In Vivo Demonstration of a Late Depolarizing Postsynaptic Potential in CA1 Pyramidal Neurons

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Submitted 19 July 2004; accepted in final form 10 October 2004


INTRODUCTION

The hippocampus plays a fundamental role in certain forms of learning and memory and exhibits extraordinary vulnerability to pathological insults such as epilepsy and cerebral ischemia. Each region of the hippocampal formation is linked by an excitatory tri-synaptic pathway (Andersen et al. 1969). The major excitatory neurotransmitter in the hippocampus is glutamate (Roberts et al. 1981). The action of glutamate is mediated through ionotropic and metabotropic receptors (Hicks et al. 1987). The ionotropic glutamate receptors consist primarily of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate, and N-methyl-α-aspartate (NMDA) receptors. AMPA and kainate receptors mediate fast excitatory postsynaptic potentials (EPSPs), whereas NMDA receptors mediate slower-rising and slower-decaying EPSPs.

The excitability of hippocampal pyramidal neurons is also influenced by the feedback recurrent (Andersen et al. 1963, 1964) and the feedforward (Alger and Nicoll 1982a) GABA receptor-mediated synaptic inhibition. GABA mediates its action through both ionotropic GABA_A and metabotropic GABA_B receptors, which underlie the fast and slow phases of inhibitory postsynaptic potentials (IPSPs), respectively (Sivilotti and Nistri 1991).

It is traditionally believed that, in the adult mammalian CNS, GABA assumes an inhibitory role, keeping neuronal excitability under control (Macdonald and Olsen 1994). However, this oversimplified notion has been challenged by many in vitro studies that use adult animals (Stein and Nicoll 2003). In those studies, exogenous and synaptically released GABA was shown to mediate a long-lasting depolarizing potential that enhanced neuronal excitability under certain conditions (Alger and Nicoll 1982b; Andersen et al. 1980; Avoli 1992; Grover et al. 1993; Gullede and Stuart 2003; Perreault and Avoli 1988; Staley et al. 1995; Taira et al. 1997; Thalmann et al. 1981; Wong and Watkins 1982).

The exact mechanisms of the depolarizing effect of GABA in adult animals have been under active investigation. Some studies suggest that there are two types of GABA_A receptors on hippocampal pyramidal neurons. The hyperpolarizing responses reflect the activation of synaptic receptors, which are highly concentrated on the pyramidal cell soma and initial segment, whereas depolarizing responses reflect the activation of extra-synaptic or dendritic receptors (Alger and Nicoll 1982a,b; Gullede and Stuart 2003). Other studies show that a collapse in the transmembrane Cl^- gradient, as well as outflux of HCO_3^- through GABA_A receptor channels, might also play a role (Kaila 1994; Staley et al. 1995).

Despite the wealth of literature describing the depolarizing action of GABA in the mature mammalian central neurons, it is not yet clear whether this occurs in vivo. Thus the physiological relevance of the GABA_A-mediated depolarization in adult animal has been challenged. We address this issue using sharp-electrode intracellular recording and staining in vivo in CA1 pyramidal neurons of adult rats. This approach offers the advantage of preserving neuronal circuitry as well as minimizing the perturbation to the cytoplasmic content. We found a long-lasting depolarizing postsynaptic potential that resembles the effect of GABA described in vitro but has an additional NMDA receptor–mediated component. These data support the view that GABAergic interneuron networks can have an excitatory role in vivo in information processing in mature CNS.

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METHODS

All procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Indiana University Institutional Animal Care and Use Committee. Male adult Wistar rats (200–300 g, Charles Rivers) were used in this study. The rats were anesthetized with 1–2% halothane mixed with 33% oxygen and 66% nitrogen gas. In some animals, one femoral vein was cannulated for subsequent intravenous administration of dizocilpine maleate (MK-801, 2 mg/kg) and/or picrotoxin (PTX, 2 mg/kg). These drugs were purchased from Sigma (St. Louis, MO) and dissolved in 0.9% saline in experiments.

