Noradrenergic modulation of excitability in acute and chronic model epilepsies

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There is mounting evidence of an important role for the monoamine neurotransmitter norepinephrine (NE) in long-term neuronal plasticity and epileptogenesis. Depletion of forebrain NE has been shown to accelerate the rate of induction of kindled epilepsy (1,2), but impairs long-term potentiation (LTP) of perforant path synapses (2,3), an apparent contradiction between the excitatory actions of NE in the dentate gyrus (DG) and retardation in kindling rates that normal noradrenergic transmission seems to entail. At the same time, growing evidence indicates that glutamate receptors of the N-methyl-D-aspartate (NMDA) subtype are also key factors in development of hyperexcitability in a number of model epilepsies, including kindling, and are important in induction of LTP. In view of these findings, we have explored consequences of some of the important actions of NE in acute, chronic, and computational models of epilepsy.

Methods

Combined slices of hippocampus (HC) and entorhinal cortex (EC) (400 μm thick) were prepared from adult male Wistar rats (150–250 g) with a McIlwain tissue chopper. After orienting the HC carefully to preserve connections to the EC, slices were cut from the ventral pole and only those from the ventral half were used. Slices were maintained at 35 ± 0.5°C pH 7.4 at the interface between artificial cerebrospinal fluid (ACSF; perfusion rate 2 ml/min) and humidified 95% O2:5% CO2. ACSF composition was (mM): NaCl 126, KCl 5, NaH2PO4 1.25, MgSO4 2, CaCl2 2, NaHCO3 26, glucose 10. In the Mg2+ free solutions MgSO4 was omitted, but due to contamination with other salts, the concentration of Mg2+ in this medium was probably from 1 to 80 μM. When used, n-2-amino-5-phosphonovalerate (AP5; Cambridge Research Biochemicals) was added to the perfusate to yield a final concentration of 30 μM, and NE bitartrate (Sigma) was always added immediately before bath perfusion at a 50-μM concentration.

Extracellular recording electrodes were made of theta glass, with one barrel (reference) containing 150 mM NaCl, and the other containing Ca2+ or K+-sensitive ion-exchange resin (or occasionally three-barreled electrodes sensitive to both), as described previously (14). Intracellular recording electrodes were filled with 4 M K-Acetate (50–120 MΩ). Stimulus-evoked changes in extracellular [Ca2+]o and [K+]o were recorded in the granule cell body layer of the DG and/or layer IV/V of the EC. In the DG, stimuli were applied via the perforant path and in the EC, via bipolar electrodes in the subiculum.

Male Wistar rats under Na-pentobarbital anesthesia (180–220 g) were stereotactically implanted either in the hippocampal commissural pathway (coordinates...
1.8 mm posterior to bregma, on the midline and 4.2 mm below the cortical surface), or the amygdaloid nucleus (2.5 mm posterior to bregma, 3.6 mm lateral to the midline and 7.8 mm below the cortical surface). After 7–10 days' recovery, kindled rats were stimulated daily (60 Hz for 1 s; 100–150 μA) until five consecutive stage V seizures (32) were elicited. Sham-stimulated rats were handled the same as kindled, but not stimulated. Kindled rats and sham-stimulated controls were killed 24 h to 6 weeks after the last stimulus and slices were prepared. Experiments were conducted in parallel on kindled and control slices in the same chamber, and one to two slices from each animal were used on each day. Because no significant differences were observed as a function of time after last kindling stimulus, results were pooled for all kindled rats. Averages are mean ± SEM.

Low [Mg²⁺]₀-induced epileptiform bursting

The reduction of extracellular Ca²⁺ or Mg²⁺ concentrations ([Ca²⁺]₀ and [Mg²⁺]₀) has long been known to enhance neuronal excitability, an action presumed to be due to decrease of membrane surface charge screening, leading to facilitation of inward currents (10, 27). More recently, Mg²⁺ has been found to exert a voltage-dependent blocking action on excitatory amino acid receptors of the NMDA type (7, 20, 28, 29). Although these receptors are not necessarily involved in normal synaptic transmission (3, 6, 23, 25), recent work has indicated that their activation may underlie plastic changes in neuronal activity (3, 13, 23, 25, 43). Additionally, because NMDA receptor antagonists such as APV have potent antiepileptic properties (8, 16, 30), activation of NMDA receptors probably contributes to epileptogenesis. In the HC, an important role for NMDA receptors in neuronal plasticity is strongly supported. The NMDA receptor antagonist AP5 blocks the production of hippocampal LTP (3, 13, 43), and also antagonizes epileptiform activity in hippocampus (8, 16, 30).

