Effects of EGTA on the Calcium-Activated Afterhyperpolarization in Hippocampal CA3 Pyramidal Cells

Abstract. *Intracellular recordings in the hippocampal slice preparation were made with electrodes filled with 0.2 molar potassium EGTA. The purpose was to investigate the role of calcium in mediating afterhyperpolarizations following bursts evoked by intracellular current pulses, arising spontaneously, or induced by the epileptogenic agent penicillin. In the first two cases the afterhyperpolarization was blocked and the associated conductance increase was reduced, suggesting that EGTA was blocking the calcium-activated potassium conductance during the hyperpolarization. In the third case the afterhyperpolarization and associated conductance increase persisted, implicating an alternative mechanism for the hyperpolarization.*

Several mechanisms undoubtedly interact to limit prolonged depolarization of neurons. The mechanisms include the rapidly repolarizing potassium conductance of the falling phase of the action potential, an increased chloride conductance due to inhibitory neurotransmitter, and a slow increase in potassium conductance which may be voltage- or calcium-dependent. The calcium-mediated conductance change has been shown to influence the neuronal firing rate in several systems. In various molluscan species, calcium-dependent potassium activations follow a burst of impulses, limiting the cell's firing frequency and duration (*1*). A slow conductance change underlies the afterhyperpolarization (AHP) seen in spinal motoneurons of the frog (*2*) and the cat (*3*) as well as in neuroblastoma cultures (*4*). In several cases, the calcium dependence of the potassium conductance was demonstrated by intracellular injection of EGTA, which binds free Ca^{2+}. This treatment abolishes the AHP and results in prolonged cell discharge (*5*, *6*).

To date, Ca^{2+}-mediated potassium conductance has not been demonstrated in mammalian cortical neurons, although such a conductance is strongly suggested (*7*). Such information is essential to explain the mechanisms underlying abnormal neuronal firing patterns during epileptiform discharge. The guinea pig hippocampal slice preparation provides an ideal system in which to investigate cellular events involved in neuronal bursting; it enables the experimenter to perform intracellular recording, permits analysis of synaptic and intrinsic membrane events, and allows rapid changing of the extracellular medium. The slice contains cell groups that exhibit spontaneous bursting (CA3 pyramidal cells) and inducible bursting (CA1 pyramidal cells) and has been extensively utilized to study epileptiform activity (*8*). We investigated the role of a calcium-mediated potassium conductance following neuronal bursts discharging spontaneously, evoked by a depolarizing current pulse, or induced by the epileptogenic agent penicillin.

Transverse slices of guinea pig hippocampus (400 μm) were prepared and maintained as described previously (*9*). Intracellular recordings were made from cells in the CA3 region by using standard electrophysiological techniques. Control recordings were obtained with electrodes containing 4*M* potassium acetate. Pyramidal cells in the CA3 region exhibit spontaneous bursting behavior consisting of 2 to 20 spikes riding on a slow wave of depolarization (*10*). The CA3 cells in our experiments produced normal bursts both spontaneously and in response to depolarizing current pulse (Fig. 1A). Conductance measurement were made during the hyperpolarizatio following each type of burst and dur ing inhibitory postsynaptic potentia (IPSP's) evoked by orthodromic activa tion (Fig. 1A). Conductance increases o 15 to 25 percent, as measured by injec tion of 50-msec, 0.5-nA hyperpolarizin current pulses, were observed durin both AHP's and IPSP's.

To investigate the role of Ca^{2+} in th generation of AHP's, we impaled CA cells with electrodes containing 0.2*M* K^+-EGTA (*6*); EGTA diffusing from th electrode presumably binds free intra cellular calcium ions. Injection of EGTA led to prolonged discharges with broa spikes from all CA3 pyramidal cells stud ied. Similar bursts occurred both sponta neously and in response to depolarizin current pulses (Fig. 1A). The AHP's tha normally follow both types of burst were abolished, and the conductanc change associated with the period after a burst was decreased (conductance in creased less than 10 percent) (Fig. 1B) The prolonged bursting in CA3 cell could be "reset" with brief hyper polarizing pulses or IPSP's and thus ap