Intracellular recording and staining in vivo

Anesthetized rats were fixed in the stereotaxic apparatus. The skull was opened to expose the recording site and to place the electrodes. Recording electrodes were pulled with a Kopf pipette puller (model 750, David Kopf Instruments, Tujunga, CA) from glass capillaries with a filament. Tip resistance ranged from 50 to 80 MΩ when filled with a solution of 5% neurobiotin (Vector Laboratories, Burlingame, CA) in 2 M potassium acetate. Bipolar stimulating electrodes (1 mm apart) were made from insulated stainless steel pins with 1 mm of exposed tip. The stimulating electrodes were placed into the contralateral commissural pathway (AP: 3.7–4.7 mm, ML: 1.5 mm, DV: 3.5 mm) and/or ipsilateral Schaffer collaterals pathway (AP: 4.7–5.7 mm, ML: 5.6 mm, DV: 3.9 mm, vertical angle: 25°; Fig. 1).

Cerebrospinal fluid was drained via a cisternal puncture to reduce brain pulsation. The animal was suspended with a clamp applied to the tail to further reduce the pulsation of the brain caused by respiratory movement. After placement of the recording microelectrode in the cortex above the hippocampus, the exposed surface of the brain was covered with soft paraffin wax. A stimulator (Master-8, A.M.P.I.) with a stimulus isolation unit (Isoflex, A.M.P.I.) was used to deliver stimulus pulses. The microelectrode was advanced slowly with a motorized micromotion controller (model ESP300, Newport Corporation, Irvine, CA) into the hippocampus at 2-μm increments to impale the CA1 pyramidal neurons. After impalement, the neurons with a stable membrane potential of at least −60 mV and action potential overshoot were selected for further recording.

Signals were amplified by a high-input impedance amplifier (Axoclamp 2B, Axon instrument, Foster City, CA). The bridge balance was monitored throughout the experiments and adjusted appropriately. Data were digitized at 4–5 kHz via a computer interface (ITC-16, Instrutech Corp., Long Island, NY) controlled by the data acquisition program (Axodata, Axon Instruments) and stored on a Macintosh computer for off-line analysis. After each successful recording, neurobiotin was iontophoresed into the cell by applying depolarizing current pulses (2 Hz, 300 ms, 1.0–1.5 nA) for 200 ms to evoke an action potential at soma. Analysis of action potentials (spike) was performed from the action potentials evoked by the rheobasic current. The spike height was measured as the difference between the resting membrane potentials and the peak of action potentials. The spike duration was measured at one-half of the peak amplitude. The decay time constant of the EPSP was derived from the decay part of the potentials. The rheobase was defined as the minimum magnitude required for a 200-ms depolarizing current pulse to evoke an action potential at soma. Analysis of action potentials (spike) was performed from the action potentials evoked by the rheobasic current. The spike height was measured as the difference between the resting membrane potentials and the peak of action potentials. The spike duration was measured at one-half of the peak amplitude of the action potentials. The spike threshold was measured at the visually determined inflection point at the onset of an action potential. The membrane time constant was calculated as the time required for the membrane potential to reach 63% of the steady-state amplitudes in response to a hyperpolarizing current pulse (~0.3 nA, 200 ms). Input resistance of the membrane was derived from the linear portion of the I-V relationship as the slope of the linear regression fitting line. For calculating the reversal potential of each postsynaptic potential, the amplitude of each component was measured and plotted as a function of corresponding membrane potential. The reversal potential was extrapolated by the intersection of the linear regression line with the membrane potential axis at which the amplitude of the component would be zero.

All values are presented as mean ± SE. ANOVA, posthoc Scheffé’s test, and paired or unpaired Student’s t-test were used to detect statistical difference (StatView, Abacus Concepts). P < 0.05 was considered to be significant.

