Epileptiform Burst Afterhyperpolarization: Calcium-Dependent Potassium Potential in Hippocampal CA1 Pyramidal Cells

Abstract. *Synaptic excitation of hippocampal cells during blockade of synaptic inhibition results in an epileptiform "burst" potential followed by a prolonged afterhyperpolarization. This afterhyperpolarization resembles the one that is seen after the epileptic interictal spike and that is considered of critical importance in preventing seizure development. The afterhyperpolarization produced in the presence of γ-aminobutyric acid antagonists is associated with a conductance increase and is inhibitory. It can occur in an all-or-none fashion after a burst, is independent of chloride, and is depressed by barium. The afterhyperpolarization has a reversal potential of −86 millivolts, and the reversal potential is strongly dependent on the extracellular concentration of potassium. The afterhyperpolarization appears to be an intrinsic, inhibitory potassium potential mediated by calcium. This finding has implications for understanding the cellular mechanisms of epilepsy.*

The interictal stage of epileptiform discharges is characterized in the cortical electroencephalograph by intermittent bursts of cellular activity called interictal spikes (*1*). Interictal spikes appear similar to the high-frequency "burst" discharges seen in various animal models (*2-6*). For instance, intracellular recordings from cells responding to the convulsant penicillin, a commonly used model of the interictal state, reveal large depolarizing potentials (paroxysmal depolarizing shifts) (Fig. 1A, left) that trigger bursts. A burst is typically succeeded by a prolonged afterhyperpolarization (AHP) that is functionally inhibitory and associated with a conductance increase (*3, 4*). Transition from the interictal to the true epileptic ictal (seizure) state is accompanied by disappearance of this AHP. The AHP has been assumed to play a key role in preventing the ictal discharge (*5*), although its identity has remained uncertain. While early evidence seemed to indicate that it is a persistent inhibitory postsynaptic potential (IPSP) (*6*), suggestions have been made that the AHP is a calcium-dependent potassium potential (*6-8*). To date there has been little direct evidence concerning this point.

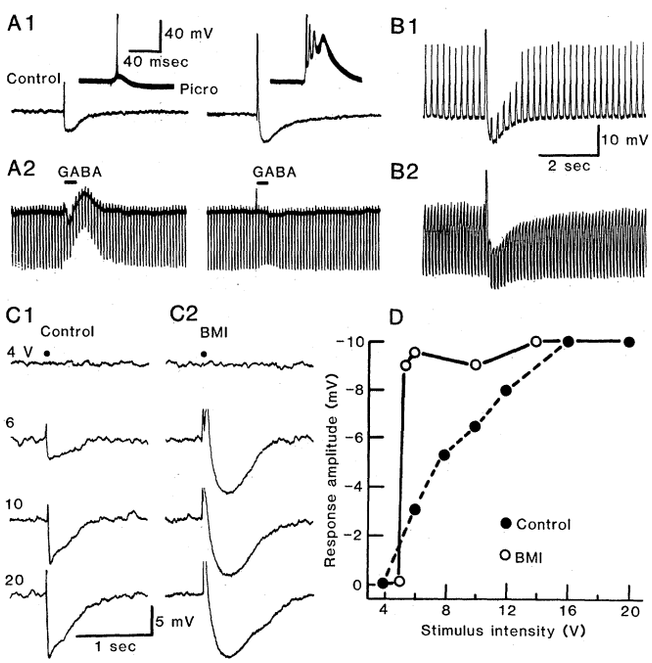

Fig. 1. Characteristics of the AHP_s in three CA1 pyramidal cells recorded with $KMeSO_4$-filled electrodes. (A) Orthodromic stimulation elicits an EPSP, action potential, and IPSP (A1, left). Iontophoretic GABA (100 nA, 2 seconds) applied through an independently positioned pipette produces a biphasic sequence of deflections (A2, left) (*14*). When the perfusion is switched to a medium containing $2 \times 10^{-4}M$ picrotoxin (*Picro*), the same stimulus evokes a "burst" potential and an AHP larger than the original IPSP (A1, right), despite the fact that the iontophoretic GABA response is nearly abolished (A2, right); resting potential, −63 mV. (B) The AHP_s in picrotoxin blocks action potentials produced by brief depolarizing current pulses passed through the recording electrode (B1). When hyperpolarizing current pulses are passed across the membrane (40-msec pulses at 10 Hz), a conductance increase accompanies the major portion of the AHP_s (B2); resting potential, −64 mV. (C and D) The IPSP in normal saline is graded with increases in stimulus intensity (C1 and closed circles in D), while the AHP_s produced by the same stimuli in $10^{-4}M$ bicuculline methiodide (*BMI*) is nearly maximal at just suprathreshold levels and does not change with increases in stimulus strength (C2 and open circles in D); resting potential, −63 mV.

