Amplification of EPSPs by Low Ni^{2+}- and Amiloride-Sensitive Ca^{2+} Channels in Apical Dendrites of Rat CA1 Pyramidal Neurons

THOMAS GILLESSEN AND CHRISTIAN ALZHEIMER
Department of Physiology, University of Munich, D-80336 Munich, Germany

Gillessen, Thomas and Christian Alzheimer. Amplification of EPSPs by low Ni^{2+}- and amiloride-sensitive Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *J. Neurophysiol.* 77: 1639–1643, 1997. Distal synaptic input to hippocampal CA1 pyramidal neurons was evoked by electrical stimulation of afferent fibers in outer stratum radiatum. Whole cell recordings from CA1 cell somata served to monitor excitatory postsynaptic potential (EPSP) envelopes after dendritic processing. To probe a functional role of low-voltage-activated Ca^{2+} current [or T current (I_T)] in the apical dendrite, EPSP recordings were combined with local application of antagonists of I_T. Dendritic application of low concentrations of Ni^{2+} (5 μM) and amiloride (50 μM) reduced EPSP amplitude measured at the soma (resting membrane potential −70 mV) by 33.0 ± 2.9% (mean ± SE, n = 27) and 27.0 ± 2.1% (n = 26), respectively. No appreciable effect on EPSP time course was observed. As expected from the voltage dependence of I_T activation, the inhibitory effect of both antagonists was strongly attenuated when EPSPs were recorded at hyperpolarized membrane potential (−90 mV). In contrast to dendritic application, somatic application of Ni^{2+} or amiloride produced only weak reduction of EPSP amplitude. Our data indicate that dendritic low Ni^{2+}- and amiloride-sensitive Ca^{2+} channels giving rise predominantly to I_T can produce substantial amplification of synaptic input. We thus propose that these channels represent an important component of subthreshold signal integration in apical dendrites of CA1 pyramidal cells.

INTRODUCTION

Apical dendrites of pyramidal neurons in hippocampus and neocortex express tetrodotoxin (TTX)-sensitive Na^+ channels, low-voltage-activated (LVA) and high-voltage-activated (HVA) Ca^{2+} channels, and different types of K^+ channels (e.g., Andreasen and Lambert 1995; Huguenard et al. 1989; Magee and Johnston 1995a,b; Markram and Sakmann 1994; Spruston et al. 1995a; Stuart and Sakmann 1994; Wong and Stewart 1992). One implication of these findings would be that propagation of synaptic signals along the dendritic tree is not only determined by the geometry and the passive properties of the dendrites (Spruston et al. 1994), but also by their active electroresponsiveness.

With the use of local TTX application onto apical dendrites of hippocampal CA1 neurons, we have recently demonstrated that dendritic Na^+ channels produce considerable amplification of excitatory postsynaptic potentials (EPSPs) (Lipowsky et al. 1996). Here we used a similar approach to investigate a possible role of LVA current [or T current (I_T)] in dendritic signal integration. The original notion that I_T should be capable of boosting synaptic signals (Deisz et al. 1991; Sutor and Zieglgänsberger 1987) gained new momentum when dendritic patch recordings and Ca^{2+} imaging experiments showed that dendritic LVA channels of hippocampal and neocortical pyramidal cells are activated by subthreshold membrane depolarization (Magee and Johnston 1995a,b; Markram and Sakmann 1994). It is unclear, however, whether the effects of dendritic I_T activation would be functionally restricted to dendritic compartments, or whether they would be also seen by axosomatic compartments, thereby influencing directly the output behavior of a neuron. To address this question, we evoked EPSPs far out on the apical dendrite and recorded their shape at the soma with dendritic I_T active or partially suppressed. Assuming that I_T is the predominant target of low Ni^{2+} and amiloride, our data provide the first evidence that this current of the apical dendrite can indeed alter the weight of excitatory signals.

