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The role of norepinephrine in epilepsy: from the bench to the bedside

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Abstract

This article provides a brief review of the role of norepinephrine (NE) in epilepsy, starting from early studies reproducing the kindling model in NE-lesioned rats, through the use of specific ligands for adrenergic receptors in experimental models of epilepsy, up to recent advances obtained by using transgenic and knock-out mice for specific genes expressed in the NE system. Data obtained from multiple experimental models converge to demonstrate the antiepileptic role of endogenous NE. This effect predominantly consists in counteracting the development of an epileptic circuit (such as in the kindling model) rather than increasing the epileptic threshold. This suggests that NE activity is critical in modifying epilepsy-induced neuronal changes especially on the limbic system. These data encompass from experimental models to clinical applications as recently evidenced by the need of an intact NE innervation for the antiepileptic mechanisms of vagal nerve stimulation (VNS) in patients suffering from refractory epilepsy. Finally, recent data demonstrate that NE loss increases neuronal damage following focally induced limbic status epilepticus, confirming a protective effect of brain NE, which has already been shown in other neurological disorders.

Keywords: Norepinephrine; Locus coeruleus; Epilepsy; Seizures; Norepinephrine system in epilepsy

1. General considerations

The role of NE in attenuating seizures represents an interesting and promising issue in modern epileptology as witnessed by a recent study, which demonstrated the need of an intact NE innervation for the antiepileptic mechanisms of VNS [1]. This procedure was recently approved to treat patients suffering from partial drug-resistant epilepsy [2–4] which is frequently triggered within limbic regions [5,6].

Initially, the impact of endogenous NE during experimental seizures was unravelled by pre-clinical studies carried out on a wide spectrum of seizure types, including systemic pentylenetetrazol (PTZ), and maximal- and low-intensity electroshock [7–9]. However, the most robust antiepileptic effect of NE was evidenced by early studies, where NE delayed the onset of amygdala kindling in rats [10–12]. These studies demonstrated that a damage to the locus coeruleus (LC), the main source of NE within the central nervous system (CNS), accelerated the rate of amygdala kindling [13,14].

At first, the antiepileptic role of endogenous NE was inferred from studies that showed deleterious effects of a damage of NE system on seizures induced by electrical stimulation or systemic administration of chemoconvulsants. Conversely, further approaches started from spontaneously epileptic rats to analyze whether alterations in the brain NE content and pattern of NE innervation occurred [15,16]. During the last decades, improvement of experimental techniques led to more sophisticated approaches to disclose the specific role of NE in epilepsy. These studies spanned from in vitro electrophysiology and receptor binding, to the latest genetic engineering of selective components of the NE system, both in vitro and in vivo, by using knock-out and transgenic mice. The latter approaches led to a deeper knowledge of the complex mechanisms by which NE tunes seizure activity. In the following paragraphs we will try to

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review these issues, showing also the difficulties in interpreting the bulk of experimental data, which at first glance might appear conflicting. The clinical relevance of these experimental studies will be outlined in the last part of this review.

2. Functional anatomy of the central NE system in relation to epilepsy

Epilepsy is by definition a cortical phenomenon. Nonetheless the influence of subcortical structures in gating and triggering seizures is well established [17]. In particular, NE-containing neurons do not extend further than the metencephalon. These nuclei belong to the rich catecholamine cell group which was first classified in the 60s by Dahlstroem and Fuxe following the method of Falck [18,19]. In their seminal work, the authors described altogether 12 cell groups in the rat CNS (A1–A12). Among these neuronal nuclei the most rostral possess dopamine (DA) as a neurotransmitter and they are located mainly in the mesencephalon, while NE nuclei do not extend beyond the pons. The most rostral NE group corresponds to the pontine nucleus named LC (A6), placed in the upper part of the floor of the fourth ventricle (Fig. 1a). It is present in all the mammalian species and it represents the major source of NE for the CNS [20–22]. The LC represents a nuclear complex, being composed of various nuclei located close to one another: the LC sensu stricto, the nucleus subcoeruleus, and scattered catecholamine neurons located close to the brachium conjunctivum (parabrachial nucleus). Catecholamine neurons belonging to this nuclear complex widely branch their terminals in such a way that a single NE axon innervates the entire cerebral cortex, running within two main ascending fiber systems: the dorsal bundle, and the much smaller rostral limb of the dorsal periventricular pathway. Other LC efferents are distributed caudally to the cerebellum, the lower medulla, and the spinal cord [21,23].

The LC complex is distinct from other scattered NE nuclei placed in the medullary ventrolateral reticular formation or within the dorsal vagal complex and the nucleus of the solitary tract. These scattered NE nuclei form the so-called ‘caudal’ NE formation, which is represented by an interconnected network of neurons sending their axons to rather restricted target areas, following a specific pattern in which distinct nuclei innervate discrete brain regions [24]. This caudal NE system is mainly involved in regulating autonomic functions, participating in neuroendocrine control [25] and, differing from the LC, does not play a significant role in epilepsy.

On the other hand, given their rich and widespread cortical projections, the NE terminals arising from the LC modulates a variety of central functions through the release of NE into several brain areas. Thus, they are involved in modulating electroencephalographic (EEG) activity [26]; regulating the sleep–waking cycle by anticipating fluctuations of EEG activity [27,28]; promoting a state of vigilance [29]; monitoring environmental stimuli with a special emphasis on alerting stimuli and orienting to novelty [30]. However, when analysing the literature on EEG changes, the activity of LC neurons does not seem to be critical in baseline conditions. Only a few papers provided evidence showing a trend of EEG changes following LC lesions [31]. We could not find such an effect [32], thus confirming what had already been described by Riekkinen et al. [33] and by Chang et al. [34]. Detailed studies carried out by Cirelli et al. [35,36] could not detect significant changes in baseline EEG even when it was extensively measured by using power spectrum analysis over several weeks following the lesion of LC neurons. On the other hand, these authors found that cortical effects of the activity of LC neurons appear to be more critical in modulating wake-related gene expression [35,36]. Stimulation of

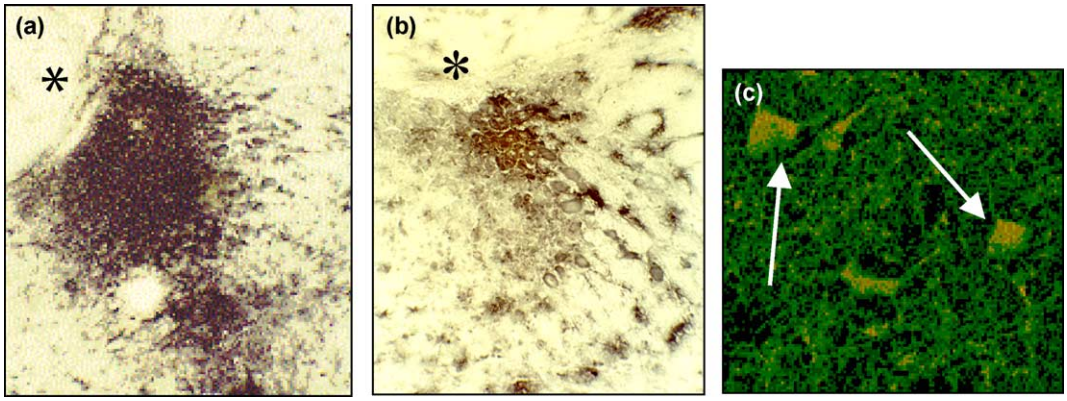

Fig. 1. Selective staining of locus coeruleus neurons in normal conditions and after DSP-4. DA-β-hydroxylase immunostaining shows NE neurons of LC, close to the floor of the fourth ventricle (*), in a control (a), and after systemic administration of DSP-4 (60 mg/kg) which is a neurotoxin selective for LC arising axon terminals (b). The marked loss of staining within the LC a few days after DSP-4 is related to the loss of axon collaterals within the nucleus, while cell bodies survive. Nonetheless, when observed several months after DSP-4, apart from loss of NE axon terminals, a degeneration of cell bodies can be observed (c), as showed by the Fluoro-Jade B technique, which stains degenerating neurons (arrows).

