Different Reactions of Control and Epileptic Rats to Administration of APV or Muscimol on Thalamic or CA3-Induced CA1 Responses

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Zhang, De Xing and E. H. Bertram. Different reactions of control and epileptic rats to administration of APV or muscimol on thalamic or CA3-induced CA1 responses. J Neurophysiol 90: 2875–2883, 2003.; 10.1152/jn.00040.2003. The physiology and pharmacology of CA1 is changed in epilepsy. There is evidence that the thalamic input to CA1 has a somewhat different physiological effect compared with the CA3 input. In this study we sought to determine whether this difference in physiology persists in epilepsy, and whether there are changes in the pharmacologic profile of these responses. Under urethane two stimulating electrodes were placed in mid to ventral CA3 and in the midline thalamus of control or epileptic rats. One glass micropipette electrode was placed into CA1 for recording. After the baseline acquisition of CA1-evoked responses to single- or paired-pulse stimulation, the stimuli were repeated with local application of either the GABAA agonist muscimol or the NMDA antagonist dl-2-amino-5-phosphonovalerate (APV). The CA1 response of epileptic rats had a smaller population postsynaptic potential (PSP) and spike amplitudes, longer PSP duration, multiple spikes, and the paired-pulse (at 20-ms intervals) facilitation in contrast to the paired-pulse depression seen in control and kindled rats. The duration of the PSP as well as the amplitude and number of spikes were reduced by administration of APV or muscimol into CA1 in both control and epileptic rats. In control rats, APV enhanced the depression induced by maximal paired thalamic or CA3 stimulation at 20-ms intervals and reduced the facilitation of threshold stimulation into a mild facilitation and reduced the facilitation of threshold stimulation. In epileptic rats neither APV nor muscimol had a significant effect on the changes of the CA1 responses induced by maximal or threshold paired stimulation. This initial in vivo study demonstrated that the physiology and pharmacology of CA1 in epileptic rats are different from control rats. Although there are physiological differences in the evoked responses that are linked to the site of stimulation in the control and epileptic group, the pharmacology in each condition is independent of the site of stimulation.

INTRODUCTION

CA1 is the major output and integrating region within the hippocampus (Andersen et al. 1972; Swanson et al. 1978). It has been shown for many years that CA3 neurons have an excitatory projection to CA1 neurons of both sides (Andersen et al. 1972; Bliss et al. 1983; Finnerty and Jefferys 1993; Ishizuka et al. 1990; Laurberg and Sorensen 1981). In addition several midline thalamic nuclei have anatomical projections to CA1 (Dolleman-Van der Weel and Witter 1996; Herkenham 1978; Su and Bentivoglio 1990; Wouterlood et al. 1990; Yanagihara et al. 1987). Recently these projections were found to be excitatory with some unique properties (Bertram and Zhang 1999; Dolleman-Van der Weel et al. 1997). The midline thalamic region has additional projections throughout the brain including to the limbic areas of entorhinal cortex and amygdala (Zhang and Bertram 2001). There is growing evidence that the midline thalamus may play an important role in some normal and pathological conditions, including certain types of memory formation and limbic seizures (Aggleton and Brown 1999; Bertram et al. 2001; Cassidy and Gale 1998; Hirayasu and Wada 1992; Juhasz et al. 1999; Miller and Ferrendelli 1990; Parker et al. 1997; Patel et al. 1988). Limbic epilepsy is frequently associated with neuronal hyperexcitability of CA1, and stimulation of CA3 induces epileptiform CA1 responses in epileptic rats (Bertram et al. 2001; D’Antuono et al. 2002; Mangan and Bertram 1997, 1998). Limbic epilepsy has also been associated with alterations in GABAergic receptor subunit composition and in the N-methyl-d-aspartate (NMDA) receptor of CA1 neurons (Kapur et al. 1989a,b; Mangan and Bertram 1997, 1998; Rice et al. 1996). Changes in these receptors can alter physiology and pharmacology of CA1 responses.

A recent description of the physiological effect of thalamic stimulation on CA1 neurons suggests that this path has a different effect compared with the “traditional” CA3 input (Bertram and Zhang 1999). In the present study we sought to determine whether these differences were also seen in epileptic rats. Moreover, we sought to determine whether and how the physiology and pharmacology of thalamic input to CA1 is changed in the epileptic state, and whether changes in this pathway are similar to the CA3 response. We compared the effects of the GABA_A receptor agonist muscimol and the NMDA receptor antagonist dl-2-amino-5-phosphonovalerate (APV) on thalamic and CA3-induced CA1 responses of epileptic animals to those responses of control animals. In addition to single evoked responses, we also tested paired stimulation at a 20-ms interval to examine the differences in responses to paired CA3 or thalamic stimulation between control and epileptic animals.

