Active Properties of Dendritic Membrane Examined by Current Source Density Analysis in Hippocampal CA1 Pyramidal Neurons

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The laminar profile of extracellular field potentials evoked by alveus or stratum radiatum stimulation was recorded at the CA1 region of guinea pig hippocampal slices. One-dimensional approximation of the current source density analysis method was applied to the data. When the alveus was stimulated, the location of inward membrane current (sink) moved from the cell body layer to the dendritic region for a distance of at least 200 μm at a velocity of 0.16 ± 0.03 m/s (n = 6). This sink movement was not blocked by either low-Ca2+ or Ca2+-free/high-Mg2+ medium, but was blocked by tetrodotoxin application restricted to the dendritic region by a local perfusion method. When the stratum radiatum was stimulated, sink movement from the dendritic region to the cell body layer was not distinct. Retrograde sink movement from the cell body layer to the dendritic region subsequent to the cell body spike was not observed. These findings indicated that the dendrites of the CA1 pyramidal neurons can generate action potentials which propagate along the dendrite and that they might primarily be mediated by Na ion.

INTRODUCTION

Hippocampal CA1 pyramidal neurons have been reported to generate action potentials at their dendrites. Small spikes distinguishable from full-size spikes were recorded intracellularly from cell bodies and were considered to be of dendritic origin.1,10,11. Recently, direct intradendritic recordings have shown spike generation at the dendrite.4,14. There are presently two hypotheses on the conductive properties of dendritic spikes. Because of the small amplitude of the spikes when recorded at the soma, Spencer and Kandel11 assumed a passive area of the membrane between the soma and the dendrite. On the other hand, Andersen and Lomo1 proposed that action potentials are initiated in the dendrites and propagated along dendrites down to the soma. This conclusion was based on the finding that synaptically evoked full-size action potentials occur without any prepotential. Analysis of the laminar record of extracellular field potentials has supported the latter opinion.4,5,12. However, extracellular field potential by itself may not accurately define the location of spike generation. To reexamine the existence of propagating dendritic action potentials, we performed current source density analysis of laminar field potentials evoked by antidromic or orthodromic stimulation using in vitro slice preparations.

Furthermore, since two distinct types of all-or-none action potentials were reported to occur in dendrites of hippocampal CA1 pyramidal neurons, one being Na+ mediated and the other Ca2+ mediated,10,14, we also examined the ionic basis for propagating dendritic spikes.

MATERIALS AND METHODS

Preparation of slices

Guinea pigs were decapitated with a guillotine, and hippocampal tissue was dissected away from the rest of the brain. Transverse slices (400 μm thick) were cut using a specially designed slicer. Slices were then transferred to an incubation chamber containing artificial cerebrospinal fluid (ACSF) consisting of (in

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mM): NaCl 124; KCl 5.0, NaH₂PO₄ 1.25, MgSO₄ 2.0, CaCl₂ 2.0, NaHCO₃ 22.0 and glucose 10.0, which was equilibrated with a gas mixture of 95% O₂ and 5% CO₂. More than 1 h later, a slice was transferred to an experimental chamber which was perfused with fresh ACSF at a rate of 2 ml/min and its temperature was maintained at 34 ± 1 °C. The slice was totally submerged in ACSF for the duration of the experiment.

Stimulation and recording

We used a pair of stimulating and a pair of recording electrodes (Fig. 1). Bipolar stimulating electrodes were constructed from two insulated tungsten wires exposed at the tip (100 μm diameter). One stimulating electrode was placed on the outer edge of the alveus to activate the CA₁ pyramidal neurons antidromically. The other stimulating electrode was placed in the stratum radiatum to activate them orthodromically. Monophasic constant current pulses were applied as the stimuli, and their intensities were those which evoked maximal responses. Glass pipettes, filled with ACSF (0.5 to 2.0 MΩ resistance), were used for recordings. One electrode was used to record extracellular field potentials at a sequential location along the axis perpendicular to the str. pyramidale in the CA1 region. The other electrode was fixed at the str. pyramidale or at the str. radiatum in the CA1 region to ensure that responses did not vary from one trial to the next. Each of the recordings was recorded on a floppy disk using a computer (NEC PC-9801).