METHODS

Transverse hippocampal slices (nominal thickness 400 μm) were prepared from ether-killed Sprague-Dawley rats 12–23 days old and maintained in a submerged chamber at room temperature (20–24°C). The artificial cerebrospinal fluid (ACSF) for preparation and storage contained (in mM) 125 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, and 10 D-glucose, gassed with 95% O_2-5% CO_2, pH 7.4. During recordings, extracellular Ca^{2+} was elevated to 3 mM and the following substances were added routinely to ACSF to isolate non-*N*-methyl-D-aspartate (NMDA)-mediated EPSPs and suppress GABAergic inhibition: the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (30 μM; RBI, Natick, MA), the γ-aminobutyric acid-A ($GABA_A$) receptor antagonist bicuculline chloride (10 μM; Sigma, Deisenhofen, Germany), and the $GABA_B$ receptor antagonist saclofen (100 μM; RBI, Natick, MA). In addition, 2 mM extracellular CsCl was present in all recordings to eliminate possible influences of the hyperpolarization-activated cation current (I_h) on EPSP envelope (cf. Spruston et al. 1995b). Whole cell patch pipettes were filled with (in mM) 140 potassium gluconate, 9 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 2 Mg-ATP, pH adjusted to 7.3 with KOH. Extracellular recordings of field EPSPs (fEPSPs) were performed with the use of microelectrodes filled with 3 M NaCl.

Whole cell blind patch recordings were performed on neurons in CA1 stratum pyramidale by means of an Axopatch 200A amplifier in conjunction with a Digidata 1200 interface with the use of pClamp 5.7 software (all from Axon Instruments, Foster City, CA). Afferent fibers in outer stratum radiatum were electrically stimulated at a frequency of 0.2 Hz with the use of a micropipette filled with modified ACSF solution ($NaHCO_3$ was replaced with equimolar HEPES/Na-HEPES solution). For dendritic or somatic drug application, a micropipette attached to a pressure application system was positioned in stratum radiatum (approximate distance from soma: 200 μm) or in stratum pyramidale (details in Lipowsky et al. 1996). To visualize the approximate spread of Ni^{2+} (5 μM)

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or amiloride (50 μM) in the tissue, both drugs were dissolved in 2% food color solution (Brauns-Heitman, Warburg, Germany). From the spread of color solution we estimated that the drugs had a radius of effectiveness of $\geq$100 μm. In control experiments ($n = 5$) dendritic pressure application of food color solution alone produced negligible reductions ($\leq$3%) of EPSP amplitude. All recordings were performed at room temperature (20–24°C). Membrane voltages are corrected for liquid junction potential (10 mV). EPSP time course was fitted with a semiempirical function of the form

$$f(t) = A*\{1 - \exp[-(t - t_0)/\tau_1]\}^2*\exp[-(t - t_0)/\tau_2]$$

where A is the EPSP amplitude, t_0 is the start time (time between stimulus artifact and onset of EPSP), τ_1 is the time constant of the rising phase, and τ_2 is the time constant of the decay phase of the signal. The double exponential was fitted with the use of a simplex algorithm to minimize the mean squared error between the data and our function. Data are expressed as means ± SE or percentage of control where appropriate. Comparative statistics were performed with the use of the two-tailed Student's *t*-test for paired data.