METHODS

Animal preparation and experimental groups

A total of 45 adult male Sprague-Dawley rats (300–450 g; Hilltop Laboratories, VA) were used in this experiment under a protocol approved by the University of Virginia IRB. Rats were housed in pairs in a temperature-controlled environment (22°C) on a 12:12-h light:dark cycle and received access to food and water ad libitum. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
approved by the University of Virginia Animal Care and Use Committee. There were 3 groups for study: animals with spontaneous limbic seizures (epileptic) (n = 20), kindled rats (n = 5), and age-matched controls (n = 20).

EPILEPTIC RATS. Animals were prepared under a protocol that has been standard in this laboratory for over 12 yr (Lothman et al. 1989, 1990). Under balanced ketamine/xylazine anesthesia, a bipolar stainless steel twisted-pair stimulating electrode was placed in the midventral hippocampus (AP, −3.6 mm; ML, −4.9 mm; DV, −5.0 mm DTP; incisor bar −3.3 mm). Ground and indifferent electrodes were placed over the cerebellum. The electrodes were secured with jeweler’s screws in the skull with dental acrylic. The electrode pins were placed in an Amphenol strip connector. After 1 wk of recovery, the animals underwent a stimulation protocol (90 min at 50 Hz, 1-ms biphasic square waves, 400-μA peak-to-peak constant current). This protocol reliably induces a mainly nonconvulsive limbic status epilepticus that lasts 8–14 h. The animals were allowed to recover from the status epilepticus and about 2–4 wk later spontaneous seizures arising in the limbic system began. Once the seizures started, they continued to recur throughout the life of the animal (Bertram and Cornett 1993, 1994). All animals used in this study were 3.5–8 mo beyond the episode of status epilepticus, a point that we previously showed represents the time when the seizure patterns are fully matured (Bertram and Cornett 1993, 1994). All epileptic animals studied had documented spontaneous seizures. Seizure occurrence was documented either by direct visualization or by continuous video-EEG monitoring (Bertram et al. 1997). Any animal that had seizures during the experiment was not included in the analysis.

KINDLED RATS. Because any observed changes of responses in epileptic rats might be the result of recurrent seizure activity, we used kindled rats as controls for the effect of recurrent seizures on the physiology. The surgery for electrode implantation of kindled rats is the same as epileptic rats. The stimulation protocol for kindling is as follows: 50-Hz biphasic square waves with 1-ms duration for 10 s every hour, 6 times per day. The amplitude was 400-μA peak-to-peak constant current. After the induction of stable kindled motor responses (about 20 stimulations) an additional 100–150 stimulations were given. Stimulus sessions were given no more frequently than every other day to provide an approximately equivalent number of seizures as in the epileptic rats. Only the primary responses were evaluated in kindled animals because we sought to determine whether the differences between control and epileptic rats were because of the epileptic condition or because of the seizures.

CONTROLS. Control rats were age matched to the epileptic rats. To control for electrode placement in the epileptic rats, half of the animals received electrodes in the midventral hippocampus. The results of the electrode controls and the naive age-matched controls were compared, and, because no differences were found, these 2 groups were combined into a single control group.

Physiology

Under urethane anesthesia (1.2 g/kg, intraperitoneally), the rats were placed on a multiaxial stereotactic frame, and body temperature was maintained at 37°C by a water blanket controlled by a rectal thermostor. The stimulating and recording electrodes for this study were inserted stereotactically using target coordinates from a standard atlas ( Paxinos and Watson 1986). Stimulating electrodes were placed in mid to ventral CA3 (5.5 mm posterior to bregma, 4.3–4.5 mm left of the midline, 4.5 mm below the dura) and in the midline thalamus (1.8–2.2 mm posterior to bregma, 0.6–0.8 mm left to the midline, 5.7–6.3 mm below the dura, with a 5° arm angle from vertical axis to avoid the sagittal sinus). This position is in the region of the reuniens and rhomboid nuclei. The CA1 recording electrode was a glass micropipette filled with 0.9% NaCl and 1% Fast Green. It was placed 5.5 ± 0.1 mm posterior to bregma, 4.8–5.1 mm right of midline, and 2.5–3.0 mm below the surface. The CA3-stimulating electrode was placed ipsilateral to the chronic hippocampal electrode, and the CA1 recording electrode was placed contralateral to the chronic hippocampal electrode. The depths of the stimulating and recording electrodes were adjusted to achieve the maximal response.