RESULTS

In vivo intracellular recording and staining were performed on 71 rats. A total of 91 neurons with stable resting membrane potentials were recorded and stained in vivo in CA1 pyramidal neurons. The brain was removed and stored in the same fixative to ensure sufficient loading. Depolarizing current pulses (2 Hz, 300 ms, 1.0–1.5 nA) for 200 ms were applied to evoke an action potential at soma. Analysis of action potentials (spike) was performed from the action potentials evoked by the rheobasic current. The spike height was measured as the difference between the resting membrane potentials and the peak of action potentials. The spike duration was measured at one-half of the peak amplitude. The decay time constant of the EPSP was derived from the decay part of the potentials. The rheobase was defined as the minimum magnitude required for a 200-ms depolarizing current pulse to evoke an action potential at soma. Analysis of action potentials (spike) was performed from the action potentials evoked by the rheobasic current. The spike height was measured as the difference between the resting membrane potentials and the peak of action potentials. The spike duration was measured at one-half of the peak amplitude of the action potentials. The spike threshold was measured at the visually determined inflection point at the onset of an action potential. The membrane time constant was calculated as the time required for the membrane potential to reach 63% of the steady-state amplitudes in response to a hyperpolarizing current pulse (~0.3 nA, 200 ms). Input resistance of the membrane was derived from the linear portion of the I-V relationship as the slope of the linear regression fitting line. For calculating the reversal potential of each postsynaptic potential, the amplitude of each component was measured and plotted as a function of corresponding membrane potential. The reversal potential was extrapolated by the intersection of the linear regression line with the membrane potential axis at which the amplitude of the component would be zero.

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potentials of –60 mV or more hyperpolarized and action potential overshoot were analyzed in this study. Among these cells, 40 neurons were intracellularly stained and identified as CA1 pyramidal neurons based on their location and morphology. The remainder were considered CA1 pyramidal neurons based on the stereotaxic parameters and their responses to the afferent stimulation.

**Synaptic responses of the CA1 pyramidal neurons to afferent stimulation**

Consistent with previous literature (Schwartzkroin 1986), low-intensity stimuli (1.5 times of the threshold intensity to evoke EPSP, 1.5 T) to the contralateral commissural pathway elicited a postsynaptic potential starting with an initial EPSP followed by an IPSP (Fig. 2A). However, with the increase of stimulus intensity to about 3 T, many neurons (62 of 86) began to exhibit a late depolarizing postsynaptic potential (L-PSP) interposed between the fast and slow IPSPs (Fig. 2B). This late component typically saturated at six times the threshold stimulus intensity (6 T). At 6-T stimulation, the amplitude of L-PSPs was 7.3 ± 0.7 mV (n = 29), and the duration was 186.2 ± 10.8 ms (n = 29). Occurrence of the L-PSP was associated with an increase in membrane conductance as indicated by the decrease in the amplitude of membrane potential deflections in response to a series of 0.5-nA hyperpolarizing current steps. L-PSP could not be induced by direct suprathreshold current injection to the soma. E: sometimes a single shock to the afferent could induce a burst of spikes from L-PSP. F: paired-pulse stimulation (interpulse interval: 50 ms) potentiated L-PSP responses and the burst of spikes could consistently be generated from the L-PSP. Except D, all traces are averages of 4 consecutive recordings, and action potentials are truncated. Scale bars in E apply to F.