central adrenergic receptors leads to changes in the state of vigilance as well as in transcription of immediate early genes (IEGs), such as *c-fos, nerve growth factor-induced A*, *nur 77*, *tis-7*, *zif-268* and *tis-21* [37,38], in basal conditions [38], during stressful stimuli [39] and specifically during seizures [40]. In line with increased activity of the nucleus in the waking state, there is a LC-dependent circadian rhythm of early gene expression, which in turn regulates the state of phosphorylation of cyclic adenosine monophosphate response element-binding proteins [35]. In contrast, during REM sleep, when the activity of the nucleus is suppressed, there is a marked reduction in the expression of these genes, this suggesting that LC is critical for inducing cerebral plasticity related to the waking state [35]. Recently, Cirelli et al. [41] by using high density microarray demonstrated that LC activity influence the expression of about 100 genes, which might participate in the neuroprotective and antioxidant activity produced by these NE neurons. These findings follow up previous research from the same group showing plasticity-related genes triggered by the activity of LC [36]. Similarly, a damage to the LC nucleus abolishes the waking-related early genes expression, and pharmacological agents that hyperpolarize NE cells, like clonidine, decrease the expression of these genes below baseline values [42]. IEGs encode for transcription factors, which in turn regulate the expression of downstream target genes. Since these genes control cell functions, the induction of IEGs represents one mechanism through which signals acting on cell membranes mediate short- or long-term biochemical and structural changes in the neuron. This explains the ability of NE to modulate synaptic plasticity as already shown in vitro during 'long-term potentiation' (a long-lasting modification of synaptic efficacy produced by repetitive stimulation of the pre-synaptic compartment) [43–45] and in vivo, where the NE innervation of the limbic cortex plays a seminal role in learning and memory [46].

The widespread nature of these effects is bound to wide areas receiving LC terminals and the pattern of NE release [47,48]. In fact, NE, apart from its classic role as a neurotransmitter, should be regarded as a paracrine agent exerting a diffuse influence on target sites, and making only a few direct synaptic contacts with post-synaptic neurons. Therefore, NE terminals, running in close contact to neurons, astrocytes, microglia, and blood vessels, produce effects on a variety of biological matrix [49,50]. This is due both to the long half-life of NE in the extra-cellular space and the profuse branching of LC axon terminals which allows a single neuron to innervate the entire cerebral cortex [47].

This is in line with the morphology of NE axons possessing varicosities ('bouttons en passage') rather than classic 'bouttons terminaux' typical of non-monoaminergic axon terminals. Finer morphological studies of NE axons arising from the LC have shown that axon terminals are thin, with small varicosities. This contrasts with NE axons arising from the medullary A1 and A2 cell groups which branch out with terminals featuring larger varicosities. It is worth while mentioning that monoaminergic axons with smaller beaded varicosities possess a lower threshold to various neurotoxic insults and at the same time they are more affected in degenerative diseases [51]. This is in line with the involvement of LC neurons in neurological disorders much more frequently than other NE nuclei.

3. Experimental tools to damage central NE pathways and methods to assess the integrity of NE innervation

Different experimental approaches could be used to obtain a selective lesion of LC-arising fibers. The first consists in micro-injecting the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) within the LC. However, this experimental procedure is subject to unavoidable site variability, it is invasive and not specific for NE neurons involving neighboring catecholamine cells (for a review, see Ref. [52]). To overcome these limits, a second approach consists in micro-injecting 6-OHDA within the dorsal catecholamine bundle [53]. However, this procedure involves only the ascending NE projections, and therefore it does not result in the total loss of LC-arising axons, leaving intact the descending NE pathways. Another method consists in administering 6-OHDA to newborn animals within the first week. During this period, the blood–brain barrier is immature and 6-OHDA can easily access the CNS. Using a selective schedule of administration (repeated injections of small doses), 6-OHDA becomes more selective for NE neurons compared with DA cells [54]. However, this experimental approach needs to be carried out in newborn animals; therefore it is not feasible in experiments performed in adults. A non-invasive method was used since the mid-70s, and consists of systemic injections of the neurotoxin *N*-(-2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4). The i.p. administration of DSP-4 causes a long-lasting loss of NE terminals arising from the LC (Figs. 1 and 2, in which a dose of DSP-4 of 60 mg/kg was used). Remarkably, this compound selectively destroys NE axons arising from LC neurons and spares extra-coeruleus NE terminals (Fig. 3). It is well established that after a single i.p. injection of this neurotoxin, NE axons degenerate in the LC target structures (Figs. 2 and 4). Furthermore, a long time after DSP-4 administration, a progressive decline in the number of LC cell bodies occurs (Fig. 1c), while the remaining NE terminals sprout to re-innervate the cerebral cortex. Following a damage produced by DSP-4 various behavioral disturbances were described [55], mostly consisting of impairment in exploratory activity [56] and alterations in active avoidance [57]. Once the NE damage is experimentally produced it is critical to carry out a quantitative assessment of the lesion [56–60]. There are several markers which indicate a damage to NE neurons including either cell bodies, axon terminals or both neuronal components.

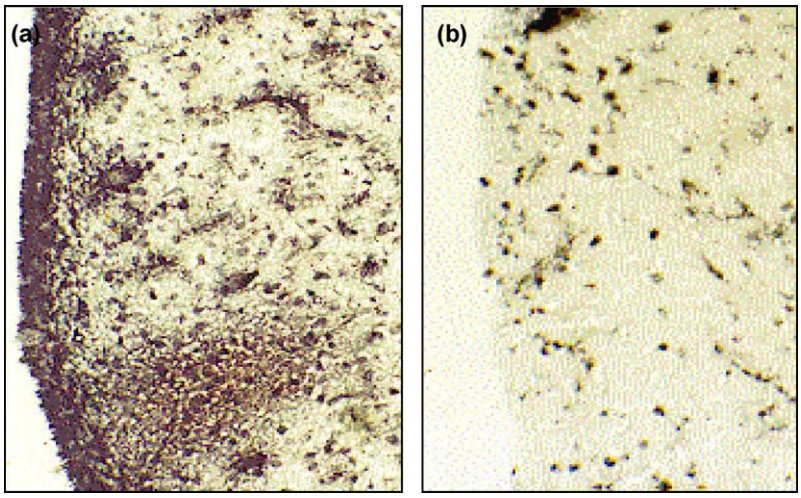

Fig. 2. NE nerve endings within the olfactory cortex. A dense pattern of immunostaining is visible in the olfactory cortex (a) by using the selective technique to stain NE axons using antibodies directed against the synthesizing enzyme DA-β-hydroxylase. This brain area contains the highest amount of NE levels compared with other cortical regions. In particular, the difference is marked when comparing the olfactory cortex with neocortical areas. Differing from isocortex, the olfactory cortex lacks any thalamic input; therefore, it was suggested that in this area the NE pathways might substitute for the functional effects exerted by thalamic afferents on cortical regions. In (b) the olfactory cortex is stained for the same antigens (DA-β-hydroxylase) after a treatment with the neurotoxin DSP-4. The dense trim of cortical NE fibers disappears especially in the deep layers of the olfactory cortex, where immunostaining is no longer present.

One method which allows a quantitative estimation, consists in measuring the loss of endogenous NE within target areas using high performance liquid chromatography coupled with electrochemical detection.

This method allows measurement of NE levels below a picomole. Generally the procedure is applied to specific brain homogenates and gives an estimation of the integrity of NE axon terminals, since the levels of NE within stored terminals far exceed those measured in the extra-cellular space. Measurement of the rate limiting enzyme in NE biosynthesis tyrosine hydroxylase (TH), despite being a useful tool to mark NE terminals in purely NE innervated regions, might reveal a fallacy in translating results to the integrity of NE terminals, since DA nerve endings contain this enzyme as well (see an example in Fig. 3). Therefore, when aiming at enzymes specific for NE nerve endings it is necessary to target the dopamine-β-hydroxylase (DBH), which is responsible for the conversion of DA to NE and allows one to distinguish NE from DA nerve endings. This measure might involve the enzyme activity, the protein content (either visualized by immunocytochemistry or measured by semiquantitative or quantitative assays). Another molecular target which might be used to visualize or to measure the amount of NE axons is the high affinity selective NE transporter (NET). This can be achieved by using autoradiography to assay (^{3}H) NE uptake or labeled compounds which selectively bind the NET like desipramine or specific antibodies (Fig. 5).

