Duration of NMDA-Dependent Synaptic Potentiation in Piriform Cortex In Vivo Is Increased After Epileptiform Bursting

A. KAPUR1 AND L. B. HABERLY1,2
1Neuroscience Program and 2Department of Anatomy, University of Wisconsin, Madison, Wisconsin 53706

Kapur, A. and L.B. Haberly. Duration of NMDA-dependent synaptic potentiation in piriform cortex in vivo is increased after epileptiform bursting. J. Neurophysiol. 80: 1623–1629, 1998. Stimulation of afferent fibers with current pulse trains has been reported to induce long-term potentiation (LTP) in piriform cortex in vitro but not in vivo. LTP has been observed in vivo only when trains are paired with behavioral reinforcement and as a consequence of kindled epileptogenesis. This study was undertaken in the urethan-anesthetized rat to determine if the reported failures to observe pulse-train evoked LTP in vivo may be related to a lesser persistence rather than lack of occurrence, if disinhibition might facilitate induction, and to examine the nature of the relationship between seizure activity and LTP. Stimulation of afferent fibers in the lateral olfactory tract with $\theta$-burst trains under control conditions potentiated the monosynaptic field excitatory postsynaptic potential (EPSP) by approximately the same extent (20.3 ± 2%; $n = 12$) as reported for the slice. However, in contrast to the slice, potentiation in vivo decayed to a low level within 1–2 h after induction (70% loss in 1.5 h, on average). The $N$-methyl-$\alpha$-aspartate (NMDA)-receptor antagonists $\alpha$-APV and MK-801 blocked the induction of this decremental potentiation. Pharmacological reduction of $\gamma$-aminobutyric acid–mediated inhibition at the recording site did not increase the duration of potentiation. In contrast, $\theta$-burst stimulation applied after recovery from a period of epileptiform bursting induced stable NMDA-dependent potentiation. Mean increase in the population EPSP was approximately the same as under control conditions (21 ± 2%; $n = 6$), but in five of six experiments there was little or no decay in potentiation for the duration of the monitoring period ($\leq 6$ h). It is concluded that seizure activity has an enabling action on the induction of persistent synaptic potentiation by stimulus trains that bypass the need for behavioral reinforcement.

INTRODUCTION

In piriform cortex in vitro, long-term potentiation (LTP) can be induced by stimulating afferent fibers with high-frequency trains of current pulses (Carpenter et al. 1994; Jung et al. 1990; Kanter and Haberly 1990). In contrast, similar trains were reported to induce little or no LTP in vivo (Racine et al. 1983; Roman et al. 1987, 1993; Stripling et al. 1988). However, when train stimulation is linked to reinforcement through a learning paradigm, LTP is induced at the afferent fiber synapse that persists for >24 h (Roman et al. 1987, 1993). Persistent potentiation was also reported at this synapse after kindling with high-frequency trains to the lateral olfactory tract (LOT) (Racine et al. 1983) or olfactory bulb (Racine et al. 1991). $N$-methyl-$\alpha$-aspartate (NMDA) dependence of the induction of LTP, a key feature with regard to mechanisms of learning (Brown et al. 1990), was demonstrated in vitro but not for the potentiation associated with behavioral reinforcement or kindling.

Experiments were undertaken to further investigate synaptic potentiation in piriform cortex in vivo by using a urethan anesthetized rat preparation. Questions that were addressed are as follows. Is the reported failure of pulse trains alone to induce LTP in vivo a result of a lack of induction or the induction of a decremental form of synaptic potentiation? Can NMDA-dependent LTP be induced in vivo as in vitro? Can seizure activity enable the induction of persistent potentiation by stimulus trains in vivo? Can LTP be enabled by disinhibition in vivo?

The results revealed that an NMDA-dependent synaptic potentiation can be induced at the afferent fiber synapse by pulse trains in vivo, but in contrast to findings in vitro the magnitude of potentiation decayed substantially <1 h of induction. However, after recovery from a period of epileptiform bursting resulting from surface application of a $\gamma$-aminobutyric acid–$\alpha$-receptor antagonist, persistent NMDA-dependent synaptic potentiation was induced by the same stimulus paradigm that failed to induce a persistent increase under control conditions. Local blockade of fast GABAergic inhibition did not increase the persistence of this potentiation, indicating that the occurrence of seizure activity rather than residual disinhibition was responsible for the increase.