EVOKE RESPONSE AND PAIRED-PULSE STIMULATION. To determine the effects of APV and muscimol on CA1 response, evoked responses to single and paired stimulation were used. Incremental stimulating intensities (2–5 V for each step) were applied in single shocks to either CA3 or the midline thalamus until the maximal response in CA1 was achieved. All analyses were performed on responses obtained at 2 stimulation intensities: 1) population spike threshold and 2) maximal response. For the paired-pulse stimulation, the conditioning and test stimulation of identical intensity were applied with an interval of 20 ms. We chose this interval because it results in a consistent depression of the second response when stimulus is at maximal response intensity in control animals. Previous work (Kapur et al. 1989a; Lothman et al. 1995) had shown that there is a reduced inhibition in CA1 associated with this rat model of epilepsy. After the application of drug, the stimulation intensities were adjusted to achieve maximal response. The measures included the amplitudes of the population PSP and spike, duration of the population PSP at its half-amplitude, and the number of population spikes. After the baseline acquisition of evoked single- and paired-pulse responses, the CA1 recording electrode was withdrawn and another that was filled with 25 μl of either t-APV or muscimol (10 mM of either compound in normal saline) was placed at the same stereotactic coordinates, after which the position of the electrode was adjusted to obtain maximal response. All electrodes had tip diameters of 10 μm to allow for diffusion of drug into the tissue immediately surrounding the electrode. Compared with the concentration of APV (Lambert et al. 1991) or muscimol (Yang et al. 1994) used in in vitro experiments, the concentration of APV or muscimol (10 mM each) used here is much higher. Because each drug was diffusing from the micropipette tip, we used concentrations well above the in vitro standard doses to ensure adequate and consistent pharmacologic effects. We recognize the potential interpretive difficulty in this approach, but in this initial study, we sought to focus on issues other than a formal dose response investigation in this preparation by providing a consistent presumed supramaximal concentration.

In addition to the same coordinates, placement was also guided by the track from the initial electrode insertion, which was identified under a surgical stereomicroscope. To allow the APV or muscimol to diffuse out sufficiently and to let the response recover from the possible effect induced by the electrode change, the stimulation was stopped for 50–100 min after inserting the electrode. Our primary results indicated that 10 min was enough to achieve the maximum effects of the drug. The data obtained with the drug containing electrode were obtained under an identical protocol to the baseline data, except that the stimulation intensity was adjusted to ensure the appropriate response amplitudes. This step was taken to ensure that any changes associated with the test compounds were not the result of a shift in the input/output (I/O) curve. After finishing the observation under APV or muscimol, the saline and Fast Green–containing electrode was returned to the same position. To ensure that any changes with drug were not the result of electrode reinsertion, repeat stimulation was performed 20 min after the reinsertion for recovery from the drug-induced changes.

At the end of each experiment, the electrode positions were marked for histological confirmation by iontophoresing Fast Green in the CA1 recording electrode into the surrounding tissue by using negative DC (20–50 μA, 5–10 min). Positive DC (10 V for 5–10 s) was passed through the negative tip of the stimulating electrodes to deposit iron from the electrode into the surrounding tissue. Then the animals were decapitated while still under anesthesia, and the brains were removed and placed into a fixative consisting of 1% potassium ferrocyanide
CA1 physiology in limbic epilepsy

RESULTS

CA1-evoked responses in control, epileptic, and kindled rats to thalamic or CA3 stimulation

Thalamic and CA3 stimulation each produced a graded response that increased in amplitude with increasing stimulation intensity, ultimately resulting in a population spike that was superimposed on the postsynaptic potential (PSP) in all rats. The spike amplitude and number also increased with increasing stimulation intensity.