Therefore, reducing [Mg²⁺]₀ is expected to enhance NMDA receptor activation, thereby helping to elucidate the role of these receptors in both normal and epileptogenic mechanisms. Indeed, reduction of [Mg²⁺]₀ in the perfusate of hippocampal slices enhances synaptically evoked potentials and facilitates generation of repetitive burst discharges (15, 24, 42). After lowering [Mg²⁺]₀, large spontaneous depolarizations have been observed in fields CA3 and CA1 of hippocampal slices (15, 42), and also in the neocortex (41). This epileptiform activity is blocked by AP5, indicating NMDA receptor involvement in its generation. In recent studies in the DG of isolated hippocampal slices, lowering [Mg²⁺]₀ facilitated the evoking of epileptiform discharges by high-frequency stimulation, but did not effect spontaneous activity (42). However, when input areas from the EC to the DG were intact, spontaneousactivity in the DG driven by the EC was observed. The similarity of these structures in the rat to those involved in temporal lobe epilepsy in humans suggests the combined EC→HC slice preparation as a useful one for the study of the role of receptors for NMDA and other neuro-modulators in the generation of low [Mg²⁺]₀-induced epileptiform activity. Therefore, we set out to characterize the role of NMDA receptors in generating low [Mg²⁺]₀-induced epileptiform bursting in EC→HC slices, as well as modulatory actions of NE on this bursting in both DG and EC.

Effects of Mg²⁺-free solution in EC

In combined EC→HC slices perfused with Mg²⁺-free medium, the washout of Mg²⁺ as measured with Mg²⁺-sensitive electrodes reached the detection limit of 0.7 mM (in the presence of 2 mM CaCl₂) in 12–20 min. Thereafter, equilibrium washout was probably achieved in 30–60 min.

Brief exposure of EC→HC slices to Mg²⁺-free medium led to marked enhancement of high-frequency stimulus-induced (subiculum; 20 Hz/10 s) decreases in extracellular Ca²⁺ (Δ[Ca²⁺]₀), increases in extracellular K⁺ (Δ[K⁺]₀), and slow negative field potentials (FP), in layer 1/2V of the EC. The effect was most pronounced with low-intensity stimuli, which were barely threshold to elicited responses in control medium, but produced much larger responses after 20 min in Mg²⁺-free medium. At higher intensities smaller enhancements were observed, but even the maximal Δ[Ca²⁺]₀, Δ[K⁺]₀, and FP were increased.
Fig. 40-1. Application of Mg\(^{2+}\)-free medium induces spontaneous epileptiform activity in entorhinal cortex layer IV/V, consisting of large $\Delta[Ca^{2+}]_o$, $\Delta[K^+]_o$, and negative field-potential shifts (FP). A: Spontaneous seizure-like events after 60 min application of Mg\(^{2+}\)-free medium. B: Another such spontaneous event on an expanded time scale. Ionic calibrations as in (A); FP and time as in (C). C: A single stimulus (arrow) evokes seizure-like events similar to those occurring spontaneously. Ionic calibrations as in (A). (Reproduced with permission from ref. 36.)

by 50–100%. At this time, single stimuli began to evoke one or more small negative potentials.

Continued perfusion with Mg\(^{2+}\)-free medium led to the development in EC layer V of large spontaneous FP transients that appeared between 30 and 90 min in 0 mM Mg\(^{2+}\), when $[Mg^{2+}]_o$ had declined to an estimated 0.05–0.2 mM. Fig. 40-1A illustrates these spontaneous seizure-like events, which exhibited a very rhythmic nature, occurring every 1–10 min. The $\Delta[K^+]_o$ responses associated with these events usually fit the characteristics of a late positive FP. One spontaneous event is illustrated on an expanded time scale in Fig. 40-1B. Once this spontaneous activity had appeared, a single stimulus was now also able to evoke a complete paroxysmal event similar in nature to the spontaneous events (Fig. 40-1C). A repetitive high frequency stimulation applied at this time elicited a spreading depression (SD) associated with prolonged (>1 min) negative FP shifts, large (>1 mM) $\Delta[Ca^{2+}]_o$ and large (>10 mM) $\Delta[K^+]_o$. During both spontaneous and stimulus-evoked seizure-like events in the EC, the slices were relatively refractory to further stimulation.

In addition to the large spontaneous paroxysmal events, small spontaneous depolarizations of population responses were often observed with frequencies of 0.01–0.1 Hz. When simultaneous extracellular recordings were made in the DG granule cell layer, smaller spontaneous depolarizations (up to 2 mV, 60 ms) were observed to closely follow the large seizure-like events in EC. A knife cut in the subiculum eliminated the spontaneous activity in the DG, but not in the EC, indicating that the EC possesses properties allowing the generation of seizure-like activity, which then drives the DG.

