HH and Rustioni A: Synaptic interactions between primary afferent terminals and GABA and nitric oxide-synthesizing neurones in superficial laminae of the rat spinal cord. J Neurosci *15*: 1363-1371, 1995.
31 Gabbott PLA and Bacon SJ: An oriented framework of neuronal processes in the ventral geniculate nucleus of the rat demonstrated by NADPH histochemistry and GABA immunocytochemistry. Neuroscience *60*: 417-440, 1994.
32 Lovick TA and Key BJ: Inhibitory effect of nitric oxide on neuronal activity in the periaqueductal grey matter of the rat. Exp Brain Res *108*: 382-388, 1996.
33 Valtschanoff JG, Weinberg RJ, Rustioni A and Schmidt HH: Co-localization of neuronal nitric oxide synthase with GABA in rat cuneate nucleus. J Neurocytol *24*: 237-245, 1995.
34 Kano T, Shimizu-Sasamata M, Huang PL, Moskowitz MA and Lo EH: Effects of nitric oxide synthase gene knockout on neurotransmitter release *in vivo*. Neuroscience *86*: 695-699, 1998.
35 de Vente J, Markerink-van Ittersum M, Axer H and Steinbusch HW: Nitric oxide-induced cGMP synthesis in cholinergic neurones in the rat brain. Exp Brain Res *136*: 480-491, 2001.
36 Lonart G, Wang J and Johnson KM: Nitric oxide induces neurotransmitter release from hippocampal slices. Eur J Pharmacol *220*: 271-272, 1992.
37 Suzuki T, Nakajima K, Fujimoto K, Fujii T, Takita M, Kanedo H, Suzuki SS and Akamatsu M: Lasting effect of NO on glutamate release in rat striatum revealed by continuous brain dialysis. NeuroReport *8*: 567-570, 1997.
38 Prast H, Tran MH, Fisher H and Philippu A: Nitric oxide-induced release of acetylcholine in the nucleus accumbens: role of cyclic GMP, glutamate and GABA. J Neurochem *71*: 266-273, 1998.

