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Responsiveness of Dendrites to the Glutamate Applied Focally with Pressure Ejector and Iontophoresis into Hippocampal Slices

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Glutamate is the most common excitatory amino acid in the brain. Responsiveness of dendrites to the glutamate greatly varies depending on the application sites. Especially, a point of the maximal response to the glutamate of the dendrite is called as 'hot spot'. In our experiment, the responsiveness of the hot spot to the glutamate was investigated in the CA1 pyramidal neuron of the rat hippocampal slice. CNQX, the antagonist of AMPA receptor, blocked 95% of membrane current to the glutamate focal application (I_{gl}). Train ejection of glutamate on one point of the dendrite increased or decreased the amplitude of I_{gl} with the pattern of train, and the changes were maintained at least for 30 min. In some cases, glutamate train ejection also induced calcium dependent action potentials. To evoke long-term change of synaptic plasticity, we adopted θ-burst in the glutamate train ejection. The θ-burst decreased the amplitude of glutamate response by 60%. However, after θ-burst glutamate train ejection, the calcium dependent action potential appeared. These results indicated that the focal application of glutamate on the neuronal dendrite induced response similar to the synaptic transmission and the trains of glutamate ejection modulated the change of AMPA receptor.

Key Words: LTP, LTD, Synaptic plasticity, AMPA receptor, NMDA receptor, θ-burst

INTRODUCTION

Glutamate, which is the most abundant excitatory neurotransmitter, activates AMPA (a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid), NMDA (N-methyl-D-aspartic acid), and kainate receptor (Mayer et al, 1984; Mayer & Westbrook, 1985) of the postsynaptic neuron. Activation of AMPA receptors locally depolarizes synaptic membrane, so that it removes Mg^{2+} block in NMDA receptor. Ca^{2+} ions account for 30% of total ion movements through NMDA receptor. Activation of NMDA receptor brings about increase of Ca^{2+} ion flux at the dendritic spine.

One of the most important mechanisms of the memory, the long term potentiation (LTP), has been well investigated, which is a long-lasting increase of excitability of synaptic transmission following high frequent synaptic transmissions (Lynch & Bliss, 1986a, b). High frequency electrical stimulation (100 Hz) onto presynaptic neuronal axon induces LTP, where 2 ~ 10 min of lower frequency stimulation (1 ~ 2 Hz) induces long term depression (LTD) (Dudek & Bear, 1992; Mulkey & Malenka, 1992). And, also prolonged low-frequency stimulation eliminates LTP. Such a process is called depotentiation of LTP (Fujii et al, 1991). Criteria for the induction of LTP are varied by the pattern of afferent activity (Mulkey & Malenka, 1992; Dudek & Bear, 1993; Mulkey et al, 1993). Moreover, there are many reports about the relation of LTP/LTD with Ca^{2+} influx through NMDA receptor. By all of the biochemical processes, LTP and LTD activate protein kinase and protein phosphatase, respectively (Shenolikar & Nairn, 1991). It has been known that 20 to 40% of protein in postsynaptic density is calcium/calmodulin-dependent protein kinase II (CaMKII) (Kelly, 1983; Kennedy, 1983), and calmodulin antagonists inhibit LTP ex-

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pression (Finn et al, 1980; Turner et al, 1982; Reymann et al, 1988). Moreover, the relation of protein kinase C (PKC) with LTP expression was reported (Akers et al, 1986; Hu et al, 1987). PKC activation increases the open probability of the ion channels, and the activities of NMDA receptors (Xiong et al, 1998). Therefore, PKC antagonists decrease both responses of NMDA and AMPA receptors. These results suggest that glutamate changes the activities of both postsynaptic NMDA and AMPA receptors, and the changes of their activities might be involved in the modulation of long term synaptic plasticity.

It is well known for LTP to have three specific characteristics such as specificity, cooperativity, and associativity. There have been many trials to elucidate the mechanism of them. However, it is difficult to find the location of LTP to be induced throughout the dendritic tree of the postsynaptic neuron. Especially, it is necessary to localize the site of LTP precisely, in order to explain the relationship between the backpropagation of action potential from the soma and associativity of LTP. However, it is not possible to track the site of LTP to be induced in the case of the electrical stimulation of presynaptic neuron for an induction of LTP. Therefore, it is important to simulate the postsynaptic responses that are evoked by the release of neurotransmitters from the presynaptic nerve ending. Although the simulation of postsynaptic response has a vulnerable point, except presynaptic mechanism of LTP, it must be important to develop the techniques specifying the location of long term changes of the postsynaptic mechanism.