**Effects of Mg\(^{2+}\)-free solution in the DG**

Prolonged perfusion of combined EC–DG slices with Mg\(^{2+}\)-free medium led to spontaneous seizure-like activity originating in the EC and propagating to the DG. Additionally, reducing $[Mg^{2+}]_o$ also acted directly in the DG to enhance excitability in response to high-frequency perforant path stimulation (20 Hz/10 s). Perfusion with Mg\(^{2+}\)-free medium (30 min) markedly enhanced $\Delta[Ca^{2+}]_o$, $\Delta[K^+]_o$, and FP in the granule cell layer elicited by repetitive stimulation. As in the EC, near-threshold stimulus intensities were affected the most, but the maximal responses were also enhanced by 50–100%. Associated with these effects were multiple epileptiform population spikes in response to single stimuli, but, as with the spontaneous activity, these multiple spikes appeared only when the EC input was intact.

With more prolonged application of Mg\(^{2+}\)-free medium, high-frequency repetitive perforant path stimulation (20 Hz/10 s) often induced in the DG granule cell layer SDs or near SDs, consisting of large (>1 mM) $\Delta[Ca^{2+}]_o$, $\Delta[K^+]_o$ (>7 mM), and a prolonged negative FP (>10 mV). Associated with
this in slices with the EC input intact was an increase in the number and amplitude of the multiple epileptiform population spikes evoked by the single stimulus, and the appearance of smaller spontaneous depolarizations (up to 2 mV, 60 ms), which closely followed the large seizure-like events seen in EC. However, at no time were large spontaneous paroxysmal events, such as those seen in the EC, observed to occur spontaneously or in response to a single stimulus, supporting the conclusion that the DG is much less prone to generation of seizure-like activity than is the EC.

The NMDA receptor antagonist AP5 blocks epileptiform activity in EC and DG

In light of reports that low Mg\(^{2+}\)-induced epileptiform activity involves NMDA receptor activation and is antagonized by AP5 (15,42), we tested the effect of AP5 on the spontaneous and single stimulus-evoked paroxysmal events in the EC. Mg\(^{2+}\)-free medium was perfused until both spontaneous and subcortical single stimulus-evoked paroxysmal seizure-like events could be reliably elicited in the EC (>90 min). When AP5 was then applied (30 \(\mu\)M; 15 min), a single stimulus, which formerly elicited a paroxysmal event, now elicited almost no responses at all. Furthermore, even multiple 20-Hz stimuli did not elicit such paroxysmal events, and even a complete high-frequency tetanus (20 Hz/10 s), which would previously have elicited an SD, was now well tolerated by the slice and elicited only normal ionic and FP responses. Blockade by AP5 of these epileptiform bursts rapidly reversed after a 15-min drug-free wash, with both spontaneous and single stimulus-evoked paroxysmal activity returning in the EC.

Similar to the actions of AP5 in the EC, evoked SDs as well as spontaneous transients in the DG were also reversibly blocked by AP5. High-frequency perforant path stimulation (20 Hz/10 s), which had previously elicited an SD in Mg\(^{2+}\)-free medium, no longer did so in the presence of AP5 (30 \(\mu\)M). Furthermore, \(\Delta[Ca^{2+}]_I\) and \(\Delta[K^+]_I\) evoked by high-frequency stimulation that was subthreshold for inducing an SD were also substantially reduced by AP5, returning to baseline amplitudes of these responses in normal medium.

NE enhances stimulus-evoked epileptiform activity in the DG

Previous studies have indicated an important role for the neuromodulator NE in the normal expression of LTP of perforant path synapses in the DG (2,38) and have shown that NE has long-lasting excitatory actions in the DG (27,39). Recently, we have found that NE can enhance activation of NMDA receptors in the DG (P. K. Stanton et al., unpublished data). Therefore, we tested the actions of NE on the epileptiform activity and high-frequency evoked epileptiform activity seen in the DG in Mg\(^{2+}\)-free medium.

First, Mg\(^{2+}\)-free medium was perfused until high-frequency perforant path stimulation (20 Hz/10 s) above a threshold intensity would reliably evoke an SD. Then NE (50 \(\mu\)M) was bath applied and the perforant path was stimulated again. Figure 40-2 illustrates such an experiment, recording intracellularly from a DG cell (RMP = -72 mV; \(R_m = 30\) M\(\Omega\)), extracellular \(\Delta[Ca^{2+}]_o\), and FP. Shown on the left (90 min, 0 mM [Mg\(^{2+}\)]\(_o\)) are the responses to a high-frequency repetitive stimulation that was below the threshold intensity for evoking any seizure-like event (100 ms: 0.4 nA constant-current pulses superimposed for intracellular resistance measurement). In the presence of NE (50 \(\mu\)M), this same intensity now evoked a seizure-like event, consisting of prolonged granule cell depolarization (30 mV), negative FP (6 mV), and large (1 mM) \(\Delta[Ca^{2+}]_o\). For stimulus intensities that already evoked such events in the absence of NE, NE markedly prolonged their duration and somewhat enhanced their amplitude. These effects were quickly reversible after a 15-min drug-free wash (Fig. 40-2, wash). Furthermore, this excitatory action of NE was blocked by the \(\beta\)-receptor antagonist propranolol (1 \(\mu\)M), consistent with other data indicating that the excitatory actions of NE in the DG are probably \(\beta\)-receptor mediated (34–37; P. K. Stanton et al., unpublished data, 38,39).