The amplitude of the maximal population PSP and spike of the CA1 response in epileptic animals after either thalamic or CA3 stimulation is significantly lower than in control and kindled rats (Fig. 1 and Table 1). There is no significant difference between control and kindled rats for either measure. The duration of the PSP of the CA1 response in epileptic animals is significantly longer than in control rats. The duration of the PSP of the thalamically induced CA1 response in epileptic rats is 11.17 ± 0.80 ms (n = 20), which is longer than that in the control rats (8.12 ± 0.39 ms, n = 19). The difference is significant (P < 0.01). The PSP duration of the CA3-induced CA1 response of epileptic rats (11.71 ± 0.74 ms, n = 20) is also significantly (P < 0.001) longer than that in the control rats (8.30 ± 0.48 ms, n = 19). In contrast to the multispike response of epileptic rats, the CA1 response to maximal thalamic or CA3 stimulation in control or kindled rats usually consists of a single high-amplitude population spike, which is frequently followed by a secondary spike that is of much lower amplitude. Rarely a very small third spike is seen (Fig. 1 and Table 2).

With paired-pulse stimulation, both conditioning and test stimulation are at the same intensity. The effects of conditioning thalamic or CA3 stimulation on test CA1 responses depend on two factors: stimulation intensity and interval between the stimuli. Stimuli were paired at intervals of 20 ms. Stimulation intensities were adjusted for each population spike threshold, at which the second (test) response is usually enhanced, or maximal population spike amplitude, at which the test response is normally depressed. In the control group, the paired-pulse stimulation at 20-ms interval at maximal population spike induces a depression (Figs. 5 and 7; responses not shown for kindled rats) of the population spike of the CA1 response to either thalamic or CA3 stimulation at maximal intensity. In kindled rats, the depression of the test response was slightly greater (test spike amplitude change: thalamic stimulation, −50.6 ± 7.7%, CA3 stimulation, −68.6 ± 2.2%, n = 5) than that in control animals (test spike amplitude change: thalamic stimulation, −15.5 ± 9.0%, compared with kindled rats, P > 0.05; CA3 stimulation, −50.0 ± 7.1%; compared with kindled rats, P > 0.05, n = 17). In contrast to the paired-pulse depression of the population spike in control and kindled rats, there is facilitation of the spike in epileptic rats (test spike amplitude change: thalamic stimulation, +7.4 ± 8.4%, compared with control rats, P > 0.05 whereas to kindled rats, P < 0.01; CA3 stimulation, +37.5 ± 8.0%, n = 20, compared with control or kindled rats, P < 0.001) (Figs. 5–7; responses not shown for kindled rats). The difference in test spike amplitude after CA3 paired-pulse stimulation in control and epileptic rats is highly significant (P < 0.001).

At population spike threshold (Figs. 5–7), all test responses

<table>
<thead>
<tr>
<th>Group</th>
<th>PSP Evoked Potential Amplitude (mV)</th>
<th>Population Spike</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thalamic</td>
<td>CA3</td>
</tr>
<tr>
<td>Control (n = 17)</td>
<td>8.1 ± 0.5</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td>Epileptic (n = 20)</td>
<td>2.9 ± 0.2*</td>
<td>2.4 ± 0.2*</td>
</tr>
<tr>
<td>Kindled (n = 5)</td>
<td>9.8 ± 1.3</td>
<td>8.9 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; t-test vs. control, *P < 0.001.

Analysis

Measurements include the half-amplitude duration of excitatory postsynaptic potential (EPSP) and the number and the amplitude of the spike. The amplitude of the primary spike was measured as the millivolt difference between the peak of the primary spike and the midpoint of the tangent line connecting the highest points of EPSP before and after the spike. For paired stimuli, the amplitude differences between the conditioning and the test primary spike (test-conditioning) were averaged in a group. All data are presented as means ± SE; Student’s t-test or paired t-test was used for group comparison. For the effects of APV or muscimol on paired-pulse induced responses, one-way ANOVA was used across the baseline and 2 drug groups.

and 4% formaldehyde. The fixed brains were frozen and sectioned at 40 μm and Nissl stained with thionine. Positions of the electrodes were then copied to images from a standard atlas as a permanent archive. Only experiments obtained with electrodes in the designated areas were included in the analysis.

FIG. 1. CA1 responses to maximal thalamic or CA3 stimulation in control, kindled, and epileptic rats. Amplitude of CA1 responses to thalamic or CA3 stimulation in epileptic animals is significantly lower than that of control and kindled rats. In addition there are a greater number of secondary population spikes in epileptic animals. For all figures, initial positive deflection in all physiological responses is a 5-mV, 1-ms calibration signal, and stimulation artifact is partially blanked. Primary population spike of each response is indicated by arrow. Tha, thalamic-induced CA1 responses; CA3, CA3-induced responses. Calibration: 2 mV, 10 ms.
in CA1 by thalamic or CA3 stimulation at 20-ms interval were enhanced. Among the epileptic rats, there was facilitation of the test response at both intensities that was essentially the same.