Despite the various sensitivity of these methods, the molecular targets and the specific procedures (biochemistry vs. morphology), results should always be handled with care. In fact, a point which needs to be clarified is that visualizing or measuring NE terminal loss within target regions does not provide us with functional information about the occurrence of NE activity loss. In fact, it is well established that even a severe damage to NE neurons does not necessarily impair the NE activity in terms of post-synaptic efficacy. In other words, there are a number of compensatory mechanisms (ranging from post-synaptic NE receptors up-regulation, adaptation of second messengers, increased pre-synaptic turn-over leading to increased NE synthesis and release per surface unit), which are able to maintain the level of extra-cellular NE at a sufficient rate to provide an appropriate stimulation, at least in basal conditions. These compensatory mechanisms following a previous damage to the NE system were demonstrated by Abercrombie and Zigmond [61] following partial injury to the NE system. These changes were attributed mostly to an increase in NE synthesis at least in the hippocampus [62]. We also found the occurrence of partial compensatory mechanisms in NE-lesioned rats using brain dialysis in the hippocampus [32]. In baseline conditions, even in the presence of a massive NE loss, rats were able to produce extra-cellular NE levels, which, during a few time intervals did not differ from controls [32]. However, in the same experiments, when seizure occurred the increase in extra-cellular NE levels was significantly suppressed in NE-lesioned rats. By using brain dialysis in the frontal cortex, Hughes and Stanford found even a paradoxical increase in NE release in partially NE lesioned rats [63]. Therefore, apart from using the markers described above, it is important to assess a decrease of extra-cellular NE levels. This might be achieved by using brain microdialysis in selected NE target areas or, indirectly, by imaging displacement of labeled NE agonists.

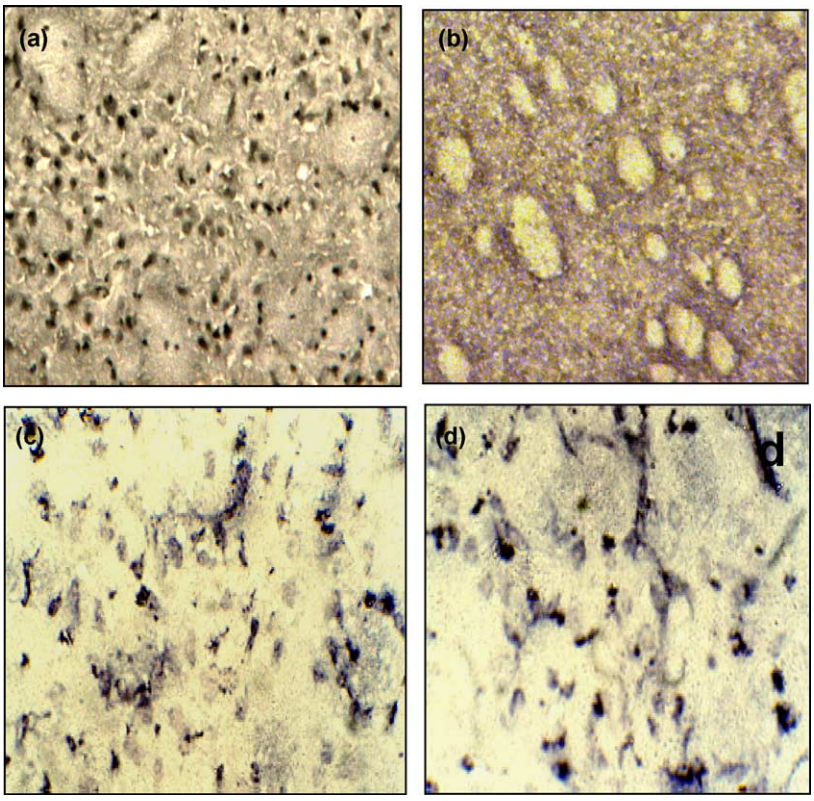

Fig. 3. Analyzing catecholamine innervation within the striatum. Catecholamine (dopamine and norepinephrine) innervation of striatum is showed by using antibodies against tyrosine hydroxylase (TH) (a), which is the rate-limiting step enzyme of catecholamine biosynthesis. However, in this way it is not possible to distinguish between nerve endings containing dopamine and those containing norepinephrine. In fact TH is contained in both NE and DA nerve endings. Therefore, in order to selectively identify the DA nerve endings it is necessary to use antibodies directed against the DA transporter (DAT) which is located exclusively on DA axons (b). Similarly, in order to observe NE nerve endings antibodies against DA-β-hydroxylase can be used showing only rare varicosities which account for the scarce levels of striatal NE (c). Thus, it is possible to carry out a comparison between the amount of DA and NE nerve endings within striatum (b and c, respectively). In this way it is evident that the dense pattern of DA nerve endings is far in excess compared with scattered NE terminals. This makes almost non-relevant the amount of NE axons in contributing to the TH immunostaining observed in the striatum and justifies the routine approach to measure the integrity of striatal DA axons by simply using TH immuohistochemistry which almost overlaps with DAT immunohistochemistry. The selective staining for NE axons, obtained by DA-β-hydroxylase immunoperoxidase (c), is not affected by the selective neurotoxin DSP-4 (d), which destroys LC arising NE axons; thus suggesting that scattered striatal NE innervation derives from other NE nuclei placed more caudally in the brainstem (A1–A2 NE areas, as described in the text).

4. NE and kindling

4.1. In vivo studies

The original work of Arnold et al. [64] sparked interest in the area of catecholamines and seizures demonstrating for the first time that catecholamine depletion accelerates amygdala kindling in rats. Since both reserpine (a non-specific catecholamine depleting agent) and 6-OHDA (which, as already mentioned, is a non-specific monoamine neurotoxin) were used, it was not possible to discern the role of each specific monoamine in sustaining the anticonvulsant effect. The caveat was solved a few years later by McIntyre et al. [65] who pre-administered desmethylimipramine (DMI), a NE uptake inhibitor, before microinfusing 6-OHDA. In this way, 6-OHDA was no longer able to be taken up by the NET and therefore could not damage NE neurons, while the neurotoxicity for the DA system was left intact. In these experimental conditions (loss of DA and maintenance of NE axons) the kindling process was no longer affected, in contrast to the marked acceleration of amygdala kindling which occurred in rats non-protected by DMI, owing to a marked NE depletion.

Further data confirming the specific involvement of NE in kindling were obtained by restricted lesions of NE pathways ascending in the dorsal forebrain bundle. These lesions produced a discrete loss of NE innervation in limbic and neocortical areas and they were accompanied by a significant increase in the kindling rate, both in the amygdala [10] and allocortical regions [66].

As one might expect, increasing NE activity delayed amygdala kindling. This was demonstrated by increasing extra-cellular NE levels following the blockade of NE uptake (DMI administration) [67], or by electrical stimulation of the nucleus LC before each kindling session [68].

A further approach to confirm the inhibitory role of NE in the development of kindling was achieved by re-establishing limbic NE release following damage to LC. This was

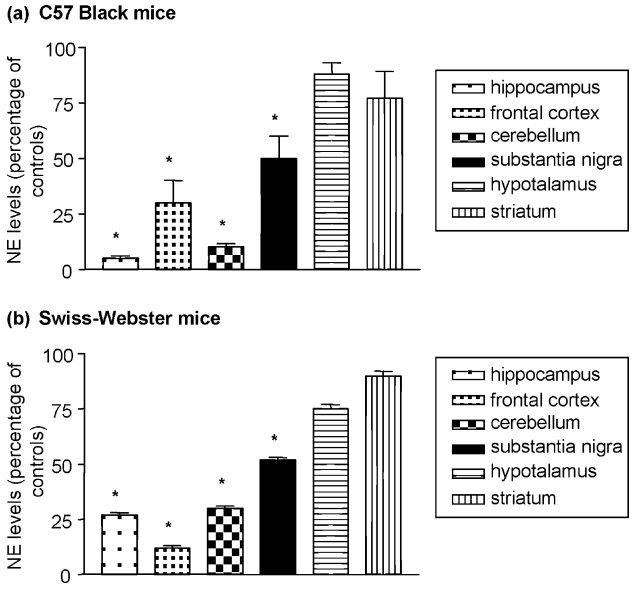

Fig. 4. Regional pattern of DSP-4 induced NE loss in two strains of mice and depleting effect of NE neurotoxin DSP-4. NE levels, expressed as percentage of controls, are showed in various brain areas of male C57Bl (a) and Swiss-Webster (b) mice, after DSP-4 neurotoxic treatment. The different amount of NE loss detectable in different brain areas depends on different density of NE terminals arising from LC which are present within each region. In both strains of mice, in front of a mild, non-significant loss of NE in hypothalamus and striatum, DSP-4 provokes a consistent reduction of NE levels in substantia nigra. A severe NE loss is detected in hippocampus, frontal cortex and cerebellum. Furthermore, in these areas the extent of NE loss appears largely depends on animal strain. Error bars are expressed as percentage of SE of the percent mean of 10 animals per group. $*P < 0.05$ compared with controls.