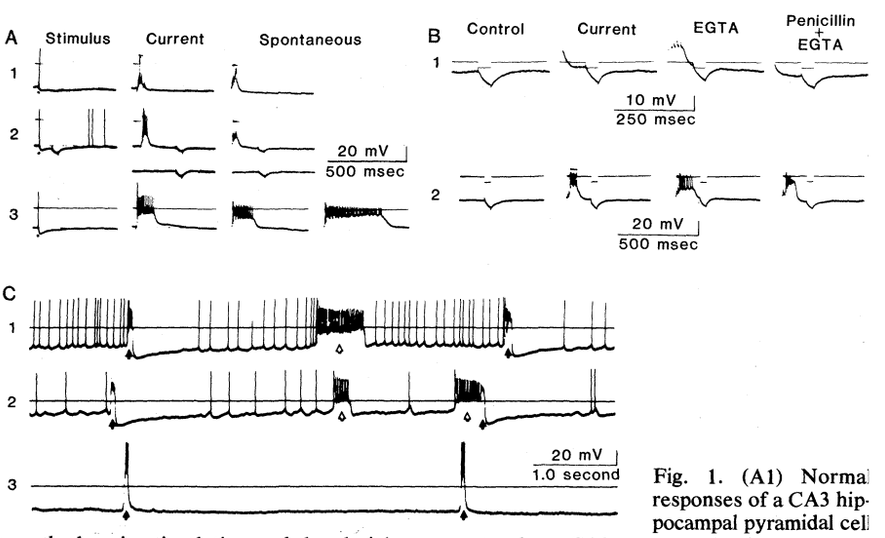

Fig. 1. (A1) Normal responses of a CA3 hippocampal pyramidal cell to orthodromic stimulation and depolarizing current pulses; CA3 neurons also burst spontaneously. Note that a hyperpolarization follows all three types of discharge. (A2) Injection of a short (50-msec) hyperpolarizing current pulse during the hyperpolarizations revealed a conductance increase (compare voltage deflection during AHP's with control responses to current injection just below). (A3) Injection of EGTA did not affect the IPSP (stimulus) but abolished the AHP following current-induced and spontaneous bursts; burst duration was also prolonged. (B1) High-gain sweeps of voltage changes produced by injection of hyperpolarizing current pulses into an EGTA-treated neuron. Little conductance change (compared to control) was noted following current-induced or spontaneous (*EGTA*) bursts (control, 10.0-mV deflection; current, 10.8-mV deflection; EGTA, 10.2-mV deflection); a conductance increase persisted during the AHP following epileptiform bursts (*Penicillin + EGTA*, 8.1-mV deflection). (B2) Lower gain sweeps show the entire response from which the sweeps in (B1) were taken. (C) Continuous records of a CA3 pyramidal neuron injected with EGTA and bathed in a medium containing penicillin. Closed arrows point to spontaneous epileptiform bursts and open arrows point to spontaneous EGTA bursts. Note that large hyperpolarizations follow epileptiform discharges but do not follow the EGTA bursts. Spontaneous spiking, including EGTA bursts, could be completely suppressed by hyperpolarizing the cell (C3); epileptiform bursts, however, continued with a constant rhythm. The epileptiform AHP, when occurring during an EGTA burst, could "reset" the membrane (C2).

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peared to be voltage-dependent. The conductance increase associated with IPSP's did not change following EGTA injections.

Addition of 2 m*M* sodium penicillin to the bathing medium led to spontaneous epileptiform bursting in the CA3 population (Fig. 1C) (*11*). Epileptiform bursts could be distinguished from spontaneous or EGTA-induced bursts by comparing their rhythmicity to the field potentials (cellular epileptiform discharge occurred synchronously, as reflected in a field potential burst) and by measuring their resistance to blockage by hyperpolarizing current (Fig. 1C). Twenty-four CA3 neurons were studied with EGTA electrodes during penicillin-induced epileptogenesis. Although the AHP during current-induced and spontaneous bursting was abolished by EGTA injection, the AHP following epileptiform bursts was unaffected in 23 cells (Fig. 1C). That is, even after intracellular injection of the calcium chelator, epileptiform bursting triggered an AHP with associated conductance increase (Fig. 1B). This AHP cannot be attributed solely to anomalous rectification (*12*) properties of the neuron.