Effects of APV or muscimol on CA1 responses evoked by thalamic or CA3 stimulation

The diffusion of DL-2-amino-5-phosphovalerate (10 mM) or muscimol (10 mM) into the recording area of CA1 reduced spike amplitude (Figs. 2–4) and number (Table 2) to maximal thalamic or CA3 stimulation in control and epileptic rats. APV and muscimol also significantly reduced the PSP duration of CA1 responses to either thalamic or CA3 maximal stimulation (Figs. 5 and 7), in some cases blocking the appearance of the test population spike. On the other hand, muscimol in control rats reduced the usual paired-pulse depression of CA1 responses or even reversed it to facilitation (Figs. 6 and 7). These drug-associated changes of spike amplitude of the CA1 responses to either thalamic or CA3 maximal stimulation–induced CA1 responses under APV or muscimol administration. However, the spike amplitude of the response after adjusting the stimulation intensity was still lower than that in the control rats, suggesting APV or muscimol really depressed the response directly.

MAXIMAL RESPONSE. In predrug baseline of control rats, the spike amplitude of test responses to maximal CA3 stimulation was depressed. However, there was no statistically significant change of the spike amplitude of the test thalamic response in control rats. In epileptic rats, there was a spike amplitude facilitation at maximal conditioning stimulation (Figs. 5–7). The differences of the spike amplitude changes of test responses to maximal CA3 stimulation between control (depression) and epileptic (facilitation) rats was significant in predrug baseline (P < 0.01). In contrast, the thalamic responses had no such difference because there was either minimal depression (control) or facilitation (epileptic).

In control rats APV enhanced the paired-pulse depression of spike amplitude of the CA1 responses to either thalamic or CA3 maximal stimulation (Figs. 5 and 7), in some cases blocking the appearance of the test population spike. On the other hand, muscimol in control rats reduced the usual paired-pulse depression of CA1 responses or even reversed it to facilitation (Figs. 6 and 7). These drug-associated changes of spike amplitude were significantly different from the predrug baseline. The changes of the spike amplitude at maximal thalamic or CA3-induced CA1 responses under APV or muscimol administration in control and epileptic rats. APV and muscimol also significantly depressed the response directly.

in control and epileptic rats. APV and muscimol also significantly reduced the population spike amplitude and number. After 20 min following reinsertion for recovery from APV-induced changes, repeat stimulation was performed to ensure any changes associated with drug were not result of electrode reinsertion. Calibration: 2 mV, 10 ms.

FIG. 2. Effects of DL-2-amino-5-phosphovalerate (APV) on thalamic or CA3-induced CA1 responses in a control and an epileptic rat. In control rats, APV resulted in reduced population spike amplitude and number. After 20 min following reinsertion for recovery from APV-induced changes, repeat stimulation was performed to ensure any changes associated with drug were not result of electrode reinsertion. Calibration: 2 mV, 10 ms.

FIG. 3. Effects of muscimol on CA1 response to maximal thalamic or CA3 stimulation in control and epileptic rats. Muscimol resulted in reduced population spike amplitude and number. Calibration: 2 mV, 10 ms.

### Table 2. Reduction of population spike number of maximal CA3- or thalamic stimulation–induced CA1 responses by APV or muscimol administration in control and epileptic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Epileptic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thalamic</td>
<td>CA3</td>
</tr>
<tr>
<td>APV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.10 ± 0.18</td>
<td>2.00 ± 0.21</td>
</tr>
<tr>
<td>APV</td>
<td>1.00 ± 0.00***</td>
<td>1.00 ± 0.00***</td>
</tr>
<tr>
<td>Muscimol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.44 ± 0.29</td>
<td>2.22 ± 0.28</td>
</tr>
<tr>
<td>Muscimol</td>
<td>1.89 ± 0.35*</td>
<td>1.44 ± 0.24***</td>
</tr>
</tbody>
</table>

Values are means ± SE. Comparing baseline and drug, paired t-test, *P < 0.05; **P < 0.01; ***P < 0.001. Compared to control, t-test, ▲P < 0.05; ▲▲P < 0.01; ▲▲▲P < 0.001.
Animals in which the test population spike was essentially blocked by APV. In epileptic animals the test spike amplitudes, which were facilitated at baseline, showed a slight additional facilitation under muscimol administration (Figs. 6 and 7). However, the changes of the paired-pulse facilitation of CA1 responses in epileptic rats by administration of APV or muscimol do not achieve significance ($P > 0.05$, one-way ANOVA across the group) (Fig. 7).