This study was undertaken to simulate synaptic transmission by focal application of glutamate at a dendrite and to trace the changes of glutamate receptor activity. The focal application of glutamate was performed on an arbitrary location of apical dendrite of pyramidal neuron.

METHODS

Preparation of hippocampal slices

Hippocampal slices were prepared from 2- to 3-week-old Sprague Dawley rats anesthetized with Equithesin (6 ml/kg, Sigma, MO, USA). After the whole body including the brain was cooled by transcardiac perfusion with ice-cold sucrose perfusion solution for 1 minute, the brain was isolated as soon as possible. The hippocampi were separated gently from the both cerebral hemispheres on iced dish and mounted on slicing chamber of vibratome (Series 1000, TPI Inc.) with cyanoacrylate in ice-cold sucrose perfusion solution. Slice cutting blade (Feather Co., Japan) was set on 20° of horizontal angle and the thickness of slices was set to 300 to 350 μm. The hippocampal slices were incubated for 30 min at 35°C in incubation solution and then stored at room temperature.

Solutions

The slice incubation solution was (in mM) 125 NaCl, 2 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 6 $MgCl_2$, and 25 glucose. The bath solution was 125 NaCl, 2 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 3 $CaCl_2$, 3 $MgCl_2$, and 25 glucose. Both of the slice incubation solution and the recording bath solution were aerated with 95% O_2 and 5% CO_2 to adjust the pH to 7.4. The sucrose perfusion solution consisted of 229 sucrose, 1.9 KCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 6 $MgCl_2$, 10 HEPES, and 10 glucose, aerated with 97.5% O_2 and 2.5% CO_2. Cesium gluconate pipette solution contained (in mM) 17.5 CsCl, 122.5 gluconic acid, 122.5 CsOH, 8 NaCl, 0.3 GTP, 2 Mg-ATP, 0.2 EGTA, and 10 HEPES, set to pH 7.2 with CsOH and osmolarity was 280 to 300 mosmol kg^{-1}. Potassium gluconate pipette solution contained (in mM) 122.5 Potassium gluconate, 5 $MgCl_2$, 0.3 GTP, 2 Mg-ATP, 40 HEPES, and 0.2 EGTA (pH 7.25 with KOH and 280~300 mosmol kg^{-1}).

TTX (tetrodotoxin, 1 mM, Tocris, UK) and/or CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, 5 μM, Tocris, UK) were included in the bath solution if they were needed to block the generation of action potentials, or to block AMPA receptors. All the chemical reagents were purchased from Sigma, except TTX and CNQX.

Whole cell patch clamp recording

Membrane currents and potentials were recorded at the soma of hippocampal CA1 neuron with whole cell patch clamp method. The hippocampal neurons were visualized by IR-DIC optics with a fixed-stage microscope (BX-50WI, Olympus, Tokyo, Japan) and monitored by IR-sensitive video camera (Hamamatsu, Photonics, Japan).

Patch-pipettes were pulled on a vertical puller (PP-

83, Narishige, Tokyo, Japan) from borosilicate tubing, filled with pipette solution, and connected to a headstage of voltage-clamp/current-clamp amplifier (Axopatch 200 A, Axon Instruments, Foster, CA, USA). Signals were amplified, low-pass-filtered at 5 kHz, and digitized online by an A/D converter (Digidata 1200, Axon Instruments). Data were recorded and processed with commercial software (pClamp 6, Axon Instruments), and stored on a personal computer. Resting membrane potential (RMP) was determined directly after obtaining whole-cell configuration. Series resistance and active membrane properties were analyzed in current-clamp mode using hyperpolarizing and depolarizing current pulses of 250 msec duration. Pipette resistance was 3~4 MΩ, and if the series resistance increased above 2 folds of the pipette resistance, experiments were stopped immediately.

Focal ejection of glutamate

To simulate postsynaptic transmission, we used pressure ejector (General Valve Co.) and iontophoresis for the fast focal applications of glutamate. The pressure ejection was controlled by the digital output from A/D converter (Digidata 1200, Axon Instruments, Foster, CA, USA). The iontophoresis could reduce ejecting time to 4 msec. Ejection current amplitude and duration were controlled by analog output from the A/D converter.

