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Rapid communication

Rapid dendritic Ca^{2+} influx is associated with induction of homosynaptic long-term depression in adult rat hippocampus

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Abstract

Homosynaptic long-term depression was induced in area CA1 of adult hippocampus by prolonged low-frequency stimulation (900 pulses at 2 Hz) in the presence of the $GABA_A$ receptor antagonist picrotoxin. Using ratio imaging with fura-2, we demonstrate that the induction of this long-term depression is associated with a rapid and transient ($\approx$ 30 s) dendritic Ca^{2+} increase ($\approx$ 500 nM) dependent on the activation of voltage-gated Ca^{2+} channels. This transient increase, by itself, was insufficient for long-term depression induction.

Keywords: Long-term depression; Ca^{2+}, dendritic; Metabotropic receptor

Prolonged low-frequency synaptic stimulation induces homosynaptic long-term depression in hippocampal slices prepared from young animals (Dudek and Bear, 1993; Mulkey and Malenka, 1992). In adult hippocampus, the same protocol of stimulation induces homosynaptic long-term depression if it is applied in combination with a pharmacological block of γ-aminobutyric acid-A ($GABA_A$)-ergic inhibition (Otani and Connor, 1996). The mechanisms underlying homosynaptic long-term depression are largely unknown. However, like long-term potentiation, the long-term depression induction requires postsynaptic Ca^{2+} (Mulkey and Malenka, 1992). It is hypothesized that long-term potentiation and long-term depression are triggered at different postsynaptic concentrations of Ca^{2+} ($[Ca^{2+}]$, e.g., Artola and Singer, 1993). Although evidence is generally consistent with this dual-threshold model (e.g., Cummings et al., 1996), no study has been conducted in the hippocampus to directly measure $[Ca^{2+}]$ changes during long-term depression induction. In the present study, we measured dendritic $[Ca^{2+}]$ changes during long-term depression induction in adult hippocampus using ratio measurements of fura-2 fluorescence.

Hippocampal slices were prepared and maintained as described previously (Otani and Connor, 1996). Conventional intracellular recordings (with 3 M K-acetate-containing micropipettes) were made from CA1 pyramidal neurons. Schaffer collateral-pyramidal cell synapses were orthodromically tested at 0.017 Hz. Long-term depression was induced by 900 pulses delivered at 2 Hz. In one group, neurons were impaled with electrodes containing 20 mM fura-2 in the tip. Fluorescence measurement and ratio imaging for intracellular $[Ca^{2+}]$ were made with the methods previously utilized in our laboratory (Petrozzino and Connor, 1994).

In the presence of picrotoxin (50 μM) in the bath, the 2 Hz stimulation induced homosynaptic long-term depression of the monosynaptic excitatory postsynaptic potential (EPSP; $-32 \pm 12\%$ at 45 min, $n = 7$, $P < 0.03$ over control, *t*-test), as we found previously (Otani and Connor, 1996). The same stimuli failed to induce long-term depression in the absence of picrotoxin ($2.8 \pm 4.7\%$, $n = 6$). This long-term depression is *N*-methyl-D-aspartate (NMDA) receptor independent, since application of DL-2-amino-5-phosphonovaleric acid (AP5, 100 μM) did not block long-term depression ($-36 \pm 6.3\%$, $n = 5$, $P < 0.02$). In a separate group of neurons, increases of $[Ca^{2+}]$ during 2 Hz stimulation were determined by fura-2 ratio imaging. In the presence of picrotoxin, long-term depression condition, 2 Hz stimulation was accompanied by rapid increases of $[Ca^{2+}]$ in medial/distal dendrites ($244 \pm 84\%$ at peak, $n = 7$, $P < 0.01$ over control) which largely decayed within

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30 s (Fig. 1). In the absence of picrotoxin, little or no $[Ca^{2+}]$ increases were observed ($35 \pm 13\%$, $n = 9$, Fig. 1). DL-APV did not block dendritic $[Ca^{2+}]$ increases seen in the picrotoxin condition ($198 \pm 62\%$, $n = 13$, $P < 0.03$). In contrast, membrane hyperpolarization (-110 mV) during 2 Hz stimuli abolished dendritic $[Ca^{2+}]$ increases ($20 \pm 14\%$, $n = 8$, $P > 0.5$ over control). In a separate group of neurons without fura-2, membrane hyperpolarization significantly attenuated long-term depression ($n = 5$, $P < 0.01$, analysis of variance). Together, these data suggest that voltage-gated Ca^{2+} channels rather than NMDA receptors contribute to the $[Ca^{2+}]$ increases and long-term depression induction.