![Synaptic response in CA1 neurons to contralateral commissural pathway stimulation.](http://jn.physiology.org/)

**FIG. 2.** Synaptic response in CA1 neurons to contralateral commissural pathway stimulation. A: representative trace showing that a weak synaptic stimuli (1.5 T) elicited an excitatory postsynaptic potential (EPSP) followed by an inhibitory PSP (IPSP). B: stronger synaptic stimuli elicited late depolarizing PSPs (L-PSPs) in addition to initial EPSPs. Amplitude of L-PSPs increased with increasing stimulus intensity and saturated at about 6-T stimulus intensity (1–6 traces are responses to 1–6 times threshold stimulus intensities, respectively). C: L-PSP (arrow) was associated with an increase in membrane conductance as indicated by the decrease in membrane potential deflections in response to a series of 0.5-nA hyperpolarizing current steps. D: L-PSP could not be induced by direct suprathreshold current injection to the soma. E: sometimes a single shock to the afferent could induce a burst of spikes from L-PSP. F: paired-pulse stimulation (interpulse interval: 50 ms) potentiated L-PSP responses and the burst of spikes could consistently be generated from the L-PSP. Except D, all traces are averages of 4 consecutive recordings, and action potentials are truncated. Scale bars in E apply to F.

**FIG. 3.** Different responses evoked from CA1 neurons. A: representative traces showing the L-PSPs evoked from the same neuron by stimulation the CC and the SC, respectively. B: input-output curves obtained from the CC and SC stimulation showed no significant difference. C: in some neurons, stimulation of CC or SC could not induce L-PSP. Traces in A and C are the average of 4 consecutive recordings, and action potentials are truncated. Scale bar in A applies to C.
pathway showed no distinguishable difference (Fig. 3B). In contrast, some CA1 pyramidal neurons (24 of 86) failed to exhibit L-PSP response regardless of the stimulation intensities (Fig. 3C).

L-PSP responses became apparent at a stimulation intensity of 3 T or higher, whereas at 1.5-T stimulation, the primary evoked potentials was the initial EPSP. We studied the kinetics of the initial EPSPs evoked at 1.5 T and examined the relationship between the initial EPSPs and the L-PSPs evoked at 6 T. We found that the duration and decay time constant of the initial EPSPs in the neurons that displayed the L-PSP were significantly larger than those of the neurons that did not display the L-PSP ($P < 0.05$), whereas the amplitude and rising slope of the initial EPSPs showed no significant difference between the 2 groups, whereas duration and decay time constant of initial EPSP in L-PSP neurons are significantly greater than those of non-L-PSP neurons ($*P < 0.05$). Numbers of cells are denoted in parentheses.

**FIG. 4.** Comparison of the initial EPSP between the neurons that display the L-PSP and other neurons. A: representative traces of the initial EPSPs from L-PSP and non–L-PSP neurons at 1.5-T stimulus intensity. Scale bar in a also applies to b and c. B: amplitude and rising slope of initial EPSP showed no significant difference between the 2 groups, whereas duration and decay time constant of initial EPSP in L-PSP neurons are significantly greater than those of non–L-PSP neurons ($*P < 0.05$). Numbers of cells are denoted in parentheses.

**FIG. 5.** Linear regression analysis between the L-PSPs elicited at 6-T stimulus intensity and initial EPSPs elicited at 1.5-T stimulus intensity. A and B: linear relationship was detected between initial EPSP decay time constant and L-PSP amplitude ($R^2 = 0.73$, $P < 0.01$, $n = 40$) and L-PSP duration ($R^2 = 0.70$, $P < 0.01$), respectively. C and D: there was no linear relationship between initial EPSP rising slope and L-PSP amplitude ($R^2 = 0.004$, $P = 0.68$) and L-PSP duration ($R^2 = 0.02$, $P = 0.34$, $n = 42$), respectively.
Electrophysiological and pharmacological properties of the L-PSP

To understand the underlying ionic mechanism of the L-PSP, we first measured the reversal potentials of different components of the postsynaptic potentials. Currents were injected into the recorded neurons to maintain the membrane potential at different levels, and the amplitudes of each postsynaptic potential were measured accordingly (Fig. 6A). The initial EPSP reversal potential was −46.1 mV (n = 18); the fast IPSP reversal potential was −65.5 mV (n = 18); the late IPSP reversal potential was −94.4 mV (n = 18); and the L-PSP reversal potential was −58.4 mV (n = 18; Fig. 6B).