39 Stewart TL, Michel AD, Black MD and Humphry PPA: Evidence that nitric oxide causes calcium-independent release of [^{3}H]dopamine from rat striatum *in vitro*. J Neurochem *66*: 131-137, 1996.
40 Lorrain DS and Hull EM: Nitric oxide increases dopamine and serotonin release in the medial preoptic area. Neuroreport *5*: 87-89, 1993.
41 Smith JCE and Whitton PS: The regulation of NMDA-evoked dopamine release by nitric oxide in the frontal cortex and raphe nuclei of freely moving rat. Brain Res *889*: 57-62, 2001.
42 Satoh S, Kimura T, Toda M, Miyazaki H, Ono S, Narita H, Murayama T and Nomura Y: NO donors stimulates noradrenaline release from rat hippocampus in a calmodulin-dependent manner in the presence of L-cysteine. J Cell Physiol *169*: 87-96, 1996.
43 Lauth D, Hertting G and Jackisch R: 3,4-Diaminopyridine-evoked noradrenaline release in rat hippocampal slices: facilitation by endogenous or exogenous nitric oxide. Brain Res *692*: 174-182, 1995.
44 Montague PR, Ganvayco CD, Winn MJ, Marchase RB and Friendlnder MJ: Role of production in NMDA-receptor-mediated neurotransmitter release in cerebral cortex. Science *263*: 973-977, 1994.
45 Guevara-Guzman R, Emson PC and Kendrick KM: Modulation of *in vivo* striatal transmitter release by nitric oxide and cyclic GMP. J Neurochem *62*: 807-810, 1994.
46 Kaehler ST, Singewald N, Sinner C and Philippu A: Nitric oxide modulates the release of serotonin in the rat hypothalamus. Brain Res *835*: 346-349, 1999.
47 Singewald N and Philippu A: Release of neurotransmitters in the locus coeruleus. Progr Neurobiol *65*: 237-267, 1998.
48 Buisson A, Lakhmeche N, Verrecchia C, Plotkine M and Boulu RG: Nitric oxide: and endogenous anticonvulsant substance. Neuroreport *4*: 444-446, 1993.
49 Boda B and Szente M: Nitric oxide synthase inhibitor facilitates focal seizures induced by aminopyridine in rat. Neurosci Lett *209*: 37-40, 1996.
50 Kabuto H, Yokoi I, Habu H, Willmore LJ, Mori A and Ogawa N: Reduction of nitric oxide synthase activity with development of an epileptogenic focus induced by ferric chloride in the rat brain. Epilepsy Res *25*: 65-68, 1996.
51 Przegalinski E, Baran L and Siwanowicz J: The role of nitric oxide in chemically- and electrically-induced seizures in mice. Neurosci Lett *217*: 145-148, 1996.
52 Herberg LJ, Grottick A and Rose IC: Nitric oxide synthesis, epileptic seizures and kindling. Psycopharmacol *119*: 115-123, 1995.
53 Urbanska EM, Drelewska E, Borowicz KK, Blaszczak P, Kleinrok Z and Czuczwar SJ: NG-nitro-L-arginine, a nitric oxide synthase inhibitor, and seizure susceptibility in four seizure models in mice. J Neural Transm *103*: 1145-1152, 1996.
54 Pereira de Vasconcelos A, Gizard F, Marescaux C and Nehlig A: Role of nitric oxide in pentylenetrazol-induced seizures: age-dependent effects in the immature rat. Epilepsia *41*: 363-371, 2000.
55 Manzoni O, Prezeau L, Marin P, Deshager S, Bockaert J and Fagni L: Nitric oxide-induced blockade of NMDA receptors. Neuron *8*: 653-662, 1992.
56 Boulton CL, Irving AJ, Southam E, Potier B, Garthwaite J and Coillingridge GL: The nitric oxide-cyclic GMP pathway and synaptic depression in rat hippocampal slices. Eur J Neurosci *6*: 1528-1535, 1994.
57 Lu W, Chen G and Cheng JS: NMDA antagonist displays anticonvulsant effect *via* NO synthesis inhibition penicillin-treated rat hippocampal slices. Neuroreport *9*: 4045-4049, 1998.
58 Tsuda M, Suzuki T and Misawa M: Aggravation of DMCM-induced seizures by nitric oxide synthase inhibition in mice. Life Sciences *60*: 339-343, 1997.
59 Ferraro G, Montalbano ME and La Grutta V: Nitric oxide and glutamate interaction in the control of cortical and hippocampal excitability. Epilepsia *40*: 830-836, 1999.
60 Getting SJ, Segieth J, Ahmad S, Biggs CS and Whitton PS: Biphasic modulation of GABA release by nitric oxide in the hippocampus of freely moving rats *in vivo*. Brain Res *717*: 196-199, 1996.
61 Kirkby DR, Carrol DM, Grossman AB and Subramanian S: Factors determining proconvulsant and anticonvulsant effects of inhibitors of nitric oxide synthase in rodents. Epilepsy Res *24*: 91-100, 1996.
62 Xiong H, Yamada K, Jourdi H, Kawamura M, Takei N, Han D, Nabeshima T and Nawa H: Regulation of nerve growth factor release by nitric oxide through cyclic GMP pathway in cortical glial cells. Mol Pharmacol *56*: 339-347, 1999.
63 Lam HHD, Bhardwaj A, O'Connel MT, Hanley DF, Traystman RJ and Sofroniew MV: Nerve growth factor rapidly suppresses basal, NMDA-evoked, and AMPA-evoked nitric oxide synthase activity in rat hippocampus *in vivo*. Neurobiology *95*: 10926-10931, 1998.
64 Calzà L, Giardino L, Giuliani A, Aloe L and Levi-Montalcini R: Nerve growth factor control of neuronal expression of angiogenetic and vasoactive factors. Proc Natl Acad Sci USA *98*: 4160-4165, 2001.
65 Penix LP, Davis W and Subramanian S: Inhibition of NO synthase increases the severity of kainic acid-induced seizures in rodents. Epilepsy Res *18*: 177-184, 1994.
66 Rigaud-Monnet AS, Pinard E, Borredon J and Seylaz J: Blockade of nitric oxide synthesis inhibits hippocampal hyperemia in kainic acid-induced seizures. J Cereb Blood Flow *14*: 581-590, 1994.
67 Rondouin G, Bockaert J and Lerner-Natoli M: L-Nitroarginine an inhibitor of NO synthase dramatically worsen limbic epilepsy in rats. Neuroreport *4*: 1187-1190, 1993.
68 Bitterman N and Bitterman H: L-arginine-NO pathway and CNS oxygen toxicity. J Appl Physiol *84*: 1633-1638, 1998.
69 Yasuda H, Fujii M, Fujisawa H, Ito H and Suzuki M: Changes in nitric oxide synthesis and epileptic activity in the contralateral hippocampus of rats following intrahippocampal kainate injection. Epilepsia *42*: 13-20, 2001.
70 Lamas S, Pérez-Sala D and Moncada S: Nitric oxide: from discovery to the clinic. TiPS *19*: 436-438, 1998.
71 Stringer JL and Erden F: In the hippocampus *in vivo*, nitric oxide does not appear to function as antiepileptic agent. Exp Brain Res *105*: 391-401, 1995.
72 Talavera E, Martinez-Lorenzana G, Corkidi G, Leon-Olea M and Condes-Lara M: NADPH-diaphorase-stained neurons after experimental epilepsy in rats. Nitric Oxide *1*: 484-493, 1997.
73 Ferraro G, Sardo P, Di Giovanni G, Di Maio R and La Grutta V: Involvement of nitric oxide in maximal dentate gyrus activation, in the rat. Pflügers Arch – Eur J Physiol, in press, 2004.
74 Quesada O, Hirsch J, Ben-Ari Y and Bernard C: Redox sites of NMDA receptors can modulate epileptiform activity in hippocampal slices from kainic acid-treated rats. Neurosci Lett *121*: 171-174, 1996.