In 17 of the fura-2-loaded neurons, simultaneous electrophysiological recordings were made. The majority of the neurons in long-term depression-inducing conditions (i.e., picrotoxin in the bath) underwent long-term depression (6/9, $-59 \pm 5.0\%$), whereas without picrotoxin, only 1 of 8 neurons showed depression. These data confirm that the $[Ca^{2+}]$ increases we observed in the presence of fura-2 are within a range sufficient for long-term depression induction. Finally, we compared the mean absolute peak value of $[Ca^{2+}]$ during the long-term depression-inducing conditions with that during long-term potentiation-inducing condition (100 Hz with picrotoxin). We found that long-term depression is associated with more moderate $[Ca^{2+}]$ increases than long-term potentiation (464 ± 66 nM vs. 1.25 ± 0.36 μM, $n = 25$ and 6, respectively, $P < 0.005$). This is consistent with an observation recently made in visual cortex (Yasuda and Tsumoto, 1996). In the present study, the measurements were made in the primary and secondary dendrites where AP5 treatment has little effect on the $[Ca^{2+}]$ increases. Much higher, AP5-sensitive increases were detected in dendritic spines and tertiary dendrites using a low-affinity Ca^{2+} indicator (Petrozzino et al., 1995). The $[Ca^{2+}]$ increases during 100 Hz stimuli observed in the present study may represent a NMDA-independent induction of long-term potentiation (e.g., Petrozzino and Connor, 1994).

Our results provide the first direct evidence in the hippocampus for dendritic $[Ca^{2+}]$ increases during long-term depression induction. Duration of the increases (≈ 30 s) is much shorter than the duration of conditioning stimuli (7.5 min). Since a conditioning this short is insufficient to induce long-term depression ($n = 5$, data not shown), additional factors must cooperate with the dendritic Ca^{2+} influx. Our more recent data indicate that synaptic stimulation of phospholipase C-coupled metabotropic (mGlu) receptors during and even after 2 Hz stimulation is necessary for long-term depression induction. We therefore propose that homosynaptic long-term depression induction in adult hippocampus involves rapid $[Ca^{2+}]$ influx and extended activation of mGlu receptors.

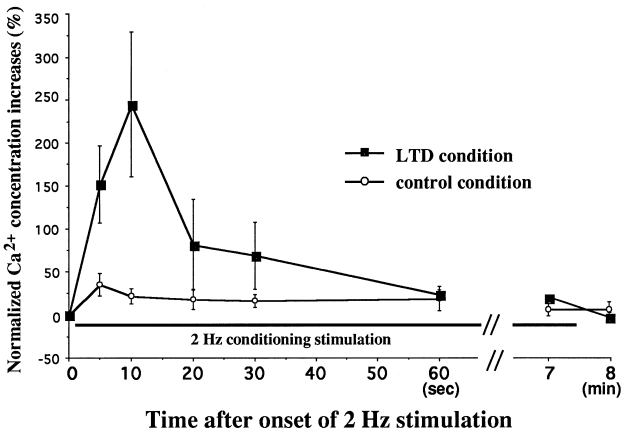

Fig. 1. Homosynaptic long-term depression induction (900 pulses at 2 Hz in the presence of picrotoxin) in adult hippocampus is associated with rapid increases of dendritic Ca^{2+} concentrations measured by fura-2 ratio imaging (filled squares). In the absence of picrotoxin, the 2 Hz stimuli do not induce long-term depression and there were no or little Ca^{2+} increases ($P < 0.01$, open circles). The Ca^{2+} increases are AP5-insensitive but sensitive to membrane hyperpolarization, suggesting the involvement of voltage-gated Ca^{2+} channels (see text). Also, the peak increase was more moderate than that during long-term potentiation-inducing stimuli (see text). Basal Ca^{2+} levels were 151 ± 12 nM and 169 ± 14 nM in control and picrotoxin groups, respectively.

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