Our studies show that EGTA blocks the AHP following spontaneous and current-induced firing. Evidence that the hyperpolarization following current-induced firing is due to a Ca^{2+}-mediated increase in potassium conductance has been derived from studies on hippocampal pyramidal cells of the CA1 region (*6*). Our results provide a direct demonstration of the existence of this Ca^{2+}-mediated increase in potassium conductance in both the CA1 and CA3 regions (*13*). Since EGTA did not block the IPSP-related hyperpolarization, it is clear that EGTA does not produce a generalized blockage of all hyperpolarizations; also, it is implied that the IPSP contributes little to normal hyperpolarizations after bursting. The observation that EGTA does not block the hyperpolarization that follows epileptiform bursts suggests that this AHP is not calcium-dependent. This finding does not support the hypothesis, proposed in previous studies of hippocampal bursting (*6, 8, 11, 14*), that the same Ca^{2+}-dependent potassium conductance mechanism is responsible for all AHP's that follow bursting.

What alternative mechanism might account for the AHP's that follow epileptiform bursts? One possibility is that these AHP's are indeed Ca^{2+}-dependent, but that EGTA diffusion is simply ineffective in reaching relevant Ca^{2+} binding sites. It is conceivable that the site involved in the generation of the epileptiform AHP is different from that involved in the production of the other AHP's. This explanation appears unlikely, however, in view of the large CA3 cell dendrites (which are located electrically close to the soma). The dendrite size renders much of the cell nearly isopotential. A second possibility is that a larger calcium influx is associated with the epileptiform burst than with the other bursts, and that EGTA did not bind sufficient Ca^{2+} quickly enough to significantly block the potassium conductance. However, since prolonging EGTA injections did not alter the amplitude of the epileptiform AHP, as might be expected if the EGTA effectiveness was dependent solely on the amount of EGTA injected, this explanation also appears unlikely. A third possibility is that the AHP is a chloride-dependent, γ-aminobutyric acid–mediated "giant" IPSP. This explanation is favored in early literature on penicillin-induced epileptogenesis (*15*). However, recent studies have shown that penicillin blocks hippocampal IPSP's (*9, 16*). A fourth possibility is that the AHP following epileptogenic bursts is a voltage-dependent potassium conductance. Experiments in our laboratory suggest that tetraethylammonium, which blocks voltage-sensitive potassium channels (*1, 17*), can indeed modify the AHP associated with epileptiform bursts (*18*).

We cannot yet ascertain the origin of the epileptiform AHP in hippocampal CA3 neurons. The evidence suggests that penicillin-induced epileptiform bursts are different from bursts evoked by current or arising spontaneously. This idea is inconsistent with some recent theories of hippocampal bursting behavior, and requires that we consider a variety of mechanisms that could be involved in controlling such activity.

Alger and Nicoll (*19*) recently investigated the mechanism underlying epileptiform AHP's in the CA1 region of the hippocampus. In their report they conclude that the AHP in CA1 is probably a calcium-dependent potential. We have two main points to make in response to their study:

1) Whereas Alger and Nicoll present convincing evidence that the AHP following epileptiform events is due to potassium conductance, the evidence that this conductance is calcium-dependent is weak. The latter conclusion is based on their experiments with barium, which they claim blocks a calcium-dependent potassium current. However, barium appears to have very complex effects (*2, 6, 20*) and is probably not specific to the calcium-dependent potassium conductance.

2) There is undoubtedly a great deal of difference between the CA1 and CA3 pyramidal cell populations (*18*). We have studied some CA1 neurons with EGTA electrodes and have found this population to be much less homogeneous than the CA3 cells. That is, while EGTA did not block the epileptiform AHP in the vast majority of CA3 cells, its effect on CA1 neurons was less predictable. In approximately 30 percent of the CA1 neurons, EGTA did block the epileptiform AHP. These results, together with those presented by Alger and Nicoll, suggest that more than one potassium conductance mechanism may be involved in the generation of epileptiform AHP's in CA1 neurons.

PHILIP A. SCHWARTZKROIN*
Department of Neurological Surgery, University of Washington, Seattle 98195

CARL E. STAFSTROM
Department of Physiology and Biophysics, University of Washington

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* Send reprint request to P.A.S.

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