Glutamate solution (250 mM) was set to pH 8.0 with NaOH, and ejected through the iontophoresis by -100 nA ejection current. Holding current was set to $+25$ nA to prevent leakage of glutamate, and $+100$ nA current was ejected by balance pipette containing NaCl to compensate the -100 nA ejection current.

RESULTS

The change of membrane potential during current injection of 0.5 nA for 250 msec was shown in Fig. 1. The change of membrane potential with current step pulse is composed of first faster increase and second slower increase. The first one is due to series resistance, and the second one is usually due to the changes in electrophysiological properties of the membrane. Using equation (1), the series resistance (R_s) was obtained by faster voltage increment ($\triangle V_{fast}$) and ejected current ($I_{ejection}$).

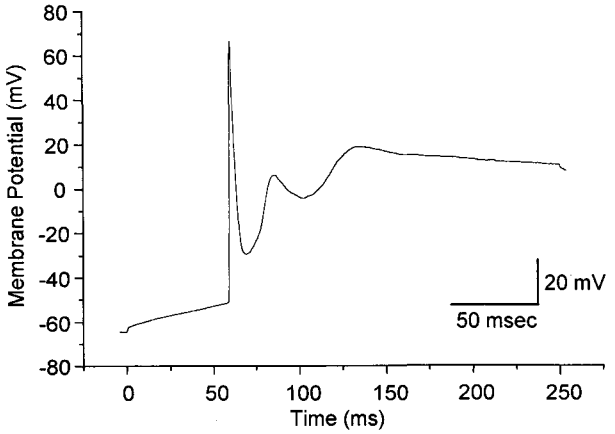

Fig. 1. Change of membrane potential shows the series resistance and several spikes in the current clamp mode. Recording pipette was filled with Cs-gluconate solution which depolarizes the membrane to 0~-30 mV. At the beginning of current ejection step like elevation of membrane potential is shown. It is proportional to the series resistance. The spike like potential induced by current ejection may be due to calcium current.

$$R_s = \frac{\triangle V_{fast}}{I_{ejection}} \quad \cdots\cdots (1)$$

Fig. 2 shows membrane current induced by glutamate focal ejection (I_{gl}) at -70 mV of membrane potential. The peak amplitude of I_{gl} was reduced up to 95% by 5 μM CNQX included in the bath solutions. This result suggested that the most of I_{gl} was composed of AMPA current. It also indicated that NMDA receptor was not activated by glutamate ejection, because it might be due to holding the membrane potential of the dendrites at -70 mV.

Fig. 3 shows the deactivation kinetics of I_{gl} induced by the pressure ejection and iontophoretic application of the glutamate. Deactivation time constants calculated from Fig. 3A and 3B were compared with time constant from outside-out recording (Spruston, et al, 1995). Deactivation curves were fitted to equation (2).

$$A_1 \exp\left\{-(t-k)/\tau_1\right\} + A_2 \exp\left\{-(t-k)/\tau_2\right\} \quad \cdots\cdots (2)$$

Fig. 4 compares I_{gl} responses before and after train ejection of 1 mM glutamate by pressure ejector. The train ejection was 100 Hz for 1 sec and duration of ejection was 4~6 msec. The membrane potential was clamped at -30 mV to activate the NMDA receptor during the train of glutamate ejection. I_{gl} increased