carried out by transplants of LC fetal cells into the hippocampus of NE-depleted rats. The LC–NE releasing grafts reversed the pro-kindling effect of the LC loss, and restored the natural time course of the hippocampal kindling process, thus confirming the marked effect of NE in counteracting kindling development [69,70]. A robust effect was also observed when fetal LC cells were grafted bilaterally into the amygdala-piriform area of NE-depleted rats [71]. In particular, transplanted rats developed kindled seizures at a much slower rate compared with NE-depleted animals [69,70]. Similar experiments performed with a transplantation of NE cells from the superior cervical ganglion failed to show the same effect [70]. This might occur since spontaneous NE release from ganglionic cells, when transplanted, is lower than LC grafted neurons [72]. In fact, the latter have a longer survival [70] after the implant, and a higher rate both in synthesizing and releasing NE [72,73] compared with cells from sympathetic ganglia. Finally, LC grafted neurons produce more axonal branching to reach the surrounding host tissue [73]. Interestingly, despite the marked effect in delaying kindling, LC grafts neither affect seizure severity nor reverse the kindling process once implanted in NE-lesioned rats who were already kindled, thus suggesting that the efficacy of NE transplants is selective for the steps involved in the kindling development [74].

4.2. In vitro studies

In addition to in vivo studies concerning NE system and the kindling process, a different approach consists in brain slice preparations to examine the epileptic 'burst response' in restricted neuronal circuits after repeated electrical stimulation performed with or without manipulation of the NE system.

By using this approach, McIntyre and Wong [12] worked on coronal slices including the amygdala-piriform/periamygdaloid regions. In these experimental conditions, neurons of the piriform and periamygdaloid cortex produce a 'burst response' following the stimulation of the adjacent amygdala. When the experiment is reproduced following previous amygdala kindling, a significant increase in the duration of the cortical 'burst response' compared with non-kindled tissue occurs. Finally, exposing the slice chamber to NE (2 μM), causes suppression of the 'burst response' in the non-kindled preparation, an effect which is observed also in the kindled slices with higher doses of NE. However, similar to in vivo conditions NE did not reverse the kindling process.

To examine the specific effects of adrenergic receptors further results were obtained by applying selective adrenergic agonists and antagonists to the slice preparation. While the α1 agonist L-phenylephrine (10 μM) had no effect on the 'burst response', the α2 agonist clonidine (1 μM) reproduced the suppressive effect of NE [12].

Taken together these results suggest an inhibitory effect of NE on seizure onset, which might be mediated by α2-adrenergic receptor subtypes.

A detailed analysis about the effects of adrenergic agonists and antagonists in seizure activity shows a slight discrepancy compared with effects described on the kindling model and will be reviewed in section 7.

5. The antiepileptic role of NE in classic seizure models

Apart from classic studies on the role of NE in the kindling process, the NE system was evaluated in various models of epilepsy. In line with data obtained in the kindling model, a damage to NE pathways produces increased seizure susceptibility, while the stimulation of LC has a protective effect against epileptic seizures.

Chen et al. [7] demonstrated that pre-treatment with the monoamine-depleting agent reserpine decreased the epileptic threshold to PTZ and caffeine in mice. Subsequent studies confirmed and extended these results [64,75]. However, a major bias is that reserpine lacks specificity, since this drug also depletes serotonin (5-HT) and DA, in addition to NE. Therefore, increased seizure susceptibility could be due to a multiple deficit of monoamines. This was solved by selectively damaging the NE system, leaving the other monoamines intact. In particular, 6-OHDA selectively microinfused in the LC destroys only NE neurons.

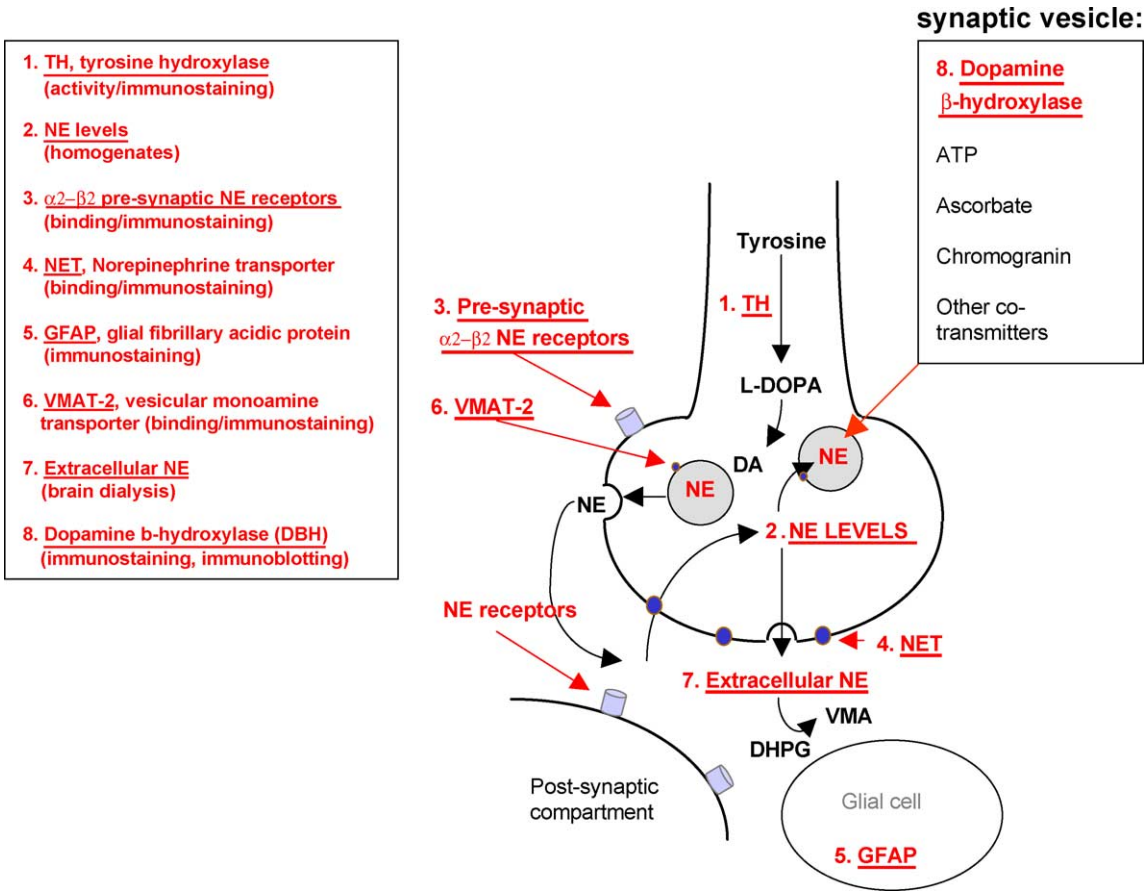

Fig. 5. How to measure the integrity of NE system: cartoon representing a NE terminal with its targets. The biosynthesis of NE starts with the aromatic amino acid tyrosine, which is converted to dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase (TH), which represents the rate-limiting biochemical step in catecholamine biosynthesis. L-DOPA is then converted to dopamine (DA), which is taken up into the synaptic vesicle by the vesicular monoamine transporter type 2 (VMAT-2) where the intravesicular enzyme dopamine-β-hydroxylase (DBH) converts DA to NE. This is the final product ready to be released together with ascorbic acid, ATP, adenosine and other various co-transmitters in the extra-cellular space where it is then able to interact either with post- or pre-synaptic NE receptors or being taken up into the axon terminal by the specific NE transporter: NET. VMAT2 thus represents a marker for all monoamine nerve terminals, TH is specific for catecholamine-containing neurons, while DBH and NET confer selectivity for NE-containing neurons. A damage to NE terminals can be also observed by increased levels of glial fibrillary acidic protein (GFAP) produced by the surrounding glial cells.