RESULTS

Distal afferents in outer stratum radiatum were electrically stimulated to record remote EPSPs of the non-NMDA type in the somata of CA1 pyramidal neurons. To study the role of dendritic I_T with minimum contamination by somatic I_T, membrane potential was set to −70 mV and stimulation strength was adjusted to obtain EPSP peak amplitudes at the soma of 7 mV on average (range 5–10 mV). Under these conditions, ~60% of all LVA channels are available, but somatic EPSP amplitude should be too small to activate these channels (Avery and Johnston 1996; Magee and Johnston 1995b). In dendritic compartments, however, EPSPs are less attenuated than they are at the soma and might well depolarize the membrane potential into the range of I_T activation. We tested this hypothesis by dendritic application of Ni^{2+} (5 μM) and amiloride (50 μM), two Ca^{2+} channel blockers that should preferably act on I_T at the given low concentrations (Avery and Johnston 1996). As shown in Fig. 1, *top traces* in *A* and *B*, both compounds produced a significant reduction of EPSP amplitude. Dendritic amiloride application reduced EPSP amplitude by 27.0 ± 2.1% (mean ± SE) ($n = 26$, $P < 0.0001$). If Ni^{2+} was injected into the dendritic region, EPSP amplitude decreased by 33.0 ± 2.9% ($n = 27$, $P < 0.0001$). The effects of both amiloride and Ni^{2+} reversed within 15–20 min of drug washout to 96.4 ± 0.8% ($n = 6$) of control and to 98.2 ± 0.5% ($n = 9$) of control, respectively. Analysis of EPSP time course indicated that neither amiloride (rise time contant: control 11. 0 ± 1.6 ms, amiloride 10.4 ± 1.5 ms; decay time constant: control 38.5 ± 2.7 ms, amiloride 39.1 ± 2.7 ms, $n = 11$, difference in both time constants not significant) nor Ni^{2+} (rise time constant: control 10. 5 ± 1.0 ms, Ni^{2+} 10.0 ± 0.7 ms; decay time constant: control 40.8 ± 2.9 ms, Ni^{2+} 45.8 ± 5.0 ms, $n = 7$, difference in both time constants not significant) caused an appreciable change in EPSP kinetics. As expected from the voltage dependence of I_T activation, hyperpolarization of membrane potential to −90 mV by somatic DC injection attenuated the effect of both antagonists (Fig. 1, *bottom traces* in *A* and *B*). Under this condition, EPSP amplitudes were reduced by 6.9 ± 2.2% ($n = 13$, $P < 0.001$) after dendritic amiloride application and by 18 ± 2.6% ($n = 10$, $P = 0.001$) after dendritic Ni^{2+} application. We ascribe the residual effect of the blockers to the nonisopotentiality of the membrane, which should allow some I_T activation out on the dendrite.

Local drug application onto the somatic region of the recorded neuron supported the above notion that, with our experimental protocol, somatic I_T should only provide a minor contribution to the observed changes in the EPSP (Fig. 2). At membrane potentials of −70 and −90 mV, somatic amiloride application reduced EPSP amplitude by 7.0 ± 1.2% ($n = 9$, $P < 0.001$) and 2.6 ± 1.3% ($n = 9$, $P > 0.08$), respectively. Somatic Ni^{2+} application decreased EPSP amplitude by 14.0 ± 2.9% at −70 mV ($n = 6$, $P = 0.01$) and by 11.9 ± 2.0% at −90 mV ($n = 4$, $P = 0.001$). The effects of dendritic versus somatic drug application on EPSP amplification at −70 mV were significantly different for both amiloride ($P < 0.0001$) and Ni^{2+} ($P < 0.006$).

In contrast to HVA channels, LVA channels have not been directly implicated in the process of transmitter release (Huguenard 1996; Reuter 1996). We nevertheless wanted to rule out that any of the observed effects were due to a presynaptic action of Ni^{2+} or amiloride. For this purpose we recorded fEPSPs in outer stratum radiatum near the site of stimulation where they should reflect the input signal before dendritic processing, and we added various concentrations of the antagonists to the bathing solution. Ni^{2+} failed to exert any influence on fEPSP amplitude at a concentration (50 μM) 10 times higher than that used for local tissue application (99.3 ± 0.2% of control fEPSP amplitude, $n = 3$). Although fEPSP recordings are by no means exclusive indicators of presynaptic events, the lack of action of Ni^{2+} on fEPSPs strongly indicates that none of the mechanisms generating the input signal (including the presynaptic ones) were affected by the blocker. Only at much higher concentrations was substantial decline of fEPSP amplitudes noted (Fig. 3), presumably due to suppression of HVA channels involved in transmitter release (Reuter 1996). Similar to Ni^{2+}, amiloride did not affect fEPSP amplitude (98.1 ± 2% of control, $n = 6$) when bath applied at a concentration (500 μM) 10 times higher than that for local tissue application.