Current source density (CSD) analysis

Current source density analysis was performed on the laminar profile of the field potential recorded in the CA1 region. We assumed that the CA1 region of hippocampal slices has translational symmetry in two dimensions and that the conductivity of the extracellular space is constant. These assumptions permit the one-dimensional approximation as described by Mitzdorf and Singer. The second spatial derivatives of the potentials were calculated according to the formula:

\[ \frac{\partial^2 \phi(z)}{\partial z^2} = \frac{\phi(z+n \Delta z)-2\phi(z)+\phi(z-n \Delta z)}{(n \Delta z)^2} \]

where \( \phi \) is the extracellular field potential, \( z \) is the coordinate perpendicular to the lamina, \( \Delta z \) is the sampling interval, \( n \Delta z \) is the differentiation grid (\( n \Delta z = 100 \, \mu \text{m} \) for our analysis). To obtain the current density, the derivatives must be multiplied by conductivity. Since our interest concerns the relative value of CSD, we regarded the second spatial derivative of the potential as the CSD.

Local perfusion

To examine the ionic dependence of evoked responses, it was necessary to apply Tetrodotoxin (TTX) locally to the dendritic region or to the somatic region. This was achieved by the following method (Fig. 1). TTX was dissolved in ACSF and loaded into a syringe (6 × 10⁻⁷ M). A broken glass pipette, connected to the syringe, was placed close to the target site. TTX was continuously pushed out onto the target site at a rate of 0.1 ml/min using a motor-driven microinjector. Another broken pipette, connected to an aspirator, was also placed near the site to continuously aspirate the TTX solution preventing its...
diffusion. This method allowed a localized blocking (shaded area in Fig. 1) of TTX-sensitive conductances long enough (more than 40 min) to record a full laminar profile without blockade of stimulus inputs, i.e. blockade of TTX-sensitive dendritic spikes or blockade of the somatic spike with intact synaptic inputs on the dendrites.

RESULTS

Alveus stimulation

Fig. 2A shows an example of a laminar profile of extracellular field potentials upon alveus stimulation. Each trace is an average of 5 records. Although it took about 20 min to record the full laminar profile, the other recording electrode fixed at the str. pyramidale ensured that the response was constant during the laminar recording. The main features of the field potentials were: (1) a large negative spike at the str. pyramidale; (2) a negative spike at the str. radiatum preceded by a positivity; and (3) two monophasic negative spikes at the str. oriens. These features were similar to those reported in in vivo experiments12.

Fig. 2A and B show the current source density calculated from the laminar field potential. It is proportional to the membrane current at respective sites. At the str. pyramidale, a large sink current (inward membrane current), which represented action potentials generated at the cell body of pyramidal neurons was observed. In the str. radiatum there was a profile of diphasic membrane currents, outward then inward (arrows in Fig. 2A). The location of a maximum inward current shifted from the str. pyramidale to the str. radiatum over a few milliseconds time period (filled arrowheads in Fig. 2B) at a velocity of about 0.1 m/s. This sink movement also appeared in the str. oriens (open arrowheads in Fig. 2B).

There are 3 possibilities as to the origin of membrane current in the str. radiatum: (1) it is a passive source or sink originating from somatic action potentials; (2) some synaptic inputs are activated by alveus stimulation, and a synaptic current is involved in it; or (3) it represents active spike generation at the dendrites.

We can eliminate the first possibility for the following reason. As shown in Fig. 2B, the sink and/or source current appeared at the str. radiatum without a substantial sink or source current at the str. pyramidale. The outward current that appeared at the str. radiatum (asterisk in Fig. 2B) is the source for the inward current that appeared within the str. radiatum itself. These currents were generated in a dendritic region, not at the soma.

To examine the second possibility of the synaptic...
current, we performed laminar recording under a low-Ca\(^{2+}\) or Ca\(^{2+}\)-free/high-Mg\(^{2+}\) condition. Monitoring antidromic and orthodromic responses evoked by alternately applied stimulation to alveus (S1 in Fig. 1) and to the str. radiatum (S2 in Fig. 1), we changed the bathing solution from a normal ACSF to a low-Ca\(^{2+}\) or Ca\(^{2+}\)-free/high-Mg\(^{2+}\) ACSF. The laminar recording was performed (Fig. 3A) after ascertaining the blockade of synaptic transmission by the disappearance of an orthodromically evoked response. A diphasic membrane current and sink movement in the str. radiatum still appeared under this condition (arrows in Fig. 3A). Similar results were obtained in all experiments in which synaptic transmission was blocked by low-Ca\(^{2+}\) (0.2 mM; \(n = 1\)) or Ca\(^{2+}\)-free/high-Mg\(^{2+}\) (4-10 mM; \(n = 5\)) ACSF.

As can be seen in Fig. 3A, the second spike appeared when low-Ca\(^{2+}\) medium was perfused. Taking into account that it did not appear when Ca\(^{2+}\)-free/high-Mg\(^{2+}\) medium was used, we believe that the lack of divalent cations made the cell membrane unstable, and hence two spikes were evoked by antidromic stimulation.