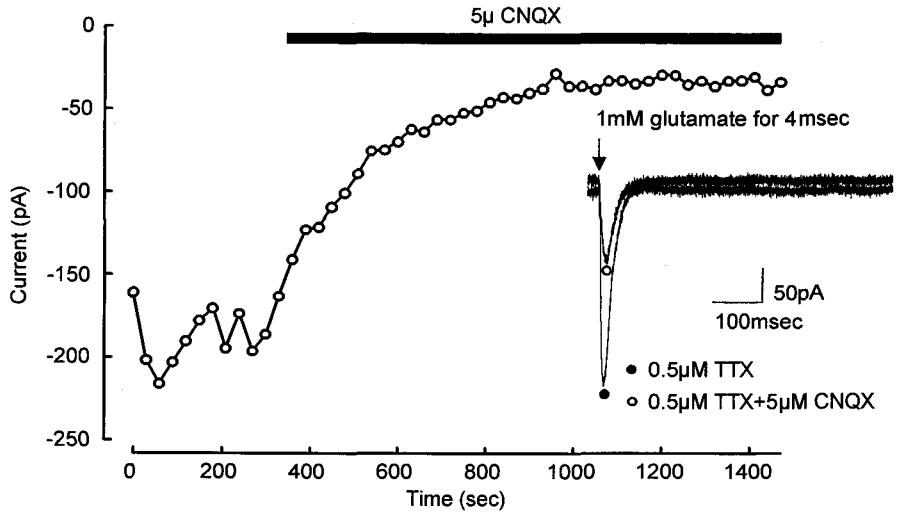

Fig. 2. I_{gl} (membrane current induced by focally ejected glutamate) was induced by 4 msec ejection of glutamate (1 mM) (n=7). Usually 4 msec glutamate ejection induced around 200 pA membrane current but variation was large from cell to cell. The peak amplitude of I_{gl} was reduced up to 95% by AMPA receptor antagonist, CNQX. This result suggests that 95% of I_{gl} is composed of AMPA current.

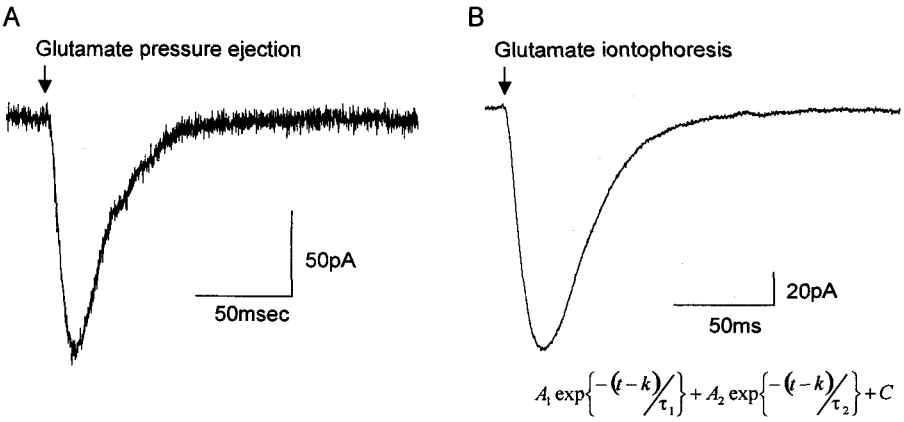

Fig. 3. Deactivation kinetics of I_{gl} induced by (A) pressure ejection and (B) iontophoresis of glutamate are shown. Inactivation process is fitted to the double exponential function. (A) Glutamate (1 mM) was ejected by pressure ejector for 4 msec. The ejection pressure and location of ejection pipette tip was controlled by monitoring the movement of target segment of the dendrite and locating the tip as close as possible to the dendrite visually. The time constants of inactivation process were 6.6 and 16.2 msec. (B) Glutamate (250 mM, pH 8.0) was applied on the dendrite focally for 4 msec. Resistance of ejection pipette was above 100 MΩ and it was monitored whether the tail of ejection current occurred or not. Time constants of inactivation process were 9.6 and 18.9. There is some difference between the time constants of our experiment and Spruston's result (Spruston et al, 1995). It may be due to the recording methods. The electrical signal recorded at soma from dendritic input are contaminated by various ionic currents and large capacitance of cell membrane.