To directly elucidate the underlying mechanisms of the L-PSP, we applied the NMDA receptor antagonist MK-801 and the GABA_A receptor antagonist PTX intravenously, after eliciting a L-PSP with 6-T afferent stimulation. MK-801 (2 mg/kg) rapidly reduced the amplitude of L-PSPs from 8.6 ± 1.3 to 3.0 ± 1.3 mV (n = 8, P < 0.01). The reduction ratio was 68.6 ± 13.1% (Fig. 7, A and D). On application of PTX (2 mg/kg), the amplitude of L-PSPs was rapidly reduced from

FIG. 6. Reversal potentials of different components of postsynaptic potentials evoked at 6-T stimulus intensity. A: representative traces showing postsynaptic potentials at different membrane potentials. Dashed lines indicate positions for measurement of initial EPSP, fast IPSP, L-PSP, and slow IPSP amplitudes, respectively. B1: linear regression of initial EPSP amplitude as a function of membrane potentials. Reversal potential of initial EPSP was −46.1 mV (n = 18). B2: linear regression of fast IPSP amplitude as a function of membrane potentials. Reversal potential of fast IPSP was −65.5 mV (n = 18). B3: linear regression of L-PSP amplitude as a function of membrane potentials. Reversal potential of L-PSP was −58.4 mV (n = 18). B4: linear regression of slow IPSP amplitude as a function of membrane potentials. Reversal potential of slow IPSP was −94.4 mV (n = 18).

FIG. 7. Effect of systemic administration of pharmacological blockers on L-PSPs. L-PSP was elicited with 6-T afferent stimulation. A: representative traces showing that amplitude of L-PSPs was significantly reduced by the N-methyl-D-aspartate (NMDA) channel blocker MK-801. B: application of GABA_A channel blocker picrotoxin (PTX) significantly reduced amplitude of L-PSP. C: simultaneous application of both MK-801 and PTX significantly reduced amplitude of L-PSP. D: group data summarizing reduction ratio of L-PSP amplitude after application of MK-801 or/and PTX (**P < 0.01).
proximal portion of the apical dendrites in the neurons that displayed the L-PSP (30.6
The spine density on the distal portion of the apical dendrites, the spine density in the neurons that displayed the L-PSP had a MK-801–sensitive component, we hypothesized that neurons that displayed the L-PSP might receive a relative lower input resistance of neurons that do not display L-PSP is due to more severe injury caused by microelectrode impalement.

Morphology of the L-PSP neurons and non–L-PSP neurons

To test if the difference in electrophysiological properties of recorded neurons had any relationship to their difference in anatomical locations within hippocampus, we plotted the recording sites with microscopy after staining. We found that the recording sites were randomly distributed within the CA1 region, and the distribution pattern of the morphologically identified L-PSP neurons and non–L-PSP neurons had no detectable difference. Both types of the cells scattered fairly homogeneously within the CA1 region (Fig. 8A). Since the L-PSP had a MK-801–sensitive component, we hypothesized that neurons that displayed the L-PSP might receive a relatively higher excitatory drive. Since the excitatory inputs of the CA1 pyramidal neurons mainly impinge on the dendritic spines, we compared spine density between the neurons that displayed L-PSP and those neurons that did not. On the basal dendrites, the spine density in the neurons that displayed the L-PSP (34.5 ± 2.8, n = 12) was significantly higher than that of the neurons that did not display the L-PSP (27.1 ± 2.9, n = 5, P < 0.05; Fig. 8, B and C). The spine density on the proximal portion of the apical dendrites in the neurons that displayed the L-PSP (37.3 ± 3.9) was significantly higher than that of the neurons that did not (31.6 ± 2.3, P < 0.05; Fig. 8C). The spine density on the distal portion of the apical dendrites in the neurons that displayed the L-PSP (30.6 ± 3.6) was also higher than that of the neurons that did not (28.9 ± 3.4), but no statistical significance was detected (Fig. 8C).