METHODOLOGY

Adult male Sprague-Dawley rats weighing 190–300 g were anesthetized with urethan (1.4 g/kg ip initial dose with supplements as necessary to maintain a surgical level of anesthesia). The animals were tracheotomized, and their external carotid, occipital, and pterygopalatine arteries were ligated or cauterized to minimize bleeding. The lateral aspect of the cranium was exposed by removing the left eye, upper portion of the mandible, and musculature of the face. Burr holes were made in the skull over the LOT for stimulation and over the anterior or posterior piriform cortex for recording. The dura was removed at the recording site. At the termination of the experiments animals were killed with an overdose of urethan.

A tungsten microelectrode was inserted through the dura into the LOT to stimulate afferent fibers. Selective stimulation of afferent fibers was confirmed by observing the response to paired pulses separated by 30–50 ms. Because dysynaptic response components are blocked over this interval by the inhibitory postsynaptic potential (IPSP) evoked by the first pulse, selective activation of afferent fibers can be confirmed by the absence of a dicrotic population excitatory postsynaptic potential (EPSP) in response to the second pulse (slowly conducting association fibers mediate a 2nd peak that is not blocked when they are monosynaptically activated). Glass micropipettes filled with 2 M NaCl were used for extracellular recording. After positioning the electrodes, the cortex was cov-
and initial peak of this field potential component, which will therefore be referred to as the population EPSP (pEPSP). Two computer-derived pEPSP parameters were used to monitor efficacy at the afferent fiber synapse, slope of the rising phase (termed pEPSP slope) and peak amplitude. Both measures yielded equivalent results, but all data presented were derived from measurements of slope. LTP was defined as pEPSP potentiation that displayed little decay for \( \geq 1 \) h. To quantify the time course of potentiation, pEPSP slope was averaged over 10-min intervals. Maximal potentiation was defined as the average percent increase over control amplitude during the 10-min interval after \( \theta \)-burst stimulation. Averages for subsequent 10-min intervals were expressed as percentages of the maximal level. Means for different conditions were compared with the two-tailed, unpaired \( t \)-test.

Epileptiform (seizure) activity, defined by the presence of large abnormal field potential components with a stereotyped waveform and discrete threshold, was induced by applying a drop of saturated picrotoxin (Sigma) in saline on the surface of the exposed cortex. Five to 20 min later, the cortex was repeatedly flooded with saline to accelerate recovery. Abnormal activity elicited by this procedure consisted of spontaneous and stimulus-evoked “interictal-like” events rather than sustained “ictal-like” discharges.

Local disinhibition was achieved without evoking epileptiform activity by applying (–) bicuculline methiodide (Sigma; 10 mM in saline) or picrotoxin (Sigma; saturated in saline) through the recording pipette (method of Steward et al. 1990) either by diffusion or by application of \( \leq 50 \) nA of iontophoretic current. Tip diameters of pipettes were 10–16 \( \mu \)m OD. To confirm the presence of disinhibition, multunit activity was monitored by filtering the field potential signal at a bandwidth of 500–5 kHz with four-pole high- and low-pass filters.

To block NMDA receptors, \( \text{D}(-)-2\text{-amino-5-phosphonovaleric acid (D-APV, Cambridge Research Biochemicals) was topically applied as a 0.4- to 3.5-mM solution in saline, or (} (+)-MK-801\text{ hydrogen maleate (Research Biochemicals) was injected intraperitoneally (0.5 or 1.0 mg/kg) 3–5 h before the start of the experiment.}

**RESULTS**

**Theta-burst stimulation induces decremental NMDA-dependent synaptic potentiation under control conditions**

Theta-burst stimulation of the LOT induced a consistent increase in pEPSP slope and peak amplitude (Fig. 1). Maximum potentiation was reached \( \leq 2 \) min after the train and averaged 20.3 ± 2\% (mean \( \pm \) SE, \( n = 12 \)). This degree of potentiation is comparable with that observed at the afferent fiber synapse in vitro with the same stimulus paradigm (Kanter and Haberly 1990).

To confirm that the monosynaptic EPSP was potentiated, paired test pulses separated by 50 ms were used in some experiments. Previous study has shown that disynaptic excitatory and inhibitory components are blocked in response to the second of a pair of pulses separated by 50 ms, thereby isolating the monosynaptic EPSP (Haberly 1973b; Haberly and Bower 1984; Ketchum and Haberly 1993a). Changes in the slope and peak of the response to the second stimulus of the pair (Fig. 1C) were comparable with those observed in the response to single pulses (inset, Fig. 1A), confirming that potentiation was induced in the monosynaptic EPSP.