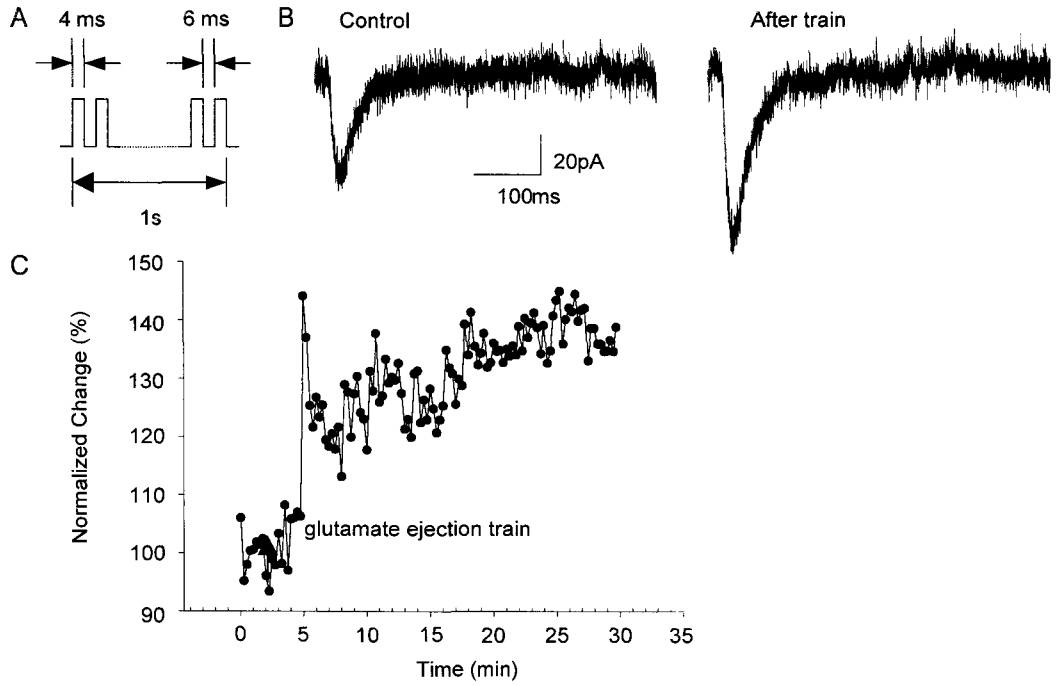

Fig. 4. Normalized changes of I_{gl} induced by pressure application of glutamate on the dendrite of CA1 neuron. (A) Train protocol is shown. Glutamate was ejected for 4 msec and duration of intermission was 6 msec. Glutamate ejection train continued for 1 sec and the membrane potential of target neuron was held at −30 mV in order to release NMDA receptor from Mg^{2+} blockage. Before and after the train glutamate was ejected every 15 sec and the potential was held at −70 mV. (B) Membrane current trace after train is the 7th one from the train of glutamate ejection. (C) Peak responses to the glutamate ejection after the train was normalized to the mean peak response before the train (n=12).

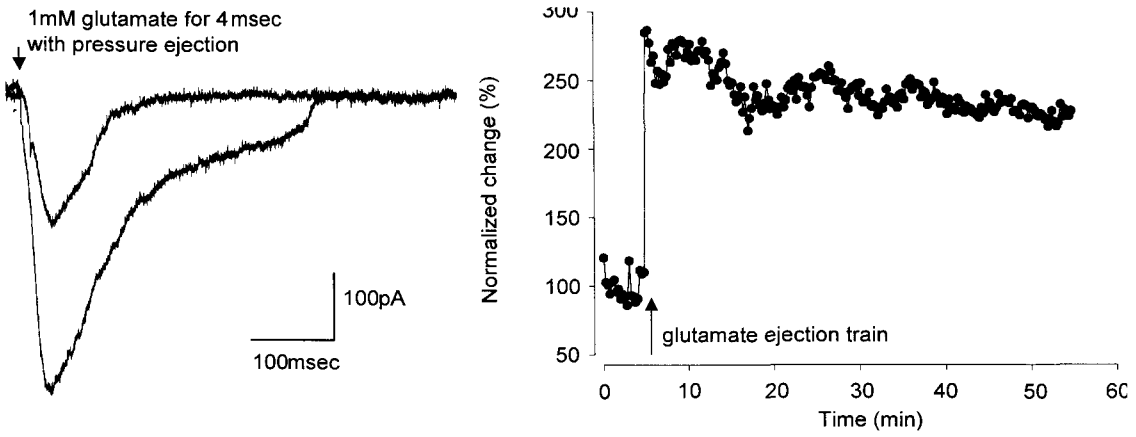

Fig. 5. Glutamate ejection train occasionally induced the membrane current presumably associated with the dendritic calcium dependent action potentials. The membrane current induced by calcium dependent action potentials take place on the dendrite that is 100 μm apart from soma. Before the glutamate ejection train there were no calcium dependent action potentials but after it every single pressure ejection of glutamate induced calcium dependent action potential.

over 30% (n=8) and the increments of them lasted over 30 minutes.