NE antagonizes low Mg\(^{2+}\)-induced seizure-like activity in the EC

Our data concerning the excitatory actions of NE on low Mg\(^{2+}\)-induced epileptiform activity in the DG is consistent with previous data assigning an overall
Fig. 40-2. Norepinephrine (NE) enhances low Mg²⁺-induced stimulus-evoked spreading depressions (SD) in dentate granule neurons. Intracellular, extracellular FP and Δ[Ca²⁺]ᵢ recordings are shown after 90 min in Mg²⁺-free medium, 15 min after addition of NE (50 μM) to the perfusate, and 15 min after washout of NE. A low-intensity high-frequency stimulation (20 Hz/5 s) was selected that was subthreshold for evoking an SD before addition of NE. After 15 min in NE, the same stimulation now evoked a prolonged depolarization seen both intracellularly (intra) and extracellularly (FP), associated with enhancement of Δ[Ca²⁺]ᵢ. This effect was reversible (15-min wash). (Reproduced with permission from ref. 36.)

In contrast to the data from the DG, bath application of NE (50 μM) produced a potent blockade of the seizure-like activity generated in EC layer IV/V. Fig. 40-3 illustrates a typical experiment in which Mg²⁺-free medium was again perfused until reliable spontaneous and single stimulus-evoked paroxysmal seizure-like activity was observed in the EC (Fig. 40-3A). Then, NE (50 μM) was bath applied and the stimuli were reapplied. NE was only slightly less potent than AP5 (30 μM) in completely blocking the seizure-like activity, as evidenced by the fact that spontaneous activity disappeared after 12–15 min in NE, as opposed to 8–10 min in AP5. After 15 min in NE, we tested responses (Fig. 40-3B) to 1, 5, 10, and 20 times the original single stimulus given at a frequency of 20 Hz, none of which elicited a paroxysmal seizure-like event. Similar to the action of AP5, even a 20-Hz/10-s high-frequency stimulation no longer evoked an SD. This action of NE was also readily reversible, as shown in Fig. 40-3C after a 15-min wash, when both spontaneous and single stimulus-evoked paroxysmal events had returned.

Interestingly, this anticonvulsant action of NE was not blocked by the β antagonist propranolol (1 μM), but was blocked instead by the α₁-receptor antagonist prazosin (1 μM), indicating that noradrenergic anticonvulsant actions in the EC are probably α₁-receptor mediated, whereas excitatory actions of NE in the DG are primarily β-receptor mediated.

**Kindling-induced alterations in noradrenergic systems**

Among chronic models of the development of epilepsy, one of the most popular since its discovery has been the kindling model. In kindling, the brief,
In contrast to the DG, NE blocks low Mg\(^{2+}\)-induced epileptiform activity in EC. A: [Ca\(^{2+}\)], [K\(^{+}\)], and negative field-potential shifts (FP) in the EC occurring spontaneously or evoked by a single stimulus after 10 min in Mg\(^{2+}\)-free medium [time base as in (C)]. B: Fifteen minutes after addition of NE (50 μM) to the perfusate, the spontaneous activity in EC layer IV/V was completely blocked, and neither a single (arrow, 1X), 3 (5X), 10 (10X), nor 20 (20X) stimuli (20 Hz) could evoke seizure-like events like those in (A). [Calibrations as in (A); time base as in (C)]. C: Return of spontaneous and single stimulus-evoked epileptiform activity after 15 min washout of NE (slice remained in Mg\(^{2+}\)-free medium). [Calibrations as in (A)]. (Reproduced with permission from ref. 36.)

daily application of high-frequency stimulation eventually leads to the development of sustained seizure activity. There is mounting evidence of a modulatory role for NE in the expression of many forms of plasticity. In the hippocampal formation, NE produces a long-lasting potentiation of DG evoked FPs, (27,30), and enhances ionic fluxes associated with high-frequency perforant path stimulation (34). Intra-cellular studies indicate that NE can greatly enhance repetitive spike firing both in hippocampal pyramidal cells (12,13) and in DG granule cells (P. K. Stanton et al., unpublished data), parallel to a β-receptor-mediated block of the burst-induced late afterhyperpolarization (AHP). At the same time, studies have shown that depletion of NE impairs the development of high-frequency stimulus-induced LTP of perforant path synapses (2,38). Moreover, we have recently shown that NE is likely to induce long-lasting plasticity by enhancing activation of glutamate receptors of the NMDA subtype (35, P. K. Stanton et al., unpublished data), and that plasticity induced by daily high-frequency stimulation (kindling) of DG input fibers unmasks an NMDA component of dentate synaptic transmission (25,23). This led us to search for alterations in neuronal sensitivity to NE after kindling, changes that might influence production, and/or persistence of neuronal plasticity.