Amplitude of the potentiated population EPSP decreased to near baseline over the posttrain monitoring period in 10 of 12 experiments (Fig. 1, A and C). In pooled data from
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FIG. 2. Time course of decay in potentiation in the control condition and after local disinhibition at the recording site. Decay is illustrated as percent of maximum potentiation (defined as average for 0–10 min from burst application relative to average baseline for preceding 10-min period). Open squares are means for control experiments (n = 12); solid symbols are means for local disinhibition experiments in which bicuculline or picrotoxin was applied through the recording pipette (n = 8). Rate of decay for control and local disinhibition conditions was not significantly different (P > 0.5, 2-tailed, unpaired t-test). Error bars are SE.

All experiments (Fig. 2, open squares) potentiation was decreased by an average of 70% at 1½ h after train application.

To determine if induction of the observed decremental synaptic potentiation required activation of NMDA receptors, β-burst stimulation was applied in the presence of topically applied d-APV (0.4–3.5 mM in saline) (Fig. 3A; n = 3) or intraperitoneal MK-801 (0.5 and 1 mg/kg, n = 2; not illustrated). Both treatments blocked the induction of potentiation in all experiments, indicating dependence on NMDA receptors. The effect of d-APV was reversible (Fig. 3B), confirming that the loss of potentiation was a consequence of drug action.

FIG. 3. Induction of potentiation at the afferent fiber synapse is N-methyl-d-aspartate dependent. A: mean slope of the pEPSP (n = 3, ±SE) in the presence of topically applied (-)-2-amino-5-phosphonovaleric acid (d-APV). Slope is expressed relative to baseline level as in Fig. 1. Theta-burst stimulation applied at time 0, did not induce a detectable change. B: same experiments as in A but after washout of d-APV.

Duration of synaptic potentiation was unaffected by local disinhibition

Because GABA_A-mediated inhibition can restrain the induction of NMDA-mediated LTP in piriform cortex (Collins 1994; del Cerro et al. 1992; Kanter and Haberly 1993) as in other systems (Steward et al. 1990; Wigstrom and Gustafsson 1983), one possible explanation for the occurrence of train-induced LTP in vitro but not in vivo is the greater strength of IPSPs in the intact cortex (Haberly, unpublished data). Therefore the effects of disinhibition on the duration and amplitude of potentiation were examined. To avoid seizure activity that is induced when more than a small area of piriform cortex is disinhibited, GABA_A antagonists were introduced at the recording site by application through the recording pipette. This was accomplished by diffusion or iontophoresis of bicuculline or picrotoxin as described in methods. The same application procedure in slices of piriform cortex enabled the induction of associative LTP that was not observed under control conditions (Kanter and Haberly 1993). To confirm the presence of disinhibition, multiunit activity was recorded by high-pass filtering of the field potential response. As illustrated in Fig. 4B, multiunit spiking under control conditions is limited to the rising phase of the pEPSP by the onset of a GABA_A-mediated IPSP (Haberly 1973a). The presence of disinhibition was indicated by an increase in the duration of spiking (Fig. 4C) and by an increase in the deep-negative field potential component.
served under control and local disinhibition conditions. Induction of the persistent potentiation was blocked by d-APV and MK-801 (not illustrated), indicating a dependence on NMDA receptors as for the decremental form.

**DISCUSSION**

The results confirmed that NMDA-dependent synaptic potentiation can be induced in the piriform cortex in vivo as previously observed in slices. In contrast to findings for the slice, the degree of potentiation decayed over time under control conditions. However, after a period of epileptiform bursting, true LTP was induced, which persisted with little or no decay for the full observation period (≥6 h).

An important question is whether the persistent potentiation that was induced after disinhibition-evoked epileptiform activity was a consequence of the occurrence of this abnormal activity or a result of residual disinhibition at the time of the potentiating stimulus. Two arguments suggest that the persistence was a consequence of the epileptiform activity. First, there was little or no difference in the waveform of the evoked field potential relative to the preepileptiform control response at the time the potentiating train was delivered, indicating that recovery from disinhibition was largely complete. Second, local disinhibition had no effect on the persistence of synaptic potentiation. On the basis of the associated changes in the field potential, this local disinhibition appeared to be stronger at the recording site than any residual disinhibition after recovery from epileptiform activity.

**Relationship to other studies in piriform cortex**

Previous studies at the afferent fiber synapse in piriform cortex revealed an intriguing difference in the persistence of synaptic potentiation in the slice and the intact cortex. In slices, NMDA-dependent LTP evoked by θ-burst stimulation (Fig. 4A). In five of eight experiments, iontophoretic current was adjusted to a level just below that evoking epileptiform field potential components. In the presence of this local disinhibition, potentiation induced by θ-burst stimulation was similar in amplitude (21.6 ± 2.4%, n = 8) and decayed at approximately the same rate as in the control condition (Fig. 2, solid symbols).