Sometimes, after the train ejection, I_{gl} was followed by the great tail current continuing for over 200 msec, and then disappeared fast (Fig. 5, n=3). Cd^{2+}, the Ca^{2+} channel blocker, reduced I_{gl} somewhat, but blocked the tail current. This suggested that Ca^{2+} current induced action potential at the specific point of dendrite and I_{gl} was increased by glutamate train ejection.

To investigate the effects of low frequency train stimulation onto the dendrite of postsynaptic neuron, glutamate was ejected in 20 Hz train for 1 sec and the duration of ejection was 20 msec. The membrane potential was held at −30 mV during the train ejection of glutamate. I_{gl} was reduced after the low frequency glutamate train ejection (Fig. 6), and the decrease of I_{gl} was not recovered completely.

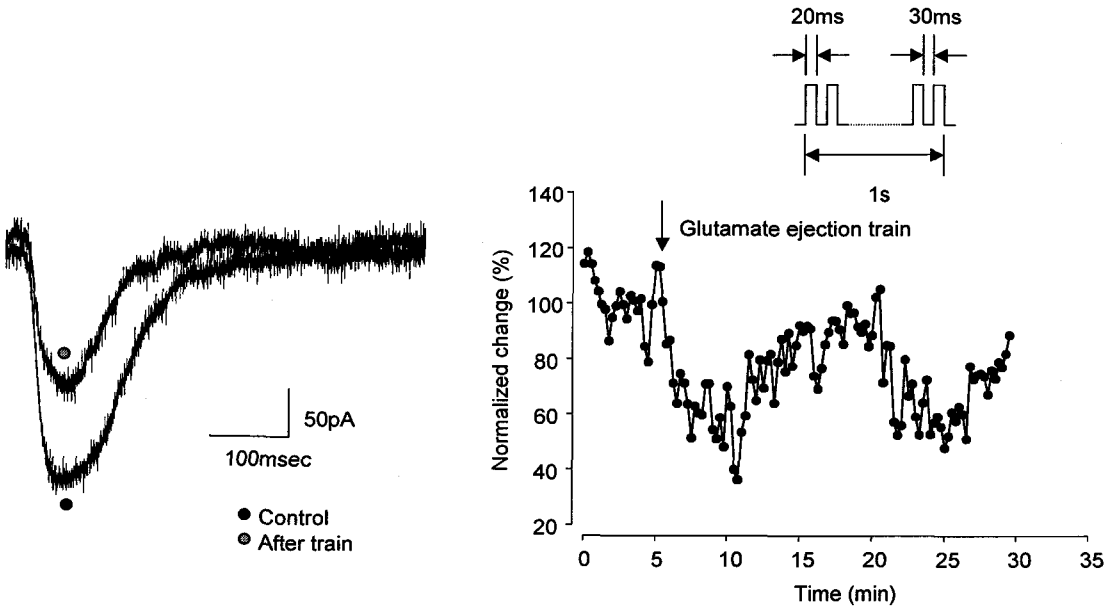

Fig. 6. Decreased I_{gl} after ejection train of glutamate was recorded from CA1 neuron (n=4). Low frequency of ejection train decreased I_{gl} by about 20~40%.