DISCUSSION

The main finding of this study is that a partly GABA A receptor–mediated long-lasting depolarization, which is termed L-PSP, can be revealed in mature CA1 pyramidal neurons in vivo by stimulating either Schaffer collaterals or the contralateral commissural pathways. Such a response can be elicited from ~70% of the recorded neurons. Both NMDA receptors and GABA A receptors contribute to the generation of the L-PSP. Approximately 30% of the recorded neurons fail to exhibit the L-PSP response under the same stimulation conditions. These neurons have a significantly lower input resistance and display differences in dendritic spine density and branching complexity.

Stimulation of afferent, but not direct suprathreshold depolarizing current injection to the soma, can evoke the L-PSP, suggesting that the L-PSP is a synaptically driven event instead of a somatic regenerative event. Consistent with this observation, the generation of the L-PSP is not dependent on the initiation of the evoked action potentials arising from the initial EPSPs. Thus it is unlikely that back-propagating action potentials play a role in L-PSP induction. The induction of L-PSps requires relatively strong stimulation intensities (>3 T), suggesting that a threshold amount of presynaptic fibers has to be activated (cooperativity). The input-output property of L-PSps reaches an asymptotic level at higher stimulation intensities (>5 T), which might reflect saturation of receptor activation. L-PSP response does not exhibit input-specificity, since both SC and CC stimulation can elicit indistinguishable response. The lack of input-specificity suggests that the L-PSP might not be specifically mediated through the CA3–CA1 connections.

The L-PSP can evoke bursts of spikes, supporting the excitatory nature of this component. It has been shown that strong excitatory synaptic activation can lead to “action potentials” generated within the dendrites, independent of the soma.
or axon initial segment (Regehr et al. 1993; Stuart et al. 1997). The burst of spikes on the L-PSP could also have a dendritic origin.

Since glutamate is the main excitatory neurotransmitter in the hippocampus, and the NMDA component of the synaptic current rises slowly to a peak (~20 ms) and decays biexponentially with time constants of 40 and 200 ms, respectively (Lester et al. 1990), the prolonged duration of the L-PSP prompted us to initially hypothesize that L-PSPs were mediated by NMDA receptors. Regression analysis indicated that the amplitude and duration of the L-PSP was positively correlated to the decay time constant, but not to the rising slope of initial EPSPs. Since, in synapses containing both AMPA and NMDA receptors, the rising slope of EPSP is mainly contributed by the AMPA component, whereas the decay of EPSP is mainly contributed by the NMDA component, the most parsimonious interpretation of this result is that the L-PSP bears certain relationship with the NMDA receptor strength. More direct evidence supporting the role of NMDA receptors in mediating the L-PSP comes from the result that MK-801, the NMDA receptor–gated channel blocker, significantly suppresses this response.

On the other hand, a possible role for GABA_A receptor involvement needs to be considered. In our study, the GABA_A receptor blocker, PTX, significantly reduced the amplitude of the L-PSP. This indicates that GABA_A receptor activation can indeed mediate a depolarizing effect in vivo.

In support of its mixed nature, L-PSPs reverse at –58.4 mV, which lies between the reversal potentials of the glutamatergic receptor–mediated EPSP and the GABAergic IPSPs. It should be noted that, as a caveat, due to the lack of pharmacological isolation in our in vivo preparation, postsynaptic potentials (i.e., excitatory and inhibitory) temporally overlap with each other to a certain degree. Thus the actual reversal potential for the EPSP should be more depolarized and the fast IPSP reversal potential should be more hyperpolarized than our measured values. Similarly, the true reversal potential of L-PSPs might be more depolarized if measured in the absence of the