In initial studies of nonadrenergic sensitivity in the DG of slices from kindled rats, we focused on extracellular measures of excitatory actions that seemed likely to underlie the enabling effect of NE in dentate long-term neuronal plasticity. NE has been shown to elicit a persistent and robust enhancement of perforant path evoked population responses (27,39). Furthermore, this effect is β\(_2\)-receptor mediated (40), and β antagonists also impair the full expression of perforant path LTP (38). An example of NE-induced potentiation is illustrated in Fig. 40-4A (CONTROL), in which NE more than doubled the population spike amplitude after 15 min bath application of NE, which persisted 30 min in the drug-free wash. In contrast, NE did not potentiate perforant path evoked FPs in slices from kindled rats (Fig. 40-4A. KINDLED). p < 0.05 Student’s t test compared with controls).

In addition, we have shown that NE can enhance the decreases in extracellular Ca\(^{2+}\) concentration and associated negative FP shifts [largely neuronal depolarization due to flow of positive charge from the extracellular space into neurons; (14)] evoked by a brief high-frequency tetanus of a type that elicits LTP (34). This is illustrated in Fig. 40-4B (CONTROL), in which NE (50 μM; 15 min) doubled the slow negative FP and Ca\(^{2+}\) influx produced by repetitive perforant path stimulation (20 Hz/10 s). Although this effect is reversible, we have proposed that both NE-induced increases in neuronal firing and Ca\(^{2+}\) influx may trigger long-term plasticity. Furthermore,
Fig. 40-4. After kindling-induced plasticity, norepinephrine (NA) no longer produced long-lasting potentiation of evoked field potentials or enhanced repetitive stimulation-induced Ca²⁺ influx in the DG; at paired-pulse evoked population potentials in kindled and control hippocampal slices before (Pre-NA), during a 15-min bath application of NA (50 μM), and after a 30-min drug-free wash. Although NA elicited a long-lasting potentiation of the population spike in control slices, this capacity was lost after kindling. Decreases in extracellular Ca²⁺ concentration and slow negative field potentials (FP) produced in the dentate granule cell layer [in different slices from (A)] by a brief, high-frequency perforant path stimulation (20 Hz/10 s). In control slices, NA (15 min, 50 μM) markedly enhanced both the Ca²⁺ influx and FP elicited by this stimulation, in a reversible fashion (Wash). In contrast, after kindling, NA no longer enhanced either the Ca²⁺ influx or FP elicited by the tetanus. (Reproduced with permission from ref. 37.)

Illustrated in Fig. 40-5A is a typical reduction in granule-cell slope conductance (i.e., increase in resistance) produced by NE (50 μM; 15 min). In contrast to data from CA1 pyramidal cells (18), we have found (25a) that the modest depolarization (2–7 mV) and resistance increase (+34 ± 6.5%) elicited in granule cells by NE are typically long lasting (>60 min), suggesting that these may reflect one of the underlying mechanisms for NE-induced long-lasting plasticity in the dentate. Figure 40-5C (CONTROL) illustrates the NE-induced enhancement of repetitive firing to a depolarizing current pulse applied through the recording electrode, an action that was reversible. Where only three action potentials fired before bath application of NE (Pre-NA), seven spikes were elicited in the presence of NE. In contrast to control neurons, NE applied to granule cells in slices from kindled rats no longer reduced slope conductance (Figure 40-5B), or increased the number of action potentials elicited by depolarizing stimuli (Fig. 40-5C; Kindled, p < 0.05 Student's t test).

as with NE potentiation of single evoked potentials, these effects are also lost in slices from kindled rats (KINDLED, p < 0.05 Student's t test).

We now turned to intracellular recordings from DG neurons to assess actions of NE on cellular excitability before and after kindling. Kindling by itself induced marked increases in granule cell repetitive firing evoked by a depolarizing current step (1 nA), control granule cells (GCS): 2.8 ± 0.76 action potentials, kindled GCS: 6.25 ± 2.3 APS, p < 0.05 Student's t test compared with controls). We have recently found that, like CA1 pyramidal cells (12, 18), NE depolarizes DG cells, increases input resistance, and increases action potential burst firing evoked either by depolarizing current injection or a synaptic tetanus (25a). These effects are associated with β₁ receptor-mediated blockade of the slow AHP that follows repetitive firing (12, 18; P. K. Stanom, unpublished data), probably by block of a Ca²⁺-activated K⁺ current underlying the late phase of this AHP (17).
Fig. 40-5. After kindling-induced plasticity, NA no longer elicits decreases in membrane slope conductance or blocks accommodation of depolarization-induced repetitive action potential firing. A: Action of bath-applied NA to reduce granule-cell slope conductance in a typical recording from a control slice. (RMP = -72 mV.) B: In contrast to (A), a typical granule cell in a slice from a kindled rat no longer exhibits reduced slope conductance with NA application. (RMP = -78 mV.) C: Responses to a depolarizing current step (150 ms; control 1.5 mA; kindled 1.0 mA) applied through the intracellular recording electrode in typical dentate granule cells in slices from control and kindled rats. Responses are shown before (Pre-NA) during 15 min NA (50 μM), and after a 30-min wash. In controls, NA markedly blocked accommodation of repetitive spike firing in depolarizing steps whereas in slices from kindled rats, NA no longer elicited such excitation of repetitive firing. (Control RMP = -70 mV; R_{in} = 30 MΩ; kindled RMP = -72 mV; R_{in} = 42 MΩ.) (Reproduced with permission from ref. 37.)