DISCUSSION

The major conclusion from our experiments is that, depending on membrane voltage, low Ni^{2+}- and amiloride-sensitive Ca^{2+} channels can produce substantial amplification of remote EPSPs, thereby compensating in part for the electrotonic attenuation imposed by the passive cable properties of the dendrites (Spruston et al. 1994). How can we be sure that the site of drug action was in fact dendritic as opposed to presynaptic or somatic? Bath application of the antagonists in conjunction with fEPSP recordings in outer stratum radiatum demonstrated that both drugs at concentrations 10 times higher than those achieved during focal application failed to impair synaptic transmission (Fig. 3), thereby excluding any presynaptic action. As for a somatic site of action, membrane potential and EPSP amplitude at the soma were unfavorable for an appreciable activation of somatic I_T. Furthermore, LVA channels appear to be more

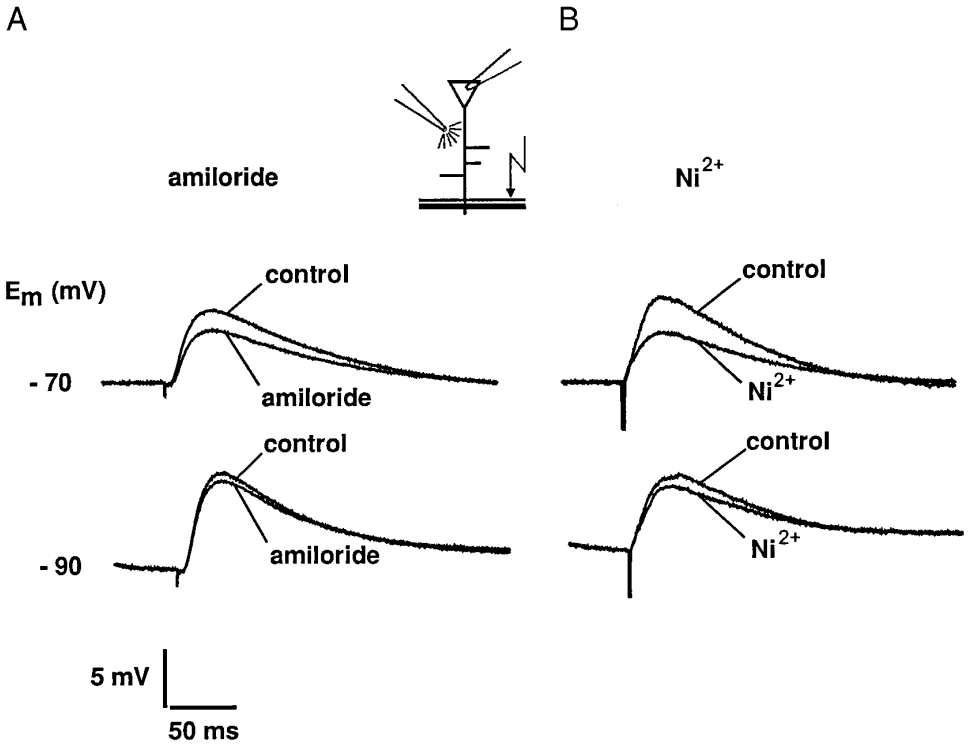

FIG. 1. Decrease of remote excitatory postsynaptic potentials (EPSPs) after local application of Ni^{2+} (5 μM) or amiloride (50 μM) to apical dendrite. Somatic EPSP recordings were obtained at 2 different membrane potentials (E_m) (−70 and −90 mV) adjusted by DC injection through whole cell pipette. Stimulus artifacts: time of stimulation. *Inset*: arrangement of whole cell pipette (*top*), pipette for local drug application (*middle*), and site of electrical stimulation (*bottom*). *A*: superimposed traces of averaged EPSPs (n = 50) recorded before and during application of amiloride. *B*: superimposed traces of averaged EPSPs (n = 50) recorded before and during application of Ni^{2+}.

densely expressed in the dendrites than in the somatic region of CA1 pyramidal neurons (Christie et al. 1995; Karst et al. 1993; Magee and Johnston 1995b). Thus the small decrease of EPSP amplitude after somatic drug application should predominantly reflect activation of dendritic I_T due to drug diffusion into adjacent regions of stratum radiatum. The fact that amiloride, which we presume diffuses less easily within the tissue than Ni^{2+}, displayed almost no effect on EPSP envelope when applied somatically (Fig. 2) further supports this notion.