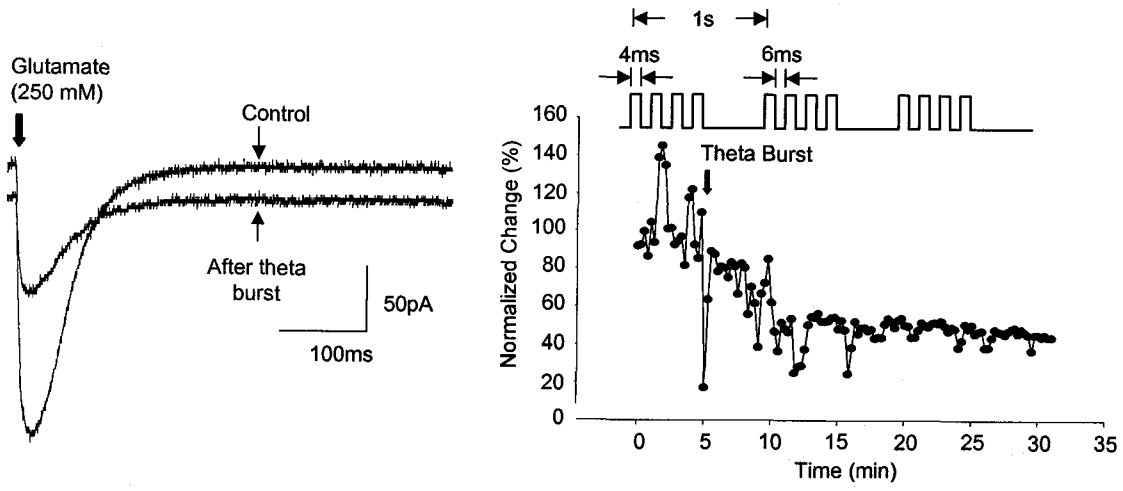

Fig. 7. Iontophoretically ejected glutamate induced the membrane currents that was depressed after θ-burst (n=9). θ-burst is the 100 Hz ejection train for 800 msec followed by 200 msec interburst interval. In this experiment, three bursts were applied consecutively.

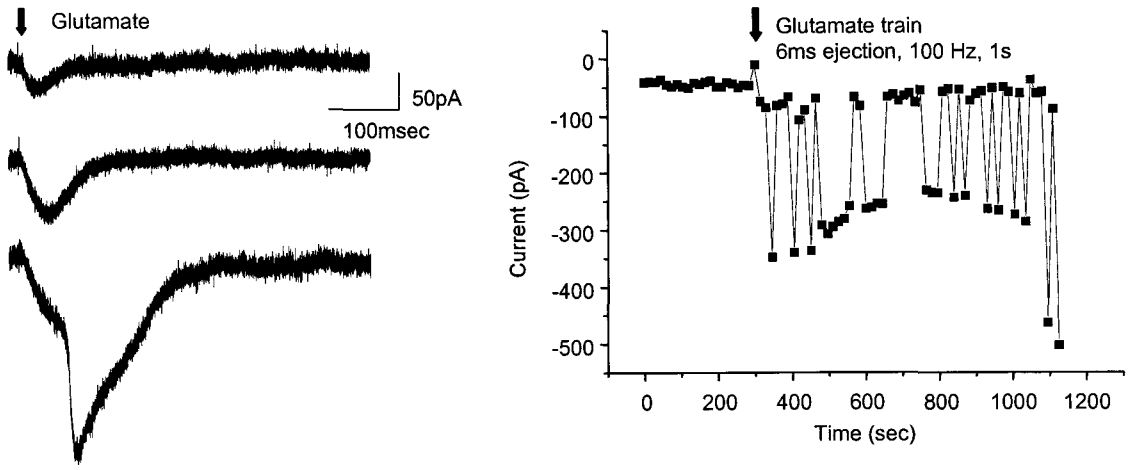

Fig. 8. Membrane current possibly associated with the dendritic calcium spike was shown after the train of iontophoretic glutamate ejection. Two case of calcium dependent action potential was observed and in one of them calcium dependent action potential was disappeared when glutamate ejection train was applied again.

For the closer simulation of synaptic transmission, iontophoresis was used to eject glutamate (250 mM), because it can exclude the mechanical influence of pressure ejector that can shift the dendrite away. The kinetics of I_{gl} induced by ionotophoretic ejection was not different from those by the pressure ejection (Fig. 3B). To induce the long-term changes of postsynaptic mechanism efficiently, we applied iontophoretic glutamate ejection train in θ-burst mode. The θ-burst was repetitions of 100 Hz of glutamate train ejection for 800 msec and 200 msec interburst interval. Fig. 7 shows changes of I_{gl} followed by three cycles of θ-bursts. The response to the glutamate was reduced to 60% by θ-bursts and the reduction of I_{gl} was not recovered until end of the experiment. The major component of I_{gl} was inferred the membrane current through AMPA receptors from the Fig. 1. Therefore, the effect of θ-burst application of glutamate means that it can modulate the activity of AMPA receptor.

Fig. 8 shows that the presumed calcium dependent action potentials were induced by θ-burst, but disappeared by reapplication of θ-burst glutamate train (n=2). These results suggested that there was a kind of switch for calcium dependent action potential.