Consistent with our extracellular data, NE no longer excited granule cells after kindling-induced neuronal plasticity. These findings could suggest either that NE has specifically lost β₁-receptor-mediated actions on granule cells, or a more general impairment in granule-cell sensitivity to NE. Therefore, as a separate index of α-receptor sensitivity, we examined the action of NE on presumed Ca^{2+}-dependent regenerative potentials in granule cells; potentials that we have recently found are attenuated by NE acting on α_1, rather than on β receptors (P. K. Stanton et al., unpublished data). Recently, some interesting features of such Ca^{2+} potentials have emerged. In normal granule cells, such potentials are unmasked only when both fast Na⁺ spikes and K⁺ conductances are blocked (25; P. K. Stanton et al., unpublished data) [in our studies, by intracellular injection of the lidocaine-derivative QX314 plus Cs⁺, to block Na⁺ spikes and K⁺ conductances, respectively (4,31)]. In contrast, after the induction of kindling, we have found that simply blocking fast Na⁺ spikes with QX314 is sufficient to reveal Ca^{2+} potentials (25). These results suggest kindling-induced alterations in the expression and/or activation of regenerative Ca^{2+} potentials, and led us to test the sensitivity of such potentials to NE...
after kindling. As with other measures of NE efficacy, the induction of kindling plasticity results in the loss of noradrenergic actions on presumed Ca\(^{2+}\)-dependent regenerative potentials (data not shown).

Computational modeling of multiple noradrenergic actions

Although multiple noradrenergic receptor effects can complexly influence granule-cell firing, the spatial distribution of these receptors may add further difficulty not easily addressed experimentally. Another complementary approach to analyzing simultaneous nonlinear systems is to employ a simplifying computational model. We employed such a granule-cell computer model to simulate the effects on granule cell firing patterns of dendritic versus somatic (loci of the principal conductances affected.

Our model consists of a multicompartiment neuron with Hodgkin-Huxley and other active ion channels, plus the Nernst-Planck equation for electrodiffusion of ions in dendritic spines. The simulator is written using structured object-oriented programming techniques that allow easy manipulation of physiologically relevant parameters within the code, and permits measurement of voltage and calcium in both somatic and dendritic compartments.

Figure 40-6 (CONTROL) illustrates a simulated burst discharge from the model granule neuron. In this run, blockade of either regenerative Ca\(^{2+}\) spikes (Alpha) or Ca\(^{2+}\)-dependent K\(^+\) conductance (Beta) was simulated over the entire cell membrane. In this simplest (a probably mostly nonphysiologic) case, \(\alpha\)-receptor activation completely eliminated burst discharge, the underlying depolarization shift, and Ca\(^{2+}\) influx. \(\beta\)-receptor activation over the entire cell markedly prolonged burst duration and doubled the number of action potentials fired. (All responses are shown from somatic potential.)

In contrast, finer gradations of action can be achieved by spatially separating \(\alpha\)-and \(\beta\)-receptor effects. Figure 40-7 illustrates responses simulating the application of NE with four possible spatial arrangements of \(\alpha\) and \(\beta\) receptors. When both actions predominate in the soma, little change is seen in spike firing pattern, but the underlying depolarization shift and Ca\(^{2+}\) influx is blocked. Both actions in the dendrite have little effect on somatic responses at all, but have large local influences on adjacent dendritic inputs. Splitting the conductances between soma and dendrites has opposing effects depending on which conductance is present in each location. Thus, NE can either markedly attenuate somatic Ca\(^{2+}\) influx (Alpha SOMA, Beta DENDRITES), or prolong bursting-induced depolarization and Ca\(^{2+}\) influx (Alpha DENDRITES, Beta SOMA).

Such computational models are becoming increasingly useful as tools revealing the behavior of even simplified nonlinear systems (such as single neurons) in ways that supply hypotheses amenable to experimental verification. For example, studies now under way are comparing dendritic and somatic Ca\(^{2+}\) influx via voltage-dependent and NMDA channels and the ways NE may regulate Ca\(^{2+}\) influx in granule neurons.