At threshold stimulation. There is paired-pulse facilitation of the spike instead of depression at 20-ms interval when the stimulation intensity is at population spike threshold as shown in Figs. 5–7. In control rats APV or muscimol significantly reduced this paired-pulse facilitation by threshold stimulation intensity ($P < 0.001$ by one-way ANOVA analysis) (Figs. 5–7). However, the results in epileptic rats were different from those in control rats. In epileptic rats, the enhanced test population spike did not significantly decrease with either APV or muscimol administration shown by one-way ANOVA (Fig. 7). As in the maximal response, APV or muscimol has much less effect on the second response of the epileptic animals.

**Discussion**

In this study, we sought to determine the differences in the CA1 responses induced by CA3 or thalamic stimulation between epileptic rats and control or kindled rats. There were several key findings. First, in epileptic rats, the CA1 response to either thalamic or CA3 stimulation has longer PSP durations, greater number of population spikes, and lower amplitudes of spikes compared with control and kindled rats. Second, we found paired-pulse facilitation of CA1 responses in epileptic rats instead of the usual depression in control and kindled rats at maximal stimulus intensity. Third, the CA1 population spike was consistently of greater amplitude when the thalamus was stimulated compared with CA3, a finding that was seen in all 3 conditions. Fourth, APV or muscimol reduces the duration of the PSP and number and amplitude of population spikes in epileptic and control animals. In addition the drugs have different effects on paired-pulse responses in control and epileptic animals. APV or muscimol has significant effects on paired responses in control rats but only minimal effects in epileptic rats. In control rats the effects of APV and muscimol on paired maximal responses are opposite: APV

![Graph showing effects of APV and muscimol on spike amplitude](image)

**Table 3. Reduction of half-amplitude duration of PSP of maximal CA3- or thalamic stimulation–induced CA1 responses by APV or muscimol administration in naive and epileptic rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thalamic</th>
<th>CA3</th>
<th>Thalamic</th>
<th>CA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>APV</td>
<td>$(n = 11)$</td>
<td>$(n = 11)$</td>
<td>$(n = 9)$</td>
<td>$(n = 9)$</td>
</tr>
<tr>
<td>Baseline</td>
<td>$8.29 \pm 0.50 \text{ mV}$</td>
<td>$8.72 \pm 0.77 \text{ mV}$</td>
<td>$11.64 \pm 1.56$</td>
<td>$11.66 \pm 1.42$</td>
</tr>
<tr>
<td>APV</td>
<td>$7.93 \pm 0.49 \text{ mV}$</td>
<td>$7.28 \pm 0.48 \text{ mV}$</td>
<td>$9.10 \pm 0.75$</td>
<td>$9.43 \pm 0.78$</td>
</tr>
<tr>
<td>Change %</td>
<td>$-15.0 \pm 3.1%$</td>
<td>$-15.3 \pm 2.8%$</td>
<td>$-18.0 \pm 4.0%$</td>
<td>$-16.4 \pm 4.1%$</td>
</tr>
<tr>
<td>Muscimol</td>
<td>$(n = 8)$</td>
<td>$(n = 8)$</td>
<td>$(n = 11)$</td>
<td>$(n = 11)$</td>
</tr>
<tr>
<td>Baseline</td>
<td>$7.89 \pm 0.66 \text{ mV}$</td>
<td>$7.75 \pm 0.39 \text{ mV}$</td>
<td>$10.78 \pm 0.77$</td>
<td>$11.75 \pm 0.75$</td>
</tr>
<tr>
<td>Muscimol</td>
<td>$6.93 \pm 0.59 \text{ mV}$</td>
<td>$5.62 \pm 0.29 \text{ mV}$</td>
<td>$10.24 \pm 0.63$</td>
<td>$10.64 \pm 0.81$</td>
</tr>
<tr>
<td>Change %</td>
<td>$-11.9 \pm 2.8%$</td>
<td>$-26.8 \pm 2.9%$</td>
<td>$-3.4 \pm 4.2%$</td>
<td>$-9.2 \pm 4.7%$</td>
</tr>
</tbody>
</table>

Values are means ± SE. Comparing control and epileptic $t$-test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Change % = (muscimol or APV – control)/control.
deepens depression whereas muscimol reverses the depression into facilitation. In control animals APV and muscimol reduce the facilitation of CA1 responses by paired stimulation at the threshold intensities. Overall the findings demonstrate that there are parallel changes in CA1 responses elicited by thalamic and CA3 stimulation. In addition epileptic animals are less sensitive to the effects of APV than control.