Since the possibility of a synaptic current was eliminated, the origin of the diphasic membrane current and sink movement in the str. radiatum could be attributed to active spike generation within the dendrites. Sink movement represents the conduction of dendritic spikes along the apical dendrites. The mean velocity of this movement was 0.13 ± 0.03 m/s (\(n = 6\)), and the distance of sink movement from the soma was 283 ± 93 \(\mu\)m (\(n = 6\)). Though not proven, the sink movement toward the str. oriens may also represent the conduction of dendritic spikes along the basal dendrites.

The above experiments also indicate that the anti-

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Fig. 3. A: laminar records of field potentials evoked by alveus stimulation in low-Ca\(^{2+}\) (0.2 mM) condition, and current source density calculated from them. Sink movement in the str. radiatum was not abolished under this condition (arrows). Blockade of synaptic transmission in this low-Ca\(^{2+}\) concentration was ascertained by disappearance of the orthodromic population spike evoked by str. radiatum stimulation. B: laminar records of field potentials evoked by alveus stimulation when TTX (6 \(\times\) 10\(^{-7}\) M) was continuously applied locally to the str. radiatum by the local perfusion technique. Calcium concentration in ACSF was 0.2 mM in this case. Sink movement in str. radiatum and the negative deflection of field potential observed in the str. radiatum was abolished (asterisks). Figs. 2 and 3 show results of experiments performed on the same slice preparation.
dromically evoked action potentials that propagate along the dendrites are mediated by the Ca ion. To examine whether the sink movement is mediated by the Na ion, we applied TIX (6 x 10⁻⁷ M) locally to the dendritic region (by local perfusion) without blocking the antidromically evoked population spike at the pyramidal cell layer. Fig. 3B shows the laminar profile of field potentials recorded when TIX was continuously applied to the str. radiatum. Synaptic transmission was blocked by the low-Ca²⁺ or Ca²⁺-free/high-Mg²⁺ ACSF in this experiment. The antidromically evoked population spike still appeared in the str. pyramidale with an amplitude comparable to that of the control (Fig. 2A) or low-Ca²⁺ (Fig. 3A) experiment. On the other hand, the negative spike at the str. radiatum was abolished by the local application of TTX (asterisks in Fig. 3BFP). During laminar recordings under local application of TTX, population spikes with a constant amplitude were observed through the other recording electrode placed at the str. pyramidale. The CSD analysis applied to this laminar profile showed a source current only at the proximal dendritic region and sink current at the str. pyramidale (Fig. 3BCSD). The sink current at str. radiatum and source current at the distal dendritic region were abolished in this condition (asterisks in Fig. 3BCSD). This current profile means that the active current of the dendritic spike and the passive source for it were blocked by TIX applied locally to the str. radiatum. After cessation of local TTX application, the sink current in the str. radiatum and the source current at the distal dendritic region recovered completely (not shown). Thus, antidromically evoked propagating dendritic spikes were blocked by TTX but were not blocked by a low-Ca²⁺ or Ca²⁺-free/high-Mg²⁺ condition. These results suggest that propagating dendritic potentials are primarily mediated by the Na ion.

**Stratum radiatum stimulation**

Fig. 4 shows the laminar profile of extracellular field potentials evoked by the str. radiatum stimulation. The main features of these field potentials were: (1) a sharp negative spike at the str. pyramidale; (2) a slow positive wave at the str. pyramidale; (3) a short-latency negative spike in the str. radiatum; (4) a longer latency negative spike in the str. radiatum; (5) a slow negative wave in the str. radiatum; and (6) a diphasic potential in the str. oriens. These features are almost identical to those reported for in vivo experiments.1,4,5

The sharp negative spike at the str. pyramidale (1 in Fig. 4), represents a summation of action potentials generated at the pyramidal neuron cell bodies. The large current sink at the str. pyramidale (a in Fig. 4) is the corresponding active membrane current. Andersen demonstrated that the short-latency negative spike in the str. radiatum (3 in Fig. 4) represents the presynaptic fiber volley. The sharp current sink with the same latency in the str. radiatum (c in Fig. 4) corresponds to the volley of presynaptic fibers such as the Schaffer collaterals. This sink appeared only in the distal radiatum, thus it appears that synaptic inputs arrive at the middle portion of the apical dendrites in this experiment.
Among the orthodromically evoked responses, our interest was focused on the longer latency negative spikes recorded in the str. radiatum (4 in Fig. 4). While this spike could be recorded in the full width of the str. radiatum, its peak latency was shortest at the distal portion of the str. radiatum. In its proximal portion, the latency of the spike became closer to that of the somatic population spike.

