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.

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 Ca2+-dependent manner. The Ca2+-calmoduline complex regulates only nNOS and eNOS; on the contrary, the iNOS shows Ca2+-independent properties (3, 8). nNOS is Ca2+-and NADPH-dependent, in fact the influx of Ca2+ 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 ìm), 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

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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

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 synthetising system.** The NO synthetising 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 \(\mu\)m (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 Purkinje 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 postsynaptic 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 GABAAergic 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 stereotaxic 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).
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
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>
<thead>
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<th>Animal</th>
<th>Structure</th>
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<td>NMDA</td>
<td>Mice</td>
<td>Gen. seizures</td>
<td>Buisson et al. (1993) (48)</td>
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<td>LTP</td>
<td>Rat</td>
<td>CA1</td>
<td>Williams et al. (1993) (88)</td>
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<tr>
<td>Kindling</td>
<td>Rat</td>
<td>Gen. seizures</td>
<td>Herberg et al. (1995) (52)</td>
</tr>
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<td>Iron ion</td>
<td>Rat</td>
<td>Cerebral cortex</td>
<td>Kabuto et al. (1996) (50)</td>
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<td>Aminopyridine</td>
<td>Rat</td>
<td>Neocortex</td>
<td>Boda et al. (1996) (49)</td>
</tr>
<tr>
<td>PTZ</td>
<td>Mice</td>
<td>Gen. seizures</td>
<td>Przegalinski et al. (1996) (51)</td>
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<td>DMCM</td>
<td>Mouse</td>
<td>Gen. seizures</td>
<td>Tsuda et al. (1997) (58)</td>
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<tr>
<td>Kainate</td>
<td>Rat</td>
<td>Hippocampus</td>
<td>Abadi et al. (1999) (89)</td>
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<tr>
<td>Basal conditions</td>
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<td>Hippocampus/cortex</td>
<td>Ferraro et al. (1999) (59)</td>
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Table I. Nitric oxide as anticonvulsant.

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.
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 GABAA 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 NMDDA 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 cooperative 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

Table II. Nitric oxide as a proconvulsant.

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<td>Rat</td>
<td>Frontal cortex</td>
<td>Pereira de Vasconcelos et al. (1995) (90)</td>
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<td>Hereditary convulsion</td>
<td>Chick</td>
<td>Forebrain</td>
<td>Sandirasagarane et al. (1996) (91)</td>
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<td>Pentyleneetrazole (PTZ)</td>
<td>Mice</td>
<td>Gen. seizures</td>
<td>Urbanska et al. (1996) (53)</td>
</tr>
<tr>
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<td>Rat</td>
<td>Pyriform cortex</td>
<td>Proctor et al. (1997) (92)</td>
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<tr>
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<td>Hippocampus</td>
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</tr>
<tr>
<td>EL mice</td>
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<td>Hippocampus</td>
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<td>PTZ-induced kindling</td>
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<td>Rat</td>
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<td>MDA</td>
<td>Rat</td>
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Nitric oxide and other neurological disorders

The role of NO has been widely investigated in several experimental models of other common neurological diseases 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 excitotoxic 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/neurotoxic 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.

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