Were our pharmacological tools sufficiently selective to allow the conclusion that the observed EPSP amplification was exclusively due to I_T activation? Several studies have shown that low micromolar concentrations of Ni^{2+} indeed

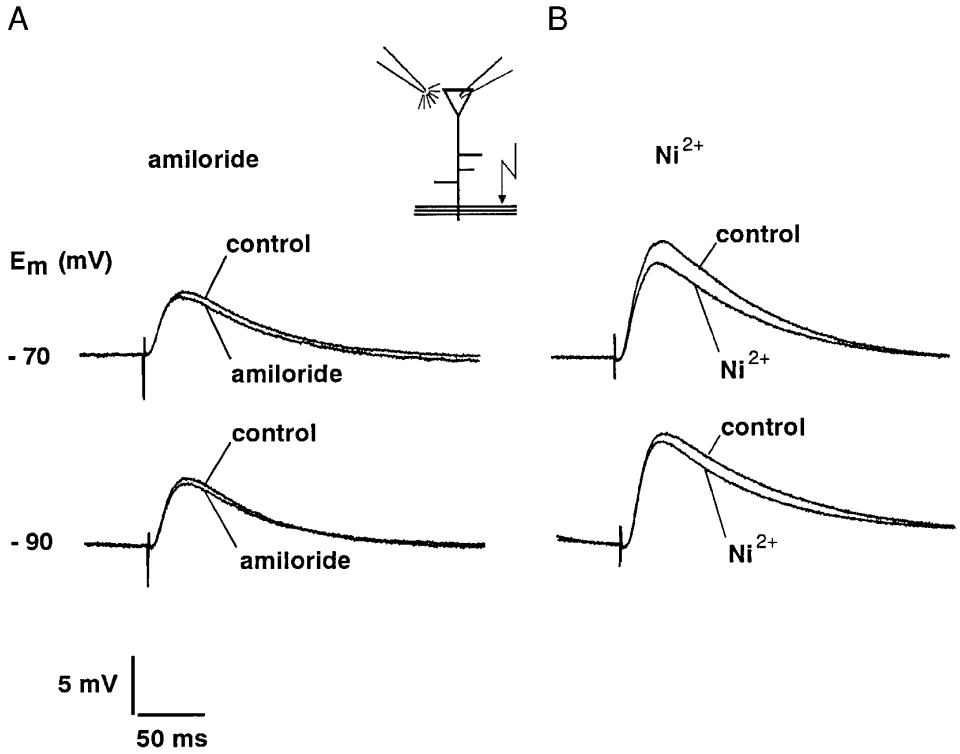

FIG. 2. Effect of Ni^{2+} (5 μM) or amiloride (50 μM) after local application in the vicinity of recorded cell somata. EPSP recordings were obtained at 2 different membrane potentials (−70 and −90 mV). Stimulus artifacts: time of stimulation. *Inset*: as in Fig. 1, but pipette for local antagonist application points toward somatic region of recorded neuron. *A*: superimposed traces of averaged EPSPs (n = 50) recorded before and during application of amiloride. *B*: superimposed traces of averaged EPSPs (n = 50) recorded before and during application of Ni^{2+}.