Alternatively, selective injection into the dorsal tegmental bundle can preserve dopaminergic neurons by pre-treatment with DA transporter blockers. As reported in section 3, further selectivity can be achieved by administering DSP-4, which produces a selective degeneration of NE projections originating from the LC [51,58,76] sparing NE axons arising from extra-coeruleus regions.

Using these selective approaches it became clear that a damage specific for the NE system produces an increased susceptibility to various epileptic stimuli.

In this way, Jerlicz et al. [77] demonstrated that bilateral lesions of LC facilitate audiogenic seizures. Similarly, Mason and Corcoran [78] showed a greater susceptibility to seizures induced by the chemoconvulsant metrazol and electroconvulsive shock in NE-lesioned rats. A facilitation of focal cobalt-induced seizures was observed in rats after removal of their forebrain NE projection from the LC [79]. Similarly, pre-treatment with DSP-4 reduced the threshold for facial and forelimb clonus obtained by corneal electroshock stimulation in rats, while maximal electroshock performed in NE-depleted rats confirmed increased susceptibility to brainstem seizures [9]. Pre-treatment with DMI, a NE reuptake inhibitor, which prevents the LC terminals from DSP-4 neurotoxicity, reversed this pro-convulsant effect [9]. On the other hand stimulation of the LC suppresses seizures induced by PTZ, amygdala kindling and focal hippocampal penicillin application [80–82]. Increased seizure susceptibility following LC lesion is not accompanied by changes in baseline EEG activity. In fact, precise studies in which EEG power spectrum of DSP-4 treated rats was extensively measured for weeks have failed to show any change compared with controls [35,36].

6. Evidence for the involvement of NE in limbic status epilepticus, and epileptic damage

The bulk of the experimental data reviewed above show that NE decreases experimental seizures, however,

the effects of this neurotransmitter in status epilepticus are much less investigated.

In a very recent paper [32] we examined the effects produced by the loss of NE terminals originating from LC on seizures induced focally, by microinfusing chemoconvulsants in the anterior extent of the piriform cortex (so-called 'area tempestas', AT) [83]. This cortical area was selected based on two main features: (1) high susceptibility to epileptogenic stimuli [84,85]; (2) high amount of NE innervation [26,32,86,87].

The high sensitivity of AT to focal microinfusions of chemoconvulsants is demonstrated in various studies [83], thus making it an optimal site to trigger limbic seizures. Microinfusing bicuculline (a GABA-A antagonist) in this brain region, elicits sporadic limbic seizures, which resemble those originally described in the amygdala-kindled rats [83,88]. Compared with other models, seizures and status epilepticus evoked focally from AT spread along the natural anatomical pathways downstream to limbic areas ruling out non-specific neurotoxicity which occurs when status epilepticus is induced by systemic chemoconvulsants. Therefore, brain damage induced in this model is solely due to the persistent synaptic activation of neurons involved in the seizure circuitry. In rats deficient for NE innervation we observed that the same dose of bicuculline microinfused in AT was able to prolong seizure frequency and duration leading to a long-lasting, self-sustaining status epilepticus (Fig. 6a). The significance of these findings extends beyond a mere decrease in seizure threshold and, analogous to the effects on kindling, allow to hypothesize that a NE deficit consolidates the epileptic circuitry thus triggering self-sustaining status epilepticus [32].

These plastic changes were evident when the trigger region was challenged by selective glutamate antagonists. While in intact rats limbic seizures are suppressed both by NMDA and non-NMDA antagonists with comparable efficacy, in NE-lesioned rats a loss of sensitivity to NMDA blockade is observed. This was demonstrated by pre-infusing the competitive NMDA antagonist amino-7-phosphonoheptanoic acid (AP-7) in NE-lesioned rats, which neither reduced limbic seizures, nor prevented the onset of limbic status epilepticus. Conversely, the non-NMDA antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]-quinoxaline-7-sulphonamide (NBQX) maintained its pharmacological efficacy preventing the onset of status epilepticus and blocking sporadic seizure activity (Fig. 6b).

As mentioned above, the olfactory cortex is a cortical area receiving abundant NE projections [26,86,87] making critical the effects of the loss of NE terminals which produce the conversion of sporadic seizures into limbic status epilepticus. However, it cannot be ruled out that NE loss in other brain regions involved in the propagation and/or gathering of seizures evoked from AT enables the status epilepticus or contributes to its maintenance.

The ability to modulate limbic status epilepticus goes beyond the antiepileptic effects of NE. In fact, limbic status

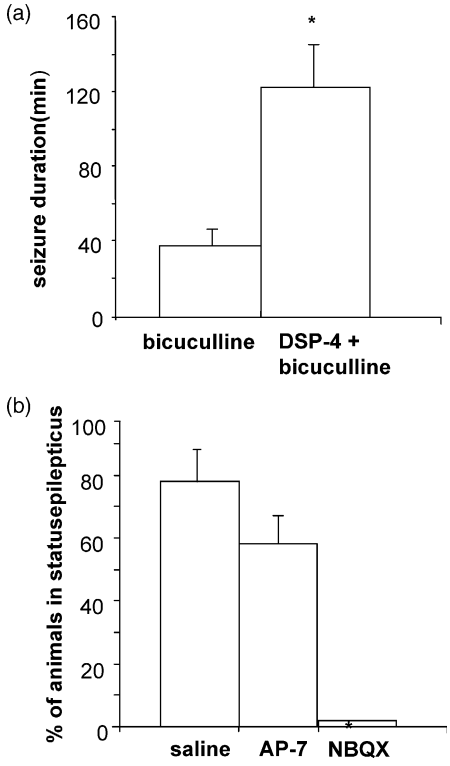

Fig. 6. Effects of NE loss on seizure duration and response to glutamate antagonists. Rats were administered DSP-4 (60 mg/kg i.p.) or saline 2 days before surgery, i.e. 3 days before bicuculline (118 pmol) microinfusion in the anterior piriform cortex (118 pmol). (a) The seizure duration, measured as the time interval during which seizure activity occurs—either sporadic or continuous—following microinfusion of bicuculline, is reported. In NE-lesioned rats (DSP-4 + bicuculline) seizure duration was significantly prolonged; $p < 0.05$ compared with bicuculline. (b) In DSP-4 (60 mg/kg i.p.)-treated rats, bicuculline induces a self-sustaining status epilepticus: rats show at least one seizure episode lasting for more than 30 min (saline group). Pre-administration of the selective non-NMDA, AMPA-preferring antagonist NBQX (520 pmol) completely prevents status epilepticus (NBQX group). Conversely, the selective NMDA receptor antagonist, AP-7 (100 pmol) while preventing episodic seizures induced by bicuculline does not reduce status epilepticus induced by bicuculline in DSP-4 treated rats (AP-7 group).

epilepticus represents a critical condition, which might lead to brain damage and death of the patient. The issue of a potential role of NE in conditioning seizure-induced brain damage was investigated by challenging rats with an intact or a NE-lesioned system with focal microinfusions in AT [32]. This study demonstrated that rats undergoing status epilepticus develop neuronal loss in various limbic regions, which is more pronounced after NE denervation (Fig. 7). In fact, when compared with other models of status epilepticus focally evoked from AT [89], neuronal damage occurring in NE-lesioned rats requires less seizure duration, suggesting specific neuroprotective effects of endogenous NE [32].