During experiments on synaptic inhibition in the hippocampal slice preparation, we observed that orthodromic stimulation in the presence of γ-aminobutyric acid (GABA) antagonists elicits a burst of action potentials and an AHP (*9*). Since the major inhibitory transmitter in the hippocampus is probably GABA (*10*), this finding strongly suggested that the AHP is not an IPSP, although various interpretations were possible. For example, the AHP could be (i) a non-GABA-mediated IPSP unmasked by GABA antagonists, (ii) an antagonist-resistant form of GABA-mediated IPSP, or (iii) a nonsynaptic potential. The experiments described in this report demonstrate that the AHP is an intrinsic, calcium-dependent potassium potential that is initiated by the preceding burst discharge.

We used the transverse hippocampal slice preparation (*11*) modified by submerging the slice at 30° to 32°C (*12*). Submerging affords stable recording conditions, and switching between various experimental media while maintaining an impalement can be performed with a "latching" solenoid valve (General Valve, Inc.). Intracellular electrodes were filled with either 2*M* potassium methyl sulfate ($KMeSO_4$), having resistances of 70 to 100 megohms at 130 Hz, or 3*M* KCl (50 to 70 megohms). Reversal potentials were determined by passing current, via a carefully balanced bridge circuit (WPI M701), through the recording electrode. Hippocampal pyramidal cells from the CA1 field of male Sprague-Dawley rats were studied. All intracellular impalements were maintained in stable condition for over 30 minutes with resting membrane potentials of at least 50 mV (mean and standard deviation, 60.6 ± 6.5 mV; $N = 64$), action potential amplitudes of 76.3 ± 7.7 mV ($N = 52$), and total neuron input impedances of 49.7 ± 14.7 megohms ($N = 45$).

Intracellular recording from a CA1 pyramidal cell with a $KMeSO_4$-filled electrode reveals an EPSP-spike-IPSP sequence in response to suprathreshold orthodromic stimulation. After the medium is switched from control to one containing bicuculline methiodide or picrotoxin ($5 \times 10^{-5}M$ to $5 \times 10^{-4}M$), the same stimulus elicits a burst potential followed by a late, slow hyperpolarization. Because this hyperpolarization is

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present in concentrations of GABA antagonists which block responses to iontophoretically applied GABA (Fig. 1A), we can eliminate the possibility that the residual hyperpolarization is due to an antagonist-resistant form of GABA response. We refer to this post-burst hyperpolarization as an AHP resulting from synaptic stimulation (AHP_s) to distinguish it from both an IPSP and the AHP resulting from depolarizing current injection (AHP_d). The AHP_s has an amplitude of 8.6 ± 3.4 mV and lasts 2.9 ± 1.2 seconds (N = 16). It is associated with a conductance increase of 36 ± 12 percent (N = 11) and is inhibitory, blocking either spontaneous or directly evoked action potentials (Fig. 1B). The AHP_s can occur in an all-or-none fashion following a burst (Fig. 1C) (*13*). This behavior is quite unlike that of synaptic potentials, such as IPSP's, whose amplitudes are continuously graded in proportion to stimulus intensity (Fig. 1, C and D).

In mammalian central nervous system neurons, IPSP's are Cl^--dependent. In hippocampal pyramidal cells, IPSP's can easily be reversed from hyperpolarizing to depolarizing potentials by allowing Cl^- to diffuse into the cells from a 3*M* KCl-filled pipette (*14, 15*). If the AHP_s were a Cl^--dependent potential, evoking it in Cl^--filled cells would be impossible (*16*). However, the AHP_s can still be produced in these conditions after switching to a perfusate with a GABA antagonist (Fig. 2A).