Overview

Our findings on low Mg\(^{2+}\)-induced epileptiform activity lead to a number of conclusions: (a) Reducing [Mg\(^{2+}\)] in combined slices of EC–HC elicits epileptiform activity in both EC layer IV/V and in the DG granule-cell layer. (b) In the EC, there are large spontaneous paroxysmal seizureslike events that are also evocable with single stimuli. (c) In contrast to the EC, the DG does not exhibit spontaneous seizureslike activity in isolation, but can be driven by activity
in the EC. The DG, like the EC, is prone to high-
frequency stimulus-induced SD, consisting of pro-
longed negative FP shifts, large Δ[Ca\(^{2+}\)]\(_o\) (>1 mM)
and Δ[K\(^{+}\)]\(_o\) (>5 mM). (d) Low Mg\(^{2+}\)-induced epil-
eptiform activity in both the EC and DG is blocked
by APS, indicating an important involvement of NMDA
receptors in its generation. (e) NE exhibits a com-
plementary modulation of epileptiform activity in two
areas. In the DG, NE enhances granule-cell excita-
ility, whereas in the EC, NE blocks both sponta-
neous and evoked epileptiform activity. (f) Although
the excitatory action of NE in the DG appears to be
β-receptor mediated, the anticonvulsant action of NE
in the EC is probably an α-receptor effect.

Data from these and other studies indicate that
reducing extracellular [Mg\(^{2+}\)]\(_o\) produces marked en-
hancements in neuronal excitability that can strongly
resemble seizure activity. A number of mechanisms
have been proposed to underlie these actions, includ-
ing (a) removal of antagonism by Mg\(^{2+}\) on pre- and
postsynaptic Ca\(^{2+}\) entry, (b) reduced surface charge
screening and associated facilitation of inward cur-
rents and action potentials (10,20,22), and (c) re-
moval of voltage-dependent Mg\(^{2+}\) blockade of NMDA
ionophores (6,20,28,29) show marked antagonism
of this epileptiform activity by the NMDA-receptor
antagonist APS. In light of the anticonvulsant prop-
erties of APS (30), it seems clear that NMDA-recep-
tor activation is important in seizure generation and
maintenance.

Under physiologic conditions, when cells are near
resting membrane potential, NMDA receptors are
inactive in synaptic transmission due to the blocking
action of Mg\(^{2+}\) on channel opening at these poten-
tials. When this blocking effect is removed, pro-
longed neuronal firing, repetitive discharges, and even
paroxysmal depolarization shifts may result from sin-
gle synaptic stimuli. Thus, stimuli that formerly eli-
cited mild depolarizations now elicit greatly enhanced
excitation and often massive activation of neuronal
aggregates, through recruitment of unblocked NMDA
receptors.

It is interesting to note that the EC possesses
properties allowing generation of spontaneous sci-
ure-like activity closely resembling that seen in status epilepticus. In contrast, the DG does not exhibit such spontaneous activity, although smaller local events driven by EC do occur. Even though the DG is characterized by a high density of NMDA receptors (26) and these receptors probably participate in long-term plastic changes (3, 13, 43), the DG seems better able to regulate their activation in low [Mg$^{2+}]_o$. Thus, greater intrinsic inhibition present in the DG may allow it to serve as a filter reducing excitatory load to the rest of the HC.

Furthermore, the DG exhibits marked differences in excitability characteristics depending on whether or not the EC remains attached. Previous work in isolated hippocampal slices (23) has shown that, if any, multiple population spike firing is observed in Mg$^{2+}$-free medium in response to single perforant path stimuli. In contrast, we observed strong multiple population spike firing and large increases in Δ[Ca$^{2+}$]$_i$ in Mg$^{2+}$-free medium when the EC is retained. Therefore, the presence of the EC and its connections with the DG may increase DG excitability rather than the antidromic stimulation of EC neurons whose firing excites DG granule cells, or through stimulation of reciprocal synaptic feedback inputs from HC to EC, or both. Consequently, interactions between EC and DG in an intact preparation may enhance the contribution of the DG to epileptiform activity.

It remains an open question whether changes in [Mg$^{2+}]_o$ large enough to unblock NMDA receptors are present during seizure activity. Studies with Mg$^{2+}$-sensitive electrodes show decreases in [Mg$^{2+}]_o$ elicited by epileptiform activity, application of excitatory amino acids, and SD (11). Heinemann et al., unpublished data). These decreases are much smaller than Δ[Ca$^{2+}$]$_i$ observed in similar conditions, indicating that Δ[Mg$^{2+}]_o$ may play a secondary role in development of seizure activity. Nevertheless, low Mg$^{2+}$-induced epileptiform activity appears to support the role of NMDA receptors in generation and maintenance of seizure activity, as well as to supply, especially in the EC, a useful model for studies of neuromodulatory control of seizure activity and screening of drugs for anticonvulsant properties.