The potential neuroprotective effects of an intact LC on seizure-induced damage might be relevant considering the deleterious effects of prolonged seizure activity on neuronal survival with an emphasis on seizures involving primarily the limbic system. The ability of NE to reduce epileptic

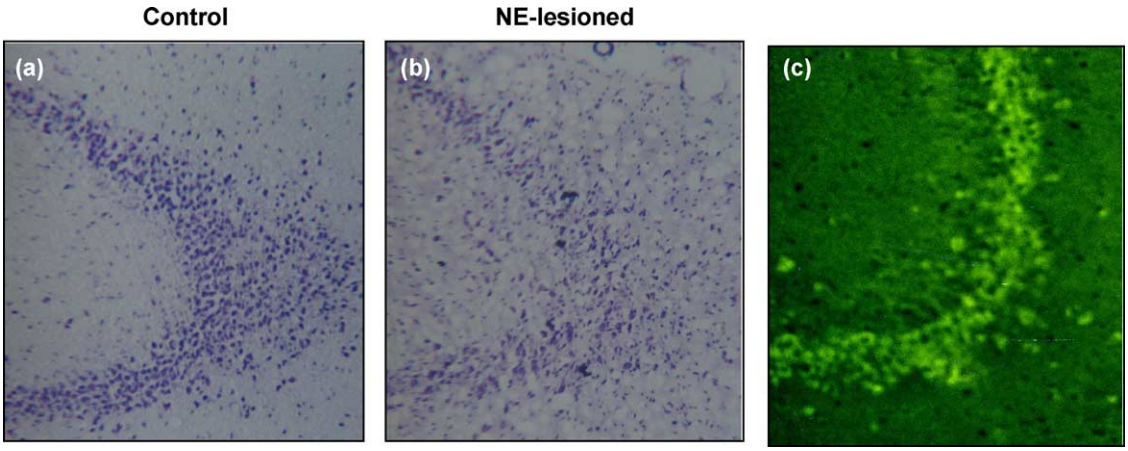

Fig. 7. Pathological effects of status epilepticus on CA3 hippocampal region. In NE-lesioned rats (DSP-4) status epilepticus induced by bicuculline was able to produce CA3 hippocampal cell loss (b) compared with controls (a) detectable using cresyl violet staining. In the same brain area of NE-lesioned rats, degenerating neurons were observed after Fluoro-Jade B staining (c).

brain damage adds to previous research demonstrating that LC neurons are critical in preventing cell loss [90,91] and promoting neuronal plasticity [35,39,92–94], including development of GABA release [95] and increased post-synaptic sensitivity to GABA [96]. In fact, the expression of specific genes associated with synaptic plasticity depends on the activity of the LC system [36,41]. In particular the expression of phosphorylated CRE-binding protein, Arc, and BDNF has been demonstrated to be low when LC neurons fire at very low rate or are silent. Conversely, these molecules increase when LC neurons fire at high rate with phasic, short bursts of action potentials following salient events [28,36,97]. These findings call for further studies aimed at exploring the specific role of NE innervation in the molecular mechanisms governing seizure susceptibility and preventing seizures-induced brain damage.

7. Proconvulsant and anticonvulsant effects of adrenoreceptor agonists and antagonists

Once established the ability of NE to produce a robust effect on seizure activity, the next step which needs to be analyzed is whether various subtypes of adrenergic receptors equally contribute to this phenomenon or they play different, or even opposite, roles in epilepsy.

Although several pharmacological studies evaluated the role of specific adrenergic receptors in experimental epilepsy, this point is still under debate due to the lack of ligands selective for specific receptor subtypes. More recently the issue has been extensively investigated by using transgenic and knock-out mice for subtypes of adrenergic receptors lacking selective pharmacological agonists or antagonists

Weinshenker and Szot [98] reviewed comprehensively these pharmacological experiments investigating the effects of adrenergic agonists and antagonists in various models of epilepsy. The pharmacological approach gave conflicting results due to different reasons: (1) a large difference exists in the distribution of adrenergic receptor between animal species, strains, and brain regions; (2) selectivity of adrenergic agonists and antagonists for different subtypes of receptors varies with the dose of the drug. For instance, clonidine might stimulate imidazoline receptors at doses even lower than those required to activate α2-adrenergic receptors [99]; therefore, in some experimental conditions a modulatory effect on seizures, which is attributed to specific adrenergic receptors, might result from a mixed non-specific binding.

Despite discrepancies, some points are well established. Studies carried out by stimulating α2-adrenergic receptors, show an anticonvulsant effect in most experimental settings in vivo, where systemic administration of clonidine produces a dose-dependent delay of the amygdala kindling [100]. This has also been observed in vitro, on brain slices containing the dentate gyrus [101]. This anticonvulsant effect might be explained by post-synaptic α2-adrenergic receptors located on glutamate nerve endings, which suppress glutamate release [102]. Conversely, kindling is accelerated by systemic administration of the α2-adrenergic antagonists idazoxan, yohimbine or rauwolscine [103].

Since the amygdala-piriform region exhibits both high NE content and high density of α2 receptors compared with other brain areas [104], this might explain the strong effects produced by clonidine in delaying amygdala kindling. Thus, as suggested by McIntyre [100], activation of α2 receptors throughout the anterior rhinencephalon may circumscribe the extension of the seizure circuitry within the stimulated hemisphere during early kindling trials. The progressive spread of seizure through the kindled hemisphere may be a result of the loss of α2 receptors which occurs during the development of amygdala kindling [105]. Lending substance to focal mechanisms, selective infusions of clonidine into the amygdala/piriform region delay kindling, while simultaneous infusion of idazoxan reverts this effect [106].

In contrast to the effects on kindling development, neither α2 antagonists nor clonidine modify seizures elicited

from previously kindled animals [103]. This evidence, along with results mentioned above, suggests that α2 receptors exert an effect on the establishment of an epileptic circuit rather than on seizure susceptibility.

The role of α1 adrenergic receptors is less studied compared with α2. However, even when considering the few available pharmacological studies, the anticonvulsant effects of α1 agonists are well established. For instance the α1 agonist methoxamine mimics the anticonvulsant effects of high doses of clonidine in rats administered with systemic chemoconvulsants, the anticonvulsant effects being blocked by prazosin [107].

Recent studies are dissecting the role of α1 receptor subtypes (with an emphasis on α1B receptors) by using knock out and transgenic mice (see below) which allow selective studies of adrenergic receptor subtypes lacking selective pharmacological ligands.

Although the role of β-adrenergic receptors in epilepsy is unclear, it seems that the main confounding factor relates to the extensive use of propanolol in different experimental models. In fact, this non-selective β receptor antagonist also exhibits a weak intrinsic activity on β receptors joined with membrane stabilization properties and local anesthetic activity [108,109]. However, the specific role played by β receptors in the epileptogenic effects of propanolol is confirmed by studies carried out using β agonists, which are anticonvulsants. For instance, in hippocampal slices taken from NE-lesioned rats, isoproterenol, a non-selective β agonist, causes a significant decrease of spikes, which is comparable to that induced by perfusing the slice with NE [110]. Again, the β1 agonist dobutamine, as well as the β2 agonist terbutaline, reduce seizures in genetically epilepsy prone rats (GEPR rats, see below) following a dose–response curve [111].

8. A possible role for NE co-transmitters in seizures susceptibility

Although this is not the aim of the present review, a bulk of neuropeptides and small molecules are contained within NE terminals and possess an antiepileptic effect. Therefore, when interpreting data obtained following a damage to NE terminals, it is critical to rule out the bias of pro-convulsant effects due to concomitant loss of co-transmitters. The same point concerns studies demonstrating the anticonvulsant effects of LC stimulation, since this implies a concomitant release of NE and its co-transmitters.

The presence of specific co-transmitters within NE nerve endings could provide for an additional effect since these peptides possess an antiepileptic effect. For instance, LC terminals contain galanin (GAL) and neuropeptide Y (NPY), co-existing with NE (for a review, see [112]). In particular, GAL is the predominant neuropeptide in LC neurons, since GAL-like immunoreactivity co-exists with NE in 80% of LC neurons, providing abundant galaninergic inputs to the hippocampus, while NPY-like immunoreactivity is present in 20–40% of LC neurons. Finally, apart from the above-mentioned peptides, it is well known that NE fiber terminals contain adenosine [113] which is constantly co-released with NE and produces an antiepileptic effect [114].

Concerning GAL, its direct injection into the hippocampus attenuates seizures in rodents [115]. By using transgenic mice overexpressing GAL (GalOE) or mice knocked-out for the GAL gene (GalKO) some relevant informations on the importance of this neuropeptide in various kinds of seizure have been obtained [116]. In particular, it has been observed that GalKO mice are significantly more susceptible to develop status epilepticus (and subsequent neuronal death). This was obtained either following systemic kainate or by repeated electrical stimulation of the perforant pathway. Again, these mice are more prone than controls to PTZ-induced seizures. The anticonvulsant properties of GAL are confirmed by opposite results obtained in mice over expressing GAL which are resistant to the same epileptic stimuli [116]. In vitro experiments in brain slices from these mice strains demonstrated that GAL inhibits glutamate release in the hippocampus, confirming previous data [117], and suggesting a potential mechanism responsible for the antiepileptic effects induced by GAL.

Similarly, NPY exerts a significant anticonvulsant effect in various models of limbic [118] as well as generalized seizures following systemic PTZ [119]. On the other hand, mice knock-out for the NPY gene show spontaneous, mild seizures and a reduced seizure threshold to PTZ [120].