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**FIG. 8.** Anatomical distribution and spine density comparison between L-PSP neurons and non–L-PSP neurons. A: non–L-PSP neurons (left, ○) and L-PSP neurons (right, ●) showed no detectable difference in patterns of their anatomical distribution. B: example light photomicrographs showing dendritic spines from non–L-PSP neurons (left) and L-PSP neurons (right). 1 and 2: spines on the basal dendrites. 3 and 4: spines on the proximal portion of apical dendrites. 5 and 6: spines on the distal portion of apical dendrites. C: quantitative comparison of spine density between L-PSP and non–L-PSP neurons. Spine density of basal dendrites and the proximal portion of apical dendrites in L-PSP neurons was significantly higher than those of non–L-PSP neurons (*P < 0.05).
FIG. 9. Morphometric comparisons of dendritic arborization between neurons that display L-PSP and neurons that do not. 

A: representative 3-dimensional neuro-lucida reconstructions of L-PSP and non–L-PSP neurons in the CA1 region. 

B: Sholl plots showing number of intersections made by basal and apical dendrites as a function of distance from the soma in L-PSP and non–L-PSP neurons. Branches of proximal apical dendrites (170–300 μm) in L-PSP neurons are significantly more than those of non–L-PSP neurons (**P < 0.01). C: summary of numeric analysis results. In basal dendrites, no significant difference was detected between L-PSP and non–L-PSP neurons in total dendritic branch ends (TDBE), total dendritic branch length (TDBL), and average dendritic branch length (ADBL). In apical dendrites, values of TDBE, TDBL, and ADBL in L-PSP neurons were significantly greater than those of non–L-PSP neurons (*P < 0.05). ADBL values were obtained by dividing the corresponding TDBL by the number of primary branches directly from the soma.

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*P < 0.05
hydropolarizing effect of its neighboring fast and slow IPSPs. Additionally, the reversal potential of more distal dendritic synapses is probably not accurately assessed with somatic current injection.

Although the L-PSP has both a MK-801–sensitive component and a PTX-sensitive component, due to the lack of an additive effect of MK-801 and PTX in blocking the L-PSP, it is unlikely that it is simply an arithmetic sum of independent NMDA and GABA_A receptor–mediated conduction. According to the feedforward inhibition theory postulated by Alger and Nicoll (1982a), a population of interneurons receive afferent excitation and make synapses on pyramidal neuronal dendrites to form the feedforward inhibition. Indeed, there is a strong excitatory drive to the GABAergic interneurons in CA1 region, which is mediated by ionotropic glutamatergic receptors (Davies et al. 1990). Furthermore, the effect of GABA on dendrites has been shown to be depolarizing (Alger and Nicoll 1979, 1982a,b; Andersen et al. 1980; Gulledge and Stuart 2003). In line with this theory, it is plausible that MK-801 mainly exerts its effect presynaptically, which leads to a reduction in the NMDA receptor–mediated excitatory drive to these interneurons and subsequent reduction in GABA release. The reduction in GABA release in turn causes less GABA_A receptor–mediated depolarization on CA1 pyramidal neurons.

Nevertheless, one should not rule out the contribution of the postsynaptic NMDA receptors on CA1 pyramidal neuron to L-PSP expression. The postsynaptic NMDA receptor activation could occur secondary to the depolarization initially caused by GABA_A receptor activation, greatly boosting the amplitude of the L-PSP. The findings that MK-801 preferentially affects the later phase of the L-PSP, whereas PTX suppresses the L-PSP fairly uniformly, fit such an explanation.