In many cells (*17*), including hippocampal pyramidal cells (*8*), a calcium-dependent potassium current [$I_{K(Ca)}$] is postulated to occur after an intracellular depolarizing current pulse produces a train of action potentials. The potential caused by this $I_{K(Ca)}$ is an AHP_d. In our experiments a 100-msec depolarizing current pulse of 0.5 to 1.0 nA, causing a deflection of 10 to 20 mV and a train of four to eight action potentials, is followed by an AHP_d of 6.6 ± 3.0 mV lasting 3.1 ± 1.3 seconds (N = 13). The AHP_d is resistant to tetrodotoxin but can be blocked by Co^{2+}, Mn^{2+}, and Ba^{2+} (*8, 18*). It is associated with a weak conductance increase. If a similar calcium-dependent potassium potential is involved in the synaptically evoked AHP_s, the AHP_s should be susceptible to blockade by calcium antagonists, but, because the AHP_s follows a synaptically initiated burst, it is not possible to test this hypothesis by using calcium antagonists that block synaptic transmission. However, while Ba^{2+} passes through Ca^{2+} channels (*19*) and antagonizes $I_{K(Ca)}$ (*20*), it does not block IPSP's (*8, 21*). In 2 m*M* Ba^{2+} and 0.2 m*M* calcium, pyramidal cells depolarize slightly and fire spontaneously, and the burst is greatly prolonged (Fig. 2B). Both the AHP_s and AHP_d are substantially and similarly reduced (approximately 60 percent reduction from control amplitudes; N = 5), emphasizing their fundamental similarity and suggesting their dependence on calcium.

There has been little direct evidence for the involvement of potassium in either the AHP_s or AHP_d in the hippocampus. We obtained such evidence by determining the reversal potentials of the AHP_s and AHP_d and demonstrating the dependence of the reversal potentials on the concentration of extracellular potassium, $[K^+]_o$. In 13 cells we obtained clear reversals of at least one of these potentials (Fig. 2C). The potentials reversed at levels that are not significantly different: for AHP_d, −87.3 ± 5.4 mV (N = 11), and for AHP_s, −84.7 ± 6.4 mV (N = 6) at normal $[K^+]_o$ (5.4 m*M*) (*22*); the combined mean is −86.4 ± 5.4 mV. In three additional cells whose Cl^- gradient had been inverted by Cl^- diffusion from a KCl-filled pipette or by perfusion with low extracellular Cl^-, the AHP_s reversed at −86.7 ± 5.0 mV, confirming the previous suggestion that Cl^- is not involved in these potentials (Fig. 2A) (*14*). In 13 other cells the AHP's could be nullified at levels from −76 to −94 mV, but not convincingly reversed. These values are all in the range expected for the equilibrium potential for potassium. Therefore, we evaluated the effects of isosmotically altered $[K^+]_o$ on the reversal potentials of the AHP_d and AHP_s (Fig. 2D). The slope of the least-squares regression line relating reversal potentials to $[K^+]_o$ is −50 mV, similar to the Nernst equation prediction of −60 mV for a purely potassium-dependent potential at 30°C. This close agreement supports the interpretation that both AHP_s and AHP_d are due to potassium currents.

Our experiments indicate that the AHP that follows a burst is not an IPSP but rather an intrinsic, calcium-dependent potassium potential. Unlike IPSP's