**Persistent NMDA-dependent synaptic potentiation is evoked after recovery from widespread disinhibition that evokes epileptiform activity**

To assess the effects of seizure activity on the subsequent induction of synaptic potentiation, a drop of saturated picrotoxin solution was applied to the exposed cortical surface followed by repeated washing with saline after the onset of epileptiform bursting. After return of the large deep-negative field potential component to the preapplication control level (requiring 1–4 h, Fig. 5A), θ-burst stimulation was applied. In contrast to control and local disinhibition conditions, the resulting potentiation persisted with little or no decrease for the duration of experiments (≥6 h) in five of six animals (Fig. 5B). Figure 6 illustrates pooled data from all experiments to compare the time course of potentiation evoked after recovery from epileptiform bursting (solid symbols; n = 6) with that evoked under control conditions (open squares; n = 12). Differences at individual time points were significant at P < 0.05–0.01 (2-tailed, unpaired t-test). Mean potentiation was 21 ± 2%, a close match to that observed under control and local disinhibition conditions. Induction of the persistent potentiation was blocked by d-APV and MK-801 (not illustrated), indicating a dependence on NMDA receptors as for the decremental form.

**Fig. 5.** Persistent potentiation (LTP) is induced when θ-burst stimulation is applied after recovery from epileptiform bursting. A: field potential evoked by an identical test pulse before, during, and after recovery from epileptiform activity induced by surface application of picrotoxin. Note the large abnormal potential in the presence of picrotoxin and recovery to a waveform that was similar to preapplication control. B: representative experiment showing persistent potentiation of the pEPSP when θ-burst stimulation was applied after recovery from epileptiform bursting. Slope is expressed relative to pretrain baseline as in Fig. 1.

**Fig. 6.** Pooled results documenting the increase in duration of synaptic potentiation induced after recovery from epileptiform bursting. Decay of potentiation for control experiments (●, n = 12; same data as in Fig. 2) and after recovery from epileptiform activity (solid symbols, n = 6). Decay is expressed as percent of initial potentiation as in Fig. 2. Error bars are SE. ∗ Difference significant at P < 0.05; ** difference significant at P < 0.01 (2-tailed, unpaired t-test).
of afferent fibers decays little during observation periods of ≈1.5 h (Kanter and Haberly 1990). In unanesthetized animals with chronically implanted electrodes, θ-burst stimulation (Roman et al. 1987) and more sustained high-frequency trains (Racine et al. 1983; Stripling et al. 1988) induce little or no LTP.

Persistent potentiation has been induced in vivo by pulse trains in piriform cortex under two conditions. First, when θ-burst trains that do not induce LTP under control conditions are paired with behavioral reinforcement, LTP is induced that persists for >24 h (Roman et al. 1987 1993). Second, when high-frequency trains to afferent fibers in the LOT (Racine et al. 1983) or olfactory bulb (Racine et al. 1991) are of sufficient strength and duration to induce afterdischarges and subsequent kindling, LTP is induced that persists for >3 mo.

Relationship between seizure activity and LTP

One interpretation of the finding that kindling induces LTP (Racine et al. 1983, 1991) is that it is the greater intensity and duration of the kindling trains that is responsible for the persistence rather than the occurrence of seizure activity. A second possible interpretation is that potentiation is induced by seizure activity and is not directly related to the train stimulation. The results of the present study support a third interpretation, that the occurrence of epileptiform activity has an enabling action on the induction of persistent potentiation by pulse trains. This interpretation follows from the temporal separation of the train stimulus from the occurrence of seizure activity in the present experiment, which allows the persistent potentiation to be attributed to application of the train. According to this interpretation, it was the onset of afterdischarge while the one-second-long kindling train was ongoing that enabled the induction of LTP by the stimulus train in the studies by Racine and coworkers.

It is therefore proposed that the occurrence of seizure discharge creates a condition whereby potentiation from stimulation that is appropriately patterned but without behavioral significance is maintained rather than decaying. In other words, the normal dependence on behavioral reinforcement for persistence of potentiation is bypassed during and after seizure discharges.

Possible mechanism for increase in LTP persistence after seizure activity

Recent studies in the hippocampal formation provide insight into processes that underlie the maintenance of synaptic potentiation. These findings suggest mechanisms whereby epileptiform discharge could increase the persistence of synaptic potentiation.