9. Genetic models based on selective breeding of spontaneously epileptic strains

Additional evidence that NE possesses a strong inhibitory effect on seizure initiation and propagation was obtained indirectly by analyzing spontaneously epileptic strains which represent genetic models of epilepsy. These consist of specific strains in which increased seizure susceptibility or spontaneous seizure activity were documented serendipitously. Isolation and selective breeding of these strains followed by extensive anatomical, neurochemical and electrophysiological studies allowed to dissect the altered brain areas, neurotransmitters, ion channels or second messengers which can induce seizures. In these studies it was frequently documented as a tight association between various alterations of the NE system and the occurrence of epilepsy. Nonetheless, since these strains often bear multiple deficits it remains uncertain a causal relationship between the phenotype of seizure susceptibility and the sole NE deficit.

In this context we will describe two classic genetic models of epilepsy: GEPR rats and tottering mice.

9.1. GEPR rats

The GEPR colonies derive from progressive inbreeding of Sprague–Dawley rats susceptible to audiogenic seizures [121]. To date, two inbred colonies of GEPR have been developed: GEPR-3 and GEPR-9. Seizure severity is higher in GEPR-9 compared with GEPR-3, as confirmed by the observation that after an acoustic stimulus GEPR-3 rats show only mild clonic seizures, while GEPR-9 exhibit massive tonic and tonic–clonic seizures [122]. Both strains possess a reduced threshold to various epileptic stimuli, such as electroshock and PTZ [123], flurothyl ether [124], limbic kindling [125].

Few years after developing the model, it was observed that there is a significant alteration of brain monoamines in GEPR, and more consistently in GEPR-9 [126,127]. In particular, the severity of the epileptic phenotype was associated with the degree of NE loss. In GEPR-9 there is a massive decrease of all typical markers of NE axons in various brain areas: reduced NE levels [15,122,128,129], DBH activity [16] and immunostaining [130], as well as NE uptake sites [16]. These massive reductions were less pronounced in GEPR-3.

Even though GEPR bear abnormalities in other neurotransmitter systems, such as 5-HT and GABA [131] only restoring NE activity reduces seizure susceptibility [132–134], thus implying a causal effect of NE deficit in producing seizure susceptibility in these rats.

9.2. Tottering mice

Tottering mice exhibit spontaneous absence seizures with cortical spike-and-wave discharges late in the development. The epileptic phenotype is associated with ataxia and intermittent myoclonus [135]. Histochemical analysis showed a significant increase in the number of noradrenergic axons in the brain regions innervated by the LC [136,137]. Despite the abundance of axon branches within target regions, cell somata in the LC were demonstrated to be hyperpolarized as a consequence of profuse branching of recurrent axon collaterals within the LC itself. This might account for the paradoxical association between epileptic phenotype and increased brain NE levels [138,139].

Again, as in the GEPRs, the abnormality in the NE system was not the unique alteration occurring in these rodents. Mutations in the gene encoding the high voltage-activated α1A calcium channel subunit were recently discovered in this model of epilepsy, accounting by itself for a possible molecular substrate pre-disposing to epilepsy in tottering mice [140].

10. NE in models of epilepsy based on selective gene manipulation

In recent years, the role of specific adrenergic receptor subtypes, the NE transporter, and enzymes specific for the NE system were investigated in genetically engineered mice, to dissect the role of these molecules in modulating seizures.

These mice were obtained from selective gene manipulation, leading to the lack or the over expression of specific genes coding for proteins selectively expressed in NE neurons or for receptors activated by endogenous NE. In this way, it is possible to investigate the role of specific NE synthesizing enzyme (i.e. dopamine β-hydroxylase, DBH) or subtypes of NE receptors in conditioning seizure susceptibility. In fact, mice lacking or over expressing these specific proteins can be challenged with a chemoconvulsant in order to understand the specific role played by these NE-related proteins in conditioning seizure susceptibility. In particular, these models allow one to overcome the uncertainties deriving from pharmacological approaches. For instance, as reviewed above, examining the role of a given NE receptor by using various concentrations of agonists/antagonists for this receptor might lead to a bias due to the lack of selectivity. Therefore, the same experiment carried out in mice, in which the same receptor is genetically silenced or is over expressed, represents a critical point to assess the role of this receptor in conditioning the expression of seizure activity. On the other hand, for certain receptor subtypes (i.e. α1B) neither selective pharmacological agonists nor antagonists exist; in this case the use of mice knock out or over expressing these receptors is the only experimental approach to understand their role on seizure activity. Again, as analyzed in the following paragraph, knocking out the enzyme DBH which synthesizes NE allows to understand the specific role of this neurotransmitter on seizure activity independently by the integrity of NE terminals.

Nonetheless, all these genetic models possess the limit of bearing these mutations as inborn deficits or abnormalities, which might modify synaptic connectivity developing abnormal brain circuitries, which by themselves, interfere with seizure expression. In this way, altered seizure activity might no longer depend on the ongoing NE activity. For these reasons it seems to be appropriate to confirm conclusions drawn from these studies by developing models in which NE transmission is genetically altered only for a narrow time window during which the chemoconvulsant is acting. Similarly, it would be useful to produce region-specific genetic manipulation to understand which brain area mediates the changes in seizure activity due to an altered NE transmission.

10.1. Dopamine-β-hydroxylase knock-out mice

These mice completely lacking endogenous NE were generated via a targeted disruption of the DBH gene, which codes for the enzyme converting DA to NE [141]. They are characterized by higher seizure susceptibility when tested for threshold to classic epileptic stimuli (flurothyl ether, PTZ, kainic acid, high decibel sounds) [142]. These DBH

deficient mice represent a robust evidence for the anticonvulsant properties of NE.

In fact, as underlined in section 8, increased seizure susceptibility due to a damage to NE neurons could also be due to a concomitant decrease of co-transmitters naturally occurring within NE terminals, which possess per se an anticonvulsant effect. The use of DBH knock-out mice demonstrated for the first time that at least a substantial part of the anticonvulsant activity produced by NE neurons is due to the presence of endogenous NE. One could further argue that mice knock out for DBH do not represent a pure model, since the absence of NE might also impair the trafficking of those peptides which require the presence of NE in the synaptic vesicles to be properly stored. For instance, purines are stoichiometrically bound to NE present in the vesicles. In light of these considerations it seems worthwhile to produce experimental models in which a NE deficit might occur only for a short time interval. This might be achieved either pharmacologically by reversible blockade of NE synthesis, or by using molecular biology aimed at transient silencing the DBH gene. Preliminary data obtained by our group demonstrate that transient inhibition of DBH activity using fusaric acid facilitate limbic seizures, thus confirming the validity of DBH knock-out mice as a strong proof that endogenous NE per se is anticonvulsant.

10.2. α1B overexpression

The α1-adrenergic receptors are less understood among the central adrenergic receptors because of the lack of selective agonists and antagonists. By creating a transgenic mouse over expressing α1B receptor it was possible to investigate the role of α1B in the onset of epilepsy [143,144]. These transgenic mice exhibit spontaneous seizures which are related to the degree of activity of the over expressed receptor. These seizures can be partially reversed in this model by terazosine, an α1 antagonist, thus indicating that authentic α1 receptor signaling sustains seizure activity. In addition to epileptic phenotype these mice show an age-progressive parkinsonian-like locomotor impairment with neurodegeneration in the cortex, hypothalamus, thalamus, cerebellum, striatum, substantia nigra. Even concerning the extensive neurodegeneration and the complex phenotype described above, this model does not represent a pure model of epilepsy; since it is not clear whether seizures are due to the brain damage or this is the consequence of reiterated epileptic events. Nevertheless, this model provides a tool which indicates a role of α1B adrenergic receptors in epilepsy albeit the absence of specific α1B pharmacological ligands.

To our knowledge the seizure sensitivity in α1B knock-out mice has not been tested until now and therefore it would be of great interest to investigate this issue.

10.3. α2 mutant mice

The use of genetically modified animals provided further evidence for the anticonvulsant role of α2 adrenergic receptors in the kindling phenomenon. Janumpalli et al. [145] observed that mice carrying a point mutation in the locus of α2-adrenergic receptor (the D79N mutant mice), possess a substantial loss of receptor function [146]; these mice were markedly prone to develop amygdala kindling. In particular, compared with wild type mice, in D79N mice the number of stimulations needed to achieve full kindling was dramatically reduced, the EEG seizure duration after the first kindling was markedly longer, and the behavioral intensity of the first kindled seizure was higher [145]. In the same paper the authors observed that administration of idazoxan (a pharmacological antagonist selective for α2 receptors) made wild-type mice prone to kindling like D79N, thus confirming the crucial role of α2 receptors in kindling development.