DISCUSSION

A dendrite forms synapses with presynaptic axon terminals but the synapses are not distributed evenly throughout the dendritic tree. Therefore, the distribution and density of hot spots, which are extremely sensitive regions to the neurotransmitter in the dendrite, are essential to understand characteristics of neuron (Jones & Baughman, 1991). Moreover, the importance of the dendritic spine has been emphasized (Minkwitz, 1976), and its distribution, density, and functions have been also studied in relation to the hot spot. In general, the distribution of the hot spot is greatly different between neurons in cultured cells and brain tissues, with respect to the kinds of neurons and the locations at the dendrites of the same neuron. In 1990, Thomson et al. applied glutamate on the various sites in the dendritic tree of cultured cortical pyramidal neuron with iontophoresis and reported that the hot spots are distributed sparsely within 100 μm from the soma. However, a study of dendritic spine of the pyramidal neurons in the brain slice showed that 2.00 ± 0.20 and 1.80 ± 0.40 ea/μm of dendritic spines are distributed at apical and basal dendrites, respectively (Andersen & Trommald, 1995; Trommald et al, 1995). In our experiment, I_{gl} could be recorded with glutamate ejection at dendrite over 100 μm from the soma, but under 100 μm, I_{gl} was too small to be measured. This result suggests that there are few hot spots at the proximal dendrites, and it agrees with the finding that there is no dendritic spine in apical dendrite near the soma (Kunz et al, 1976).

The hippocampal CA1 neuron has many branches of dendrites, so that the total membrane capacitance

is much large, and the thin pipe-shaped dendrites have a large axial resistances to signal transmission. Therefore, the dendrites would function as a passive filter (Spruston et al, 1994; Migliore, 1996; Hoffman et al, 1997) to reduce postsynaptic potential (PSP) (Meyer et al, 1997). However, various ionic channels on the dendrites do amplify the PSPs and prevent attenuation of them. Therefore, it is regarded that the postsynaptic current is composed of ionic currents through the receptors generating the PSPs and booster ionic currents through membrane potential dependent ion channels of the dendrites (Bouron et al, 1999; Magee & Carruth, 1999). In our experiment, 95% of I_{gl} was an AMPA current and the rest might be composed of the ionic currents through the other type of ionotropic glutamate receptors and voltage-dependent ion channels on the dendrites.

In the case of applying whole cell voltage clamp technique to the neuron, it must be considered how much area of neuronal membrane can be held at desired potential and also how fast the membrane potential can be clamped. Because the cell such as a neuron has large membrane area and highly branched shapes, it is impossible to hold the membrane potential of the input site on the dendrite rapidly at desired value as well as to prevent the generation of action potential by holding the membrane potential upon the excitatory signal input. In our experiment, glutamate was applied with the pressure ejector or iontophoresis on a point of the dendrite far from the soma. It was difficult to clamp the membrane potential of the input site and to prevent the generation of action potential in the neuron where the glutamate applied focally. TTX prevented the generation of action potential in the cell whose membrane currents were recorded as well as presynaptic neurons.

Synaptic plasticity is changed by presynaptic and postsynaptic modifications - changes in the amount of quanta to be released (Gottschalk et al, 1998; Kelly & Lynch, 1998; Nishizaki et al, 1999), and properties of postsynaptic receptors (Hanse & Gustafsson, 1992; Kauer, 1999). Generally, the long term change of synaptic plasticity (LTP or LTD) is assumed to occur with both of them. While the role of NMDA receptor in the LTP/LTD is regarded to be important, that of AMPA receptor is not well known (Fedorov et al, 1997). In this study, glutamate ejection trains increased or decreased I_{gl} composed of AMPA current by 95%, and this change continued for a quite long time. Activation of AMPA receptor depolarizes the membrane locally and it releases NMDA receptors from Mg^{2+} blockage. It suggests that the increase of AMPA current allows greater number of NMDA receptor to be activated. Therefore, high frequency-synaptic input shows that the changes of AMPA receptor activity take part in the changes of synaptic plasticity.

Ca^{2+} dependent action potentials could be evoked by focal iontophoresis of glutamate (Schwindt & Crill, 1999), by current injection through a dendritic recording electrode (Kim & Connors, 1993; Larkum et al, 1999) or by synaptic stimulation (Kim & Connors, 1993; Schwindt & Crill, 1998; Larkum et al, 1999). Iontophoretically applied glutamate on the dendrite 178 to 648 μm from the soma increases the calcium concentration and induces the regenerative Ca^{2+} dependent potentials (Oakley et al, 2001). Occurrence of Ca^{2+} dependent action potential after the train of glutamate focal application suggests that it plays a role in the associativity of LTP.

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