Perhaps the most interesting result presented here concerns the complementary noradrenergic regulation of seizure activity in the EC and DG. Previous work has supported a role for NE in expression of LTP in the DG (2, 38), and shown excitatory properties of NE in this area (27, 34, 39). In light of this, it was unclear how normal noradrenergic transmission could also be antiepileptogenic (21). Now, we find that NE has unique complementary actions permitting the suppression of synaptic inputs from the EC and retarding epileptogenesis in this area, while enhancing throughput in the DG filter to the HC. By simultaneously reducing noise in the input and boosting the gain of the dentate filter, NE seems able to improve signal-to-noise ratio across the hippocampal network even more effectively than previously postulated (33).

It seems likely that NE can modulate learning and memory (5, 9, 19, 40), long-term hippocampal plasticity (2, 35, 38, 39), and seizure generation and maintenance (21). The presence of NE has a potent associative effect on the magnitude of hippocampal plasticity (2, 38) and may thereby allow this modulator to exert an enabling function in the association of synchronized inputs during learning. At the same time, epileptiform reverberant activity in neuronal aggregates may be dampened by NE through its anticonvulsant properties. It seems likely that pharmacologic intervention in noradrenergic transmission may influence both disorders of memory consolidation and retrieval, and disorders of reverberant activity in neuronal aggregates in epilepsy.

Our results with the kindling model of epileptogenesis indicate that large depletions in DG neuronal sensitivity to NE are associated with kindling of either the hippocampal commissures or the amygdala. This is potentially significant in the context of multiple possible roles for NE in epileptogenesis in the DG and EC. In studies relating NE to kindling and LTP, an interesting paradox has emerged. While NE increases dentate granule cell excitability (34–37; P. K. Stanton et al., unpublished data; 38, 39), depletion of NE accelerates the rate of kindling (1, 21), suggesting that NE suppresses kindling development. Studies indicate that α$_2$ receptors likely mediate this antiepileptogenic action of NE (11). However, our acute studies indicate that NE has a predominantly β, excitatory action on dentate granule cells, but a potent α$_2$ inhibitory effect on EC epileptiform activity. Our finding that kindling is associated with marked
downregulation of both \( \alpha_1 \) and \( \beta_1 \) noradrenergic receptor sensitivity supports the notion that receptor and/or area-specific actions of NE may simultaneously amplify repetitive signals and discourage recurrent paroxysmal activity. This also leads to the prediction that concomitant downregulation of \( \alpha_2 \)-receptor sensitivity may contribute to development of kindling-induced seizures.

Studies have shown that depletion of NE reduces perforant path-DG LTP (3,9), that NE enhances repetitive stimulation-induced depolarizations, \( \text{Ca}^{2+} \), K- fluxes (34), and efficaciously long-lasting potentiation of perforant path synapses (27,39), and that \( \beta \)-receptor antagonists impair both noradrenergic potentiation (27,39) and LTP (38). These all make it clear that the full expression of long-term neuronal plasticity in the DG requires NE. However, our findings indicate that a prior history of long-term plastic changes in DG excitability renders the dentate incapable of exhibiting NE-induced plasticity. Furthermore, intracellular measurement of NE actions on dentate granule cells show that prior induction of long-term plasticity renders them insensitive to either \( \beta \)-receptor-mediated actions on passive membrane properties, enhancement of repetitive firing, blockade of the AHP, or \( \alpha \)-receptor-mediated blockage of regenerative \( \text{Ca}^{2+} \) potentials, indicating a general decrease in sensitivity to NE.

Interestingly, we have shown that potentiation of evoked potentials and ionic fluxes produced by NE is blocked by the NMDA receptor antagonist AP5 (P. K. Stanton et al., unpublished data). Furthermore, we have recently found that kindling mimics a prominent NMDA component in dentate synaptic potentials (24,25). This leads us to suggest that NE, which seems to induce plastic changes by enhancing NMDA receptor activation, may lose this capacity once NMDA receptors are upregulated, perhaps as a "write protect" mechanism against further modification of synaptic strength. Future studies in the developing brain may find that, before the activation of mechanisms protecting against further long-term changes in synaptic strength, NE-induced long-term plasticity, as well as plastic actions of other neuromodulators, are more general phenomena. Exploring the mechanisms underlying downregulation of noradrenergic sensitivity may open new approaches to learning disorders and epilepsy in both young and aged brain.

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References


43. Wiegandt H, Fruehmann H. A possible correlate of the post-

Discussion

Ben-Ari: Could you elaborate on the effects of norepinephrine in control conditions on the AHP and the calcium spikes? What happens first? Do you first block the potassium conductance and then the calcium spikes or do you first block the calcium spikes and then the AHP?

Stanton: That would be difficult to say. I really cannot answer that for the following reason. Normally in dentate granule cells it is very difficult to evoke a calcium spike, so it requires the intracellular application of QX314 as well as cesium chloride. The question of time course could possibly be pursued in the kindled state; studies by myself and Dr. Mosy have suggested an upregulation of these calcium potentials, so that after kindling you no longer need to block potassium currents. You can simply evoke them in granule cells by blocking sodium currents. In the development of kindling, studies remain to be done to determine which noradrenergic effects are lost first, alpha or beta.