A small proportion of the CA1 neurons failed to exhibit the L-PSPs regardless of the stimulation intensities and the stimulation pathways (contralateral commissural path or ipsilateral Schaffer collateral path). This group of neurons shows indistinguishable resting membrane potentials and action potential properties compared with the group of neuron exhibiting L-PSPs. However, the input resistance and membrane time constant of these neurons were significantly lower. In theory, these passive membrane properties determine the temporal and spatial summation of the synaptic potential (Koester and Siegelbaum 2000), thus one can argue that the neurons with smaller input resistance and time constant are not able to amplify and propagate the remotely generated synaptic potentials and therefore fail to display a L-PSP. However, this possibility is not supported by the fact that the failure to elicit a L-PSP is not dependent on the stimulation intensities in these neurons. Rather, the failure to exhibit a L-PSP in these neurons is more likely due to the difference in their synaptic inputs. Since L-PSP generation is dependent on NMDA receptors and GABA_A receptors, the failure to generate a L-PSP response could result from the lack of enough glutamatergic excitatory synaptic input to these neurons, as well as the lack of innervations from feedforward interneurons. Such an explanation is supported by the discernible difference in spine density and the proximal apical dendrite branch pattern between the L-PSP neurons and non–L-PSP neurons. Since spines are the site of glutamatergic synaptic contact on the hippocampal pyramidal neurons (Harris and Kater 1994), the difference in spine expression levels between these two groups of neurons implies that they receive different levels of excitatory input. In addition, previous studies have shown that the proximal dendrites of pyramidal neurons can initiate regenerative local spikes, including sodium, calcium, and NMDA receptor–mediated spikes (Larkum et al. 2001; Schiller et al. 2000), it is likely that these morphological features might correlate to the generation of the L-PSP.

L-PSPs may have important functional implications. Since the hippocampus is implicated in learning and memory formation and hippocampal long-term potentiation (LTP) is postulated to be an underlying cellular substrate (Bliss and Collingridge 1993; Eichenbaum 1994), we speculate that the L-PSP could play a potentially important role in synaptic integration and activity-dependent synaptic plasticity. First, prolonged synaptic depolarization would lead to more Ca^{2+} influx through NMDA receptors and voltage-gated calcium channels, activating a variety of Ca^{2+}-dependent signal transduction cascades that are critically involved in information processing and storage. Second, prolonged synaptic depolarization could promote dendritic spike initiation and back-propagation of somato-axonal action potentials, both of which are critical for spike-time-dependent long-term synaptic plasticity (Hoffman et al. 1997; Magee and Johnston 1997; Watanabe et al. 2002). Indeed, the spiking activities elicited from the L-PSP resemble previously well-documented complex spike bursting that is typical of hippocampal pyramidal cells (Ranck 1973). This bursting may represent an important form of information coding in the hippocampus (Lisman 1997). In agreement with this notion, it has been shown that strong stimulation to the Schaffer collateral at theta frequencies can trigger the complex spike bursting, which enables the induction of LTP (Thomas et al. 1998). It is important to note that the GABA_A receptor-mediated depolarization will relieve the Mg^{2+} block of the NMDA receptor, thus facilitating NMDA receptor-dependent plasticity (Ben-Ari et al. 1997). Furthermore, the dendritic spike initiation may be facilitated by the cooperative activation of both NMDA and depolarizing GABA_A receptors (Larkum and Zhu 2002; Schiller et al. 2000). In addition to the implication in physiological situation, the depolarizing effect of GABA has also been implicated in the pathogenesis of epilepsy. There is evidence that interictal epileptic activity in the human temporal lobe is critically dependent on the excitatory action of GABA (Cohen et al. 2002).

These results provide the first in vivo evidence for the existence of GABA_A-mediated excitation under normal physiological conditions in mature neurons. These findings suggest that GABA may play a far more sophisticated role than its postulated inhibitory role in controlling neuronal excitability.

Acknowledgments

We thank Drs. Roger A. Nicoll, Amiel Rosenkranz, and Andreas Frick for critical comment on this manuscript.

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Grants

This work was supported by American Heart Association Grants 0070048 to Z. C. Xu and 0110275Z to Y. Fan, 0320081Z to B. D. Zou, and 0120566Z to Z. Pang.
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