There are a number of technical issues that could affect the interpretation of the results. First, we have to consider the effect of electrode reinsertion. After finishing the observation under APV or muscimol, the saline and Fast Green–containing electrode was returned to the same position. Repeat stimulation was performed 20 min after the reinsertion, and this step demonstrated recovery from the drug-induced changes. The second issue is high concentrations of the drugs. In this initial study of drug administration, we sought to focus on issues other than a formal dose response investigation in this in vivo preparation by providing a consistent very high concentration (10 mM each) compared with the concentration of APV (Lambert et al. 1991) or muscimol (Yang et al. 1994) used in in vitro experiments. We used these high concentrations because each drug was diffusing out from the micropipette tip (diameter about 10 μm), and we sought to ensure adequate and consistent pharmacologic effects. In addition, there is the effect of the unknown concentration gradient from the electrode tip to the surrounding area. We recognize the interpretive difficulty by using this approach, given that the actual delivered dose is unclear and there are concentration gradients from the pipette tip to the surrounding area. The neuronal circuits surrounding the pipette are very complex, and the drug is applied to the local circuit, so that the drug had its effects on the pyramidal
neurons as well as the interneurons. The dosage and effect of APV or muscimol depend on the application method and the animal preparation. In slice low concentration (0.1–0.25 μM) APV induced epileptiform burst discharges (Gorji and Speckmann 2001), whereas higher dosage (50–100 μM) blocked the NMDA component of CA1 response and partially blocked the induction of epileptic activity (Fountain et al. 1998). The amount of APV or muscimol that diffused out to the surrounding area is unclear, and there was doubtless a concentration gradient. In this study we found that the effects of the drugs were consistent and reversible. To explore the issue further a dose response study is needed, which is more difficult in vivo.

Interpreting the effect of global application of a drug to a particular brain region has two sides. On the one hand, because so many different synapses and cell types are affected, it becomes impossible to understand the real mechanisms underlying the alterations in response after drug application. On the other hand, the changes represent the net effect of the drug on a neural system in which the excitatory and inhibitory cells are both affected by the drug. In this situation, a proexcitatory compound could have a greater effect on the inhibitory cells, which would result in an overall diminished excitatory response. Conversely, if an inhibitory compound had a greater effect on inhibitory neurons, the net effect could be a disinhibition with enhanced excitatory responses. The difficulty is compounded by the potential-variant sensitivity to the drugs by the different receptor subtypes. Drugs with similar mechanisms of action could have different effects depending on which receptors in which cells they were more effective.

Clearly the interpretation of regional application is mechanistically problematic, but it provides a better sense of what can happen with the global application of a compound to a local or regional circuit. In this study, the application of the GABA_A receptor agonist and the NMDA antagonist resulted in the reduction of the number of population spikes, especially in the epileptic animals. These drugs also caused a reduction in the amplitude of the population spike and a mild reduction in the duration of the population PSP. The drugs had opposite effects on the paired-pulse studies in the control animals: muscimol surprisingly caused an increase in the amplitude of the second or test population spike, whereas APV caused a further amplitude reduction. One can hypothesize several possibilities for why these two drugs with different mechanisms of action but similar effects on the primary responses had such different effects: they each affected the population of neurons differently so that muscimol caused a net disinhibition by inhibiting the interneurons more, whereas APV affected the primary neurons more, so that there was an enhanced inhibition. It is also possible the muscimol, because it slightly reduced the primary response, changed the response by shifting the I/O relationship to one of facilitation. Because the stimulus intensities were adjusted to ensure that the primary response was maximal, we have at least ensured that the primary response was on the top end of the curve. If the second response is altered because of a shift in the primary response rather than a direct effect on the mechanisms controlling paired-pulse response alterations, it is still an effect. The real answer will come only by determining all of the components of each circuit, examining how the drug affects each component individually, and then modeling how these altered individual responses can sum up to the global regional response.