One mechanism is suggested by experiments that indicate the presence of a “metabotropic switch” that must be activated before persistent potentiation can be induced (Bortolotto et al. 1994). After activation, this enabling process persists for ≈6 h. When the activation process was blocked by a metabotropic receptor antagonist, NMDA-dependent synaptic potentiation could still be evoked but decayed with a time course comparable with that observed in this study. This process could also be blocked by kinase inhibitors, indicating a requirement for phosphorylation as reported for LTP (Colley et al. 1990; Malenka et al. 1989; Malinow et al. 1989). It can be speculated therefore that epileptiform activity triggers the metabotropic switch, either by activating metabotropic glutamate receptors or by a downstream second messenger pathway.

A second possible mechanism involves platelet activating factor (PAF). PAF has been shown to accumulate intracellularly during seizure activity (Kumar et al. 1988), and intracellular application of PAF can enable the induction of LTP by low-frequency stimulation of presynaptic axons that is otherwise ineffective (Kato et al. 1994).

Studies of the effects of blocking protein synthesis on LTP provide a third possible mechanism (Frey and Morris 1997; Nguyen et al. 1994). Of particular relevance is the finding that the cascade leading to protein synthesis can be set in motion by strong tetanic stimulation and the resulting product utilized up to 4 h later for the induction of persistent potentiation by a weak tetanus (Frey and Morris 1997). It can be speculated that the period of epileptiform activity in the current preparation triggered synthesis of the same “persistence factor” as the strong artificial tetanus.

Finally, recent studies linking neurotrophic factors to both LTP and seizure activity provide a fourth potential mechanism (Gall and Isaacson 1989; Gall et al. 1997; Lu and Figurov 1997; Nawa et al. 1997). Findings for brain-derived neurotrophic factor (BDNF) are particularly intriguing with regard to interpretation of the present results; seizure activity rapidly enhances expression of BDNF messenger ribonucleic acid (mRNA) in many limbic areas including piriform cortex (Dugich-Djordjevic et al. 1992a,b; Kornblum et al. 1997), and BDNF can increase the magnitude and persistence of pulse train-evoked LTP (Figurov et al. 1996). The recent finding that inhibition of protein synthesis in vivo has only a modest effect on seizure-induced increase in a BDNF mRNA transcript (Lauterborn et al. 1996) suggests that mediation is direct via immediate early genes and therefore represents an independent process from that under investigation by Frey and Morris.

It is proposed that, in contrast to hippocampus where pulse trains alone can induce sustained potentiation, in piriform cortex activation of an additional process is required for persistence. The occurrence of seizure activity in a time window that extends from before train presentation (current results) up to the time of train delivery (Racine et al. 1983, 1991) would appear to spuriously activate this additional process.

Why LTP in vitro but not in vivo?

If an active process is required for the induction of LTP in piriform cortex in vivo, an intriguing question is why LTP can be readily induced in slices. The urethan used for these
experiments does not appear to be a factor because LTP was reported in the hippocampus in the presence of this anesthetic agent and pulse trains fail to induce LTP in unanesthetized animals (Racine et al. 1983; Roman et al. 1987; Strippling et al. 1988). If a complex signaling cascade is involved, it is conceivable that biochemical or anatomic disruption associated with slice preparation results in a persistent activation, e.g., a loss of phosphatase activity so that phosphorylation would not be required, or interruption of a neuromodulatory pathway that might suppress phosphorylation. Alternatively, bursting activity associated with slice preparation might activate metabotropic receptors or the synthesis of PAF. Another possible explanation, an increase in persistence as a consequence of a disruption of inhibitory circuitry in the slice, is discounted by the observed lack of increase in duration of potentiation during local disinhibition in vivo.

**Significance**

Confirmation of the hypothesis that epileptiform bursting enables the induction of LTP in vivo in the absence of appropriate behavioral context would have implications for epilepsy as well as learning-related synaptic plasticity. An increase in excitability from inappropriate synaptic potentiation could contribute to the development or progression of epilepsy. In addition, the presence of a ceiling on the extent of synaptic potentiation suggests that the induction of stable synaptic potentiation by behaviorally irrelevant activity during and after seizure activity could reduce the capacity for further synaptic potentiation and therefore contribute to the learning impairment that was demonstrated after kindling (Sutula et al. 1995).

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Present address for A. Kapur: Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Address for reprint requests: L. B. Haberly, Dept. of Anatomy, University of Wisconsin, 1300 University Ave., Madison, WI 53706.

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