11. Clinical correlates for the involvement of NE in epilepsy

Data obtained from animal models of epilepsy since 1980s converge to document an effect of seizure-attenuation produced by the NE system, thus calling for further clinical studies on the role of NE in epilepsy. In fact a significant gap between data obtained in experimental neurology and the clinical outcome of the antiepileptic role of NE still exists and the bulk of pre-clinical studies are not paralleled by a substantial number of clinical investigations. In fact, only a few papers examined the role of NE in epileptic patients and the lack of specificity of several drugs does not allow to extrapolate a specific role of NE in human seizures. Nonetheless, the few data obtained from recent clinical studies converge with basic research providing evidence for NE as a seizure-attenuating neurotransmitter.

For instance, in line with the anticonvulsant role of α1-adrenergic receptors, Briere et al. [147] found a decrease in α1 receptors density in the epileptic tissue obtained from patients undergoing temporal lobectomy due to refractory partial epilepsy. This suggests a reduced sensitivity to NE within the epileptic focus, thus implying an impairment of inhibitory mechanisms focally, within the epileptic tissue. Other studies examined the concentration of NE within the human cortex of patients with neo-cortical and mesial temporal lobe epilepsy. These studies demonstrated a specific alteration of NE levels depending on the type of seizures and the brain region, thus lending substance to the hypothesis of an impairment of NE pathways in human epilepsy [148].

Further evidence is related to the ability of several antiepileptic drugs to increase NE activity. For instance, valproate and phenytoin increase NE levels in various brain regions [149,150]. In the case of valproate this is accompanied by enhanced expression of TH in the LC [151]. Carbamazepine produces a dose-dependent increase in the firing rate of LC [152], concomitant with higher NE levels in various brain areas [153]. Again, the efficacy of

both phenobarbital and phenytoin in preventing seizures induced by maximal electroshock is attenuated by damaging NE neurons [154]. Altogether, these data suggest that antiepileptic drugs may potentiate endogenous NE activity as part of their mechanism of action.

A further correlation between the NE system and epilepsy is offered by psychiatric disorders. Epidemiological studies indicate that depression is more frequent in epileptic patients [155,156], and a NE deficit was reported in humans with depression [157], as well as in patients with epilepsy [158,159]. This explains the common belief that a NE deficit might underlie both seizure susceptibility and mood disorders [158]. This is confirmed by the efficacy of antiepileptic drugs in the management of affective disorders, while there is some evidence that antidepressants possess antiepileptic effects [160–162]. In particular this is true for the selective 5-HT reuptake inhibitors, which demonstrated anticonvulsant properties both in humans and animal models [160–162]. This antiepileptic effect has been attributed to the increased 5-HT transmission, even though some molecules of the group also act by inhibiting the NE reuptake. However, the selective role of NE reuptake inhibitors, such as reboxetine, remains to be established. This is partly due to the fact that this is a novel drug to treat major depression; therefore, specific studies focused on its effects on epileptic threshold are still lacking, although clinical trials document both the absence of seizures in non-epileptic patients and the lack of exacerbation of seizures in epileptic patients [163,164]. On the other hand, tricyclic antidepressants may produce or exacerbate seizures in epileptic patients [165,166]. This pro-convulsant effects of tricyclic antidepressants has been related to overdosing and it is attributed to the blockade of α1-adrenergic receptors [165].

Again, confirming common NE substrates for epilepsy and mood disorders, VNS, which was recently approved as a novel therapy to treat patients with refractory epilepsy, has now been extended for the treatment of refractory depression [167]. The stimulation of the vagus nerve is known to produce EEG changes as reported by Rutecki [168]. Based on original findings, the use of VNS started on animal models of seizures, as those induced by maximal electroshock [169] and then became a useful tool both for treating psychiatric conditions and refractory epilepsy in humans [4]. As reported by George et al. [4] VNS has been used in 15,000 patients worldwide, offering a new perspective to treat refractory seizures safely, for prolonged time intervals. This treatment is based on the implantation of a generator, the NCP (neurocybernetic prosthesis) subcutaneously, connected with bipolar electrodes placed around the left vagus nerve [170]. In particular, VNS is indicated for treating medically refractory epilepsy including Lennox–Gastaut syndrome [3,4,171] and this antiepileptic effect may persist for several months post-implantation as demonstrated in a clinical study involving 154 patients affected by refractory epilepsy [172]. The mechanisms of action of VNS as an antiepileptic treatment have been investigated extensively by using c-fos expression [173], pharmacological experiments [174], as well as lesion experiments [1]. These studies demonstrated the need of vagal afferents to the nucleus of the solitary tract (NTS) [174], which is inhibited during stimulation of the vagus nerve. The inhibition of the nucleus of the solitary tract and other interconnected brainstem nuclei [173] is in line with the hypothesis that these nuclei act as a gating site to control seizure spreading [17,174]. The inhibition of the NTS leads to increased activity of locus coeruleus neurons which represents the most critical brainstem nucleus responsible for the antiepileptic effect of VNS. In fact, the efficacy of VNS requires the integrity of NE LC neurons [1]. This is demonstrated by blocking LC activity transiently (by microinfusions of local anesthetics) or permanently (by microinfusions of the neurotoxin 6-OHDA). In both cases, the loss of activity of LC neurons abolishes the anticonvulsant effects due to VNS [1]. The lesion of LC needs to be bilateral in order to suppress the anticonvulsant efficacy of VNS [1]. These data demonstrate that the antiepileptic effects of VNS require: (1) the presence of intact projections from the nucleus of the solitary tract, the visceral afferent nucleus of the vagus nerve [173], joined with (2) intact connections with LC which is then markedly activated and is responsible for the antiepileptic effects of VNS.

12. Final considerations

Based on data coming from basic and clinical research, it is evident that endogenous NE plays an antiepileptic effect. This is more pronounced when considering seizures spreading along the limbic system. The antiepileptic effects of NE extend beyond a mere increase in seizure threshold and affect mostly the development of an epileptic circuitry. This plasticity-dependent antiepileptic mechanisms are enlightened by:

(1) The robust influence of NE in preventing the kindling of limbic structures where the antiepileptic role of NE was first described;
(2) The ability of NE to prevent the conversion of sporadic into continuous (status epilepticus) limbic seizures.

In addition, the ability of an intact NE activity in the limbic system is important to reduce the damage produced by a long-lasting status epilepticus.

Altogether these phenomena should be considered as an example of the role of NE in the limbic system, which goes beyond a mere anticonvulsant activity and involves various physiological and pathological processes taking place within limbic regions.

13. Consequences beyond the pure epileptology

Moving from plasticity-dependent antiepileptic mechanisms exerted by endogenous NE, the review emphasized the role of this neurotransmitter in determining and maintaining long-term potentiation (a long-lasting neuronal change produced by repetition of a stimulus which is supposed to underlie memory formation). These considerations open the field to a wider range of physiological and pathological conditions. For instance, it is well known that the same limbic circuits recruited in the pathophysiology of epilepsy are also critical in the maintenance of physiological mechanisms involved in learning and memory. Limbic areas represent brain regions with lower seizure threshold, while playing a critical role in the formation of declarative memory. Since the same brain areas exhibit pronounced excitability to serve learning and memory processes, limbic epilepsy (the most common human epileptic syndrome), appears as the result of aberrant stimulation of the same circuitry which is physiologically dedicated to the processes of learning and memory. This is also confirmed by memory impairment which occurs in patients suffering from limbic seizures. In this scenario, the highest susceptibility to epilepsy of limbic structures could be the price which the limbic system has to pay to work as a highly excitable structure.

Confirming the overlap between neuronal circuitry underlying seizure activity and learning and memory, VNS in humans was reported to improve verbal memory in epileptic patients. In particular, in a protocol used by the Browning's group to test verbal retention memory, subjects were asked to read a series of paragraphs and later identify words that were highlighted in the text. In these patients, vagal stimulation carried out after reading, significantly enhanced retention memory [175], thus lending substance to the crucial role of endogenous NE beyond the mere regulation of seizure threshold.

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