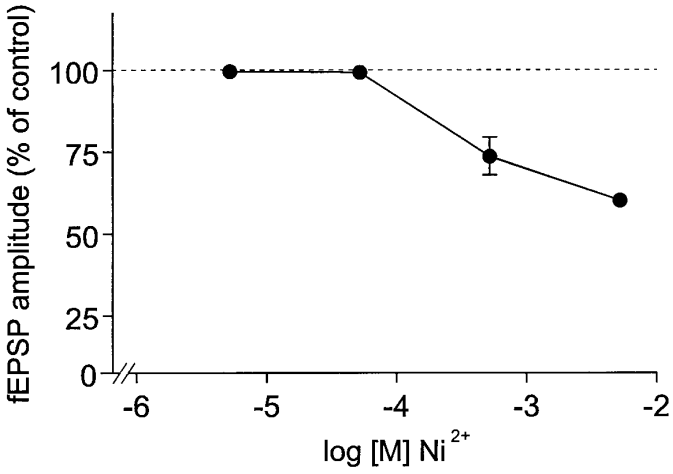

FIG. 3. Relative changes in field EPSP (fEPSP) amplitude as function of increasing Ni^{2+} concentrations in the bathing solution. Extracellular recordings were performed in outer stratum radiatum near site of stimulation. Part of the data was obtained by cumulative drug application. Error bars not shown when smaller than symbol size (50 μM) or when $n = 2$ (5 μM and 5 mM).

produce significant reduction of I_T in somatic and dendritic regions of pyramidal neurons of hippocampus and neocortex (Avery and Johnston 1996; Magee and Johnston 1995b; Markram and Sakmann 1994; Ozawa et al. 1989; Toselli and Taglietti 1992). Nevertheless, even at these low concentrations, partial effects of Ni^{2+} on other types of Ca^{2+} channels cannot be ruled out completely (Avery and Johnston 1996). It is noteworthy that apical dendrites of CA1 pyramidal neurons express a type of HVA Ca^{2+} channels (R type) that displays much higher sensitivity to Ni^{2+} than other types of HVA channels (Magee and Johnston 1995b). Although we chose recording conditions in which, at the soma, EPSPs were unlikely to recruit HVA channels, they might have done so while traveling along the dendrite where their amplitudes were less attenuated. Thus our experimental approach does not exclude some partial contribution of dendritic R-type Ca^{2+} current to EPSP amplification.

Are our EPSP recordings free of contamination by other ion currents? This holds for the NMDA receptor-mediated excitatory postsynaptic current, for inhibitory postsynaptic currents, and for I_h (see METHODS). However, we cannot exclude nonlinear interactions in the dendrite between I_T and a persistent Na^+ current (I_{NaP}). Both currents are activated below firing threshold and display a negative slope conductance in this voltage range (French et al. 1990; Takahashi et al. 1991). As shown here and in a previous study in the same preparation (Lipowsky et al. 1996), pharmacological suppression of each current produces a considerable decrease in EPSP amplitude. As a consequence, EPSP envelope will recruit less of the unblocked current and the boosting effect of the current under study might be overestimated.

Because our method is not capable of determining precisely the extent of I_T inhibition on the apical dendrite, the present study does not allow us to describe EPSP amplification by dendritic I_T in quantitative terms. Our data do indicate, however, that low Ni^{2+}- and amiloride-sensitive Ca^{2+} channels (predominantly T-type channels) of the apical dendrite are strong enough to assign additional weight to distal synaptic signals while they propagate to the soma. Thus I_T and I_{NaP} (Lipowsky et al. 1996) emerge as essential factors of signal amplification in the apical dendrites of CA1 neurons. From computer simulations (Bernander et al. 1994) we would expect that these amplifier currents would be balanced in a delicate manner by dendritic K^+ current(s) to prevent saturation of excitatory input, but experimental evidence for this hypothesis remains to be established.

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Present address of T. Gillessen: Institute of Pharmacology and Toxicology, Federal Armed Forces Medical Academy, Ingolstädter Landstr. 100, D-85748 Garching, Germany.

Address for reprint requests: C. Alzheimer, Dept. of Physiology, University of Munich, Pettenkoferstr. 12, D-80336 Munich, Germany.

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