75 Huang Z, Huang PL, Ma J, Meng W, Ayata C, Fishman MC and Moskowitz MA: Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. J Cereb Blood Flow Metab *16*: 981-987, 1996.

76 Iadecola C: Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci *20*: 132-139, 1997.

77 Moncada S, Palmer RMJ and Higgs EA: Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev *43*: 109-142, 1991.

78 Iadecola C, Zhang F, Casey R, Nagayama M and Ross ME: Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. J Neurosci *17*: 9157-9164, 1997.

79 Castillo J, Rama R and Dàvalos A: Nitric oxide-related brain damage in acute ischemic stroke. Stroke *31*: 852-860, 2000.

80 Elibol B, Söylemezoglu F, Ünal I, Fujii M, Hirt L, Huang PL, Moskowitz MA and Dalkara T: Nitric oxide is involved in ischemia-induced apoptosis in brain: a study in neuronal nitric oxide synthase null mice. Neuroscience *105*: 79-86, 2001.

81 Law A, Gauthier S and Quirion R: Say NO to Alzheimer's disease: the putative links between nitric oxide and dementia of the Alzheimer's type. Brain Res Rev *35*: 73-96, 2001.

82 Das I, Khan NS, Puri BK and Hirsch SR: Elevated endogenous nitric oxide synthase inhibitor in schizophrenic plasma may reflect abnormalities in brain nitric oxide production. Neurosci Lett *215*: 209-211, 1996.

83 Marras RA, Martins AP, Del Bel EA and Guimaraes FS: L-NOArg, an inhibitor of nitric oxide synthase, induces catalepsy in mice. Neuroreport *7*: 158-160, 1995.

84 Wallace MN, Tayebjee MH, Rana FS, Farquhar DA and Nyong'o AO: Pyramidal neurones in pathological human motor cortex express nitric oxide synthase. Neurosci Lett *212*: 187-190, 1996.

85 Demas GE, Eliasson MJ, Dawson TM, Dawson VL, Kriegsfeld LJ, Nelson RJ and Snyder SH: Inhibition of neuronal nitric oxide synthase increases aggressive behavior in mice. Mol Medicine *3*: 610-616, 1997.

86 La Grutta V and Sabatino M: Focal hippocampal epilepsy: effect of caudate stimulation. Exp Neurol *99*: 38-49, 1988.

87 La Grutta V and Sabatino M: Substantia nigra-mediated anticonvulsant action: a possible role of a dopaminergic component. Brain Res *515*: 87-93, 1990.

88 Williams JH, Li YG, Nayak A, Errington ML, Murphy KPSJ and Bliss TVP: The suppression of long term potentiation in rat hippocampus by inhibitors of nitric oxide synthase is temperature and age dependent. Neuron *11*: 877-884, 1993.

89 Alabadı JA, Thibault JL, Pinard E, Seylaz J and Lasbennes F: 7-Nitroindazole, a selective inhibitor of nNOS, increases hippocampal extracellular glutamate concentration in status epilepticus induced by kainic acid in rats. Brain Res *839*: 305-312, 1999.

90 Pereira de Vasconcelos A, Baldwin RA and Wasterlain CG: Nitric oxide mediates the increase in local cerebral blood flow during focal seizures. Proc Natl Acad Sci USA *92*: 3175-3179, 1995.

91 Sandirasegarane L, Mikler JR, Tuckek JM and Sulakhe PV: Enhanced forebrain nitric oxide synthase activity in epileptic fowl. Brain Res *735*: 311-313, 1996.

92 Proctor MR, Fornai F, Afshar JK and Gale K: The role of nitric oxide in focally-evoked limbic seizures. Neuroscience *76*: 1231-1236, 1997.

93 Murashima YL, Yoshii M and Suzuki J: Role of nitric oxide in the epileptogenesis of EL mice. Epilepsia *41*: 195-199, 2000.

94 Huh Y, Heo Y, Park C and Ahn H: Transient induction of neuronal nitric oxide synthase in neurons of rat cerebral cortex after status epilepticus. Neurosci Lett *281*: 49-52, 2000.

95 Han D, Yamada K, Senzaki K, Xiong H, Nawa H and Nabeshima T: Involvement of nitric oxide in pentylentetrazole-induced kindling in rats. J Neurochem *74*: 792-798, 2000.

96 Borowicz KK, Luszczki J, Kleinrok Z and Czuczwar SJ: 7-Nitroindazole, a nitric oxide synthase inhibitor, enhances the anticonvulsive action of ethosuximide and clonazepam against pentylenetrazol-induced convulsions. J Neural Transm *107*: 1117-1126, 2000.

Received March 12, 2004
Accepted April 21, 2004