Cragg and Hamlyn, Fujita and Sakata, and Andersen and Lømo proposed that this negative spike represents action potentials which propagate along the apical dendrites of the pyramidal neurons. If so, there must be sink movement in the str. radiatum. As shown in Fig. 4, although minor sink movement was observed in the dendritic region proximal to the soma (b to a in Fig. 4), it was not distinct in the middle layer of the str. radiatum. This finding was consistent in all the cases examined \( (n = 9) \). Thus, our results of CSD analysis do not directly support the contention that the propagating dendritic action potentials can be evoked by synaptic inputs.

Nonetheless these findings do not deny the active changes of dendritic membrane in response to synaptic inputs. We did not observe any sink movement from the cell body layer to dendritic regions subsequent to the large sink current at the cell body layer. This suggests that the properties of a dendritic membrane change actively prior to spike generation at the cell body.

In the str. oriens, a diphasic profile of the membrane current (d in Fig. 4), which corresponds to the diphasic potential in this region (6 in Fig. 4) appeared. Sink movement from the str. pyramidale into the str. oriens indicates the propagation of action potentials along the basal dendrites and/or axons of pyramidal neurons.

**DISCUSSION**

By applying the current source density analysis on extracellular field potentials evoked by alveus stimulation, we confirmed that the apical dendrite of the hippocampal CA1 pyramidal neuron generates action potentials and that they can be propagated along the dendrite from cell body to dendrites.

Dendrites of hippocampal CA1 pyramidal neurons are known to generate Na\(^{+}\)-mediated and Ca\(^{2+}\)-mediated action potentials. We have shown that the sink movement in the apical dendritic region evoked by alveus stimulation was not blocked in low-Ca\(^{2+}\) or Ca\(^{2+}\)-free/high-Mg\(^{2+}\) condition but was blocked by TTX. These findings indicate that propagating dendritic spikes are primarily mediated by the Na ion, not by the Ca ion. Similar results were obtained by Jefferys in dentate granule cells. He showed that when activated orthodromically or antidromically, granule cell dendrites were invaded by action potentials and that the action potentials could be blocked by TTX.

The intrasomatic recordings taken from pyramidal neurons by Spencer and Kandel showed that about 25% of the CA1 pyramidal cells exhibit small spikes (fast prepotential, FPP), which were distinguishable from full-size spikes. They speculated that FPP originates at some point within the dendrite and that a passive area of membrane lies between the active membrane responsible for the FPP and the soma. This conception differs from our interpretation, based on the sink movement in response to antidromic activation, that an active membrane lies along the full length of the proximal dendrites.

There are several early reports in which the action potentials were concluded to propagate along the apical dendrite of CA1 pyramidal neurons all the way from dendrite to soma in response to orthodromic inputs based on the laminar record of the extracellular field potential. Andersen and Lømo arrived at the same conclusion with intrasomatic recordings. Our results support the concept that the membranes of dendrites are active all the way from soma to dendrites, but did not directly support the idea that action potentials propagate along the dendrites in response to synaptic inputs.

There are several possible mechanisms to interpret the fact that neither centripetal sink movement nor centrifugal sink movement which was clearly observed when stimulated antidromically, were brought about by synaptic inputs: (1) although pyramidal neurons generate propagating dendritic spikes, they are not synchronous. As they propagate close to the soma, spikes become well synchronized by an ephaptic interaction between neurons; (2) concomitant excitatory and/or inhibitory synaptic inputs reduce resistance of the dendritic membrane, so spike generation may be blocked or the amplitude of spikes reduced at the middle part of the dendrites; (3) when activated synaptically, dendritic spikes may
conduct centripetally in a saltatory manner due to cable properties of the dendrite; (4) orthodromic synaptic inputs may activate a non-propagating active conductance such as \( \text{Ca}^{2+} \) conductance at the distal part of the dendrite which in turn triggers propagating action potentials at the proximal part of the dendrites. Further work is required to determine which of these explanations best explains the findings, or whether another explanation must be sought.

It is well established that hippocampal CA1 pyramidal cells exhibit plastic changes of responsibility, so-called long-term potentiation (LTP). As one feature of LTP, Andersen et al. pointed out the E-S source density analysis of electrically evoked potentials. They observed an increase in population spike amplitudes even when the size of the field EPSP was kept constant and named the phenomenon E-S potentiation. They also showed that it was not merely a matter of general increase in excitability of the cell body. We have shown that the dendritic membrane has active properties. As they intervene between synaptic inputs and somatic spike generation, it is possible to imagine that the modulation of the active properties of the membrane along the dendrite is one of the underlying mechanisms of E-S potentiation.

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