The increase or decrease (facilitation or depression) in the postsynaptic response induced by paired stimulation is likely a mix of presynaptic and postsynaptic processes. The presynaptic mechanisms include changing the amount of released neurotransmitter (release probability), regulation by autoreceptors (Davies et al. 1990), and altered ionic gradients. Depression may arise from depletion (Zucker 1989) of readily releasable neurotransmitter or from receptor-mediated autoinhibition that also reduces neurotransmitter release. Short-interval facilitation depends on presynaptic calcium (Charlton et al. 1982) that can enhance neurotransmitter release. The postsynaptic mechanisms that can affect paired-pulse responses include receptor desensitization (Wachtel and Kandel 1971). It is possible that residual free calcium remaining after the first stimulus sums with calcium entering on subsequent stimuli to produce facilitation (Katz and Miledi 1968; Zucker 1989). It is likely that all of these mechanisms play a role in determining the amount of facilitation or depression. These factors are also affected by shifting the timing of the pulse interval, the strength of the stimuli, and location of the stimuli in the synaptic circuits. As the second pulse is given after the activation of interneurons, there are circuit phenomena that will also affect the second response. Pathological changes also have some effects on the paired-pulse–induced changes. In epileptic animals it is known that the usual depression that is seen after short-interval paired stimuli commonly changes to facilitation (Kapur et al. 1989a; Lothman et al. 1995).

Because inhibition is diminished in epilepsy, we chose an interstimulus interval that normally results in maximal inhibition to determine how altered this response is in epileptic animals, and how sensitive it is to pharmacologic manipulation. Because epilepsy is a disorder that is associated with a breakdown of inhibitory mechanisms at high-frequency activity, it is important to examine what happens at short-interval stimuli, and to determine how these responses might be affected by drugs. The threshold stimuli were used because we sought to determine whether the epileptic animals were more likely to have enhanced responses at these low-intensity stimuli and were thus more sensitive to minimal stimuli that were given in succession.

The responses may also be affected by the degree of drug diffusion from the tip of the micropipettes. For both drugs we used a concentration of 10 mM, a thousandfold greater than the usually maximally effective concentrations of APV and muscimol for in vitro preparations. Although the drug diffusion will be affected by these concentration differences, there are other factors that may contribute to the overall spread of effective concentrations. Some of these factors include the relative affinity of the receptors for the drugs, the number of available receptors, and the movement of water through the tissue. This latter issue could be a factor in epileptic tissue as well, given that a number of diffusion-weighted magnetic resonance images have shown that this process is altered in some regions of the brain in epileptic animals (Wieshmann et al. 1999). How this altered water diffusion could change drug efficacy is unclear, unless the movement was at a rate and in a direction that would shift the pattern of drug distribution around the recording tip. At the moment little is known about this issue, but it is likely that the movement of extracellular water within the recording site is the same for the 2 groups: toward the ventricles. Whether the differences in the rate of
movement are so different as to affect drug distribution is unclear. However, it is more likely that changes in the drug receptors have the greater effect in this particular experiment. There are multiple descriptions of altered affinity and efficacy of the receptors for the ligand in tissue from epileptic animals, and it is likely that this change plays a major role (Gibbs et al. 1997; Grooms et al. 2000; Hamilton et al. 1997; Mtchedlishvili et al. 2001).

There is also the issue of the reduced efficacy of the drugs in the epileptic animals. There are several possible explanations for this observation. First, there may be reduced diffusion of the drug in the epileptic animals. This cause cannot be completely excluded, but there were clear, consistent, and significant effects of the drugs on the primary responses including reduced PSP duration and number of population spikes. This observation, which paralleled the nature and degree of the findings in the control animals, suggests that the drugs did reach the target tissues to a similar degree in both groups. The second possibility is that the neurons from the epileptic animals have an altered sensitivity to the drugs. This explanation can be demonstrated only by formal dose response curves that are really possible only in vitro test systems. At this time an in vitro system would not allow for a separation of the thalamic and CA3 inputs. Still the drugs did affect the primary response more than in control animals. A number of other studies have demonstrated altered pharmacology in tissue from epileptic rats (Coulter 1999; Mangan et al. 1995, 1998; Mody and Heinemann 1987; Mody et al. 1988; Mtchedlishvili et al. 2001) and the findings of this study are consistent with these observations.

In summary, this initial study demonstrates the alterations that occur in the physiology and pharmacology of several pathways in limbic epilepsy. Of some interest, the thalamic input to CA1 appears to have a more potent effect on the local evoked responses compared with the effect of CA3 as determined by population spike amplitude. In control and epileptic animals, the pharmacology of the response is the same, independent of the site of stimulation, although the pharmacology of the two conditions is quite different. The observation that the basic responses in kindled animals are similar to the responses in control suggests that the changes found in the epileptic animals are related to the condition and not the consequence of recurrent seizures.

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DISCLOSURES

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