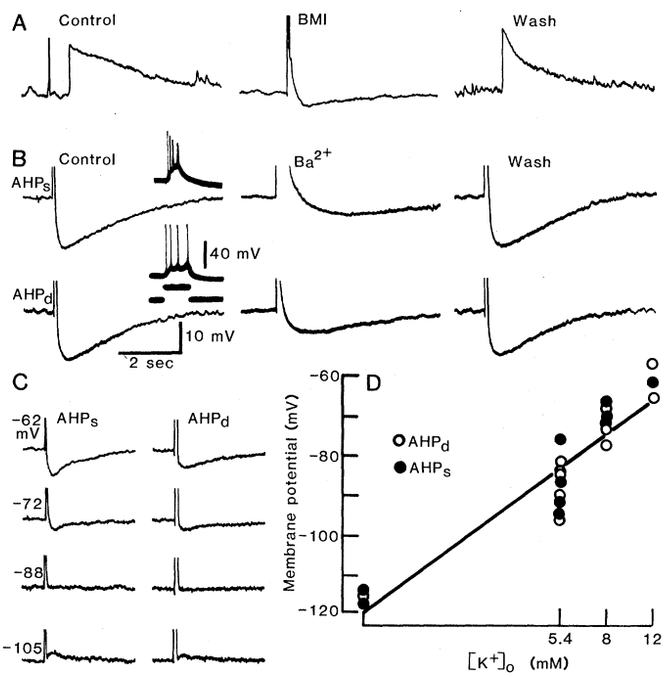

Fig. 2. Ionic mechanism of the AHP_s. (A) A CA1 pyramidal cell recorded with a KCl-filled pipette. In the presence of 10^{-4}*M* pentobarbital, orthodromic stimulation produces a pronounced depolarizing IPSP (control). After the medium is changed to one containing BMI, the response is reversibly replaced by a burst of action potentials and an AHP_s; resting potential, −55 mV. (B) Comparison of the effects of 2 m*M* Ba^{2+} on the AHP_s and AHP_d in a single cell. In the presence of BMI (*Control*), both orthodromic (top) and direct (bottom) stimuli produce large AHP's. Twenty-six minutes after the medium is changed to one containing 2 m*M* Ba^{2+} and 0.2 m*M* calcium, the cell has depolarized 15 mV and begun to fire spontaneously. Both AHP's are depressed at the new depolarized resting level, but, in order to compare their amplitudes in Ba^{2+} with control amplitudes, we hyperpolarized the cell to the original resting potential (*Ba^{2+}*). Barium effects are reversible (*Wash*). The current pulse producing the AHP_d is 0.65 nA and lasts 100 msec; control resting potential is −61 mV. (C) Reversal potentials of AHP_s and AHP_d determined in the presence of 10^{-4}*M* picrotoxin. The membrane is hyperpolarized to the indicated levels by passing current through a bridge circuit; resting potential, −62 mV. (D) Effects of extracellular potassium on reversal potentials of AHP_s and AHP_d determined for six cells. Reversal potentials were obtained for at least two levels of $[K^+]_o$ in every cell. The least-squares regression line has a slope of −50 mV per decade change in $[K^+]_o$. The deviation from the slope expected from the Nernst equation (−60 mV) is probably due to the values obtained in 1 m*M* K^+, predicted to be higher than were observed. This may be due to incomplete equilibration of 1 m*M* K^+ in the extracellular space or to a slight contribution by another ion with a relatively less polarized equilibrium potential.

in mammalian central nervous system neurons, the AHP_s can be all-or-none, is independent of Cl^-, and is reduced by barium. It has a very negative reversal potential that is similar to that of the directly activated, calcium-mediated AHP_d. Both AHP's are sensitive to changes in $[K^+]_o$. We conclude that the AHP_s is produced by the influx of calcium which underlies the burst potential in these cells (*23*). Increased intracellular calcium results in activation of a potassium conductance, allowing potassium to leave the cell and hyperpolarize it. The conductance increase and the hyperpolarization act to inhibit the cell.

If the burst and AHP_s adequately model the interictal state (*24*), then characterization of the AHP_s is relevant to understanding the transition between interictal and ictal epileptic states in emphasizing conditions that would depress the AHP_s, such as the rise in $[K^+]_o$ and the decrease in $[Ca^{2+}]_o$ associated with neuronal activity (*25*). As previously suggested (*6, 7*), attenuation of synaptic inhibition allows a burst (*2*) to be expressed in response to excitatory inputs. A burst is followed by an AHP_s, a nonsynaptic inhibitory potential that functions initially to prevent the development of epileptic discharges. Increased neuronal activity during and after bursts would elevate $[K^+]_o$ and depress $[Ca^{2+}]_o$, both actions tending to decrease the inhibitory effectiveness of the AHP_s. When a point is reached at which the remaining calcium-dependent potassium potential is inadequate to block runaway prolongation of a burst, a seizure results. Our data are in accord with this hypothesis.

In experiments performed after this report was originally submitted, we recorded from 16 CA1 cells with electrodes filled with 0.2*M* EGTA plus 2*M* $KMeSO_4$. We confirm the observation of Schwartzkroin and Stafstrom (*26*) that the current-evoked AHP_d is blocked by this treatment. The AHP_s following evoked bursts, which may be analogous to their penicillin-induced bursts, is also altered in CA1 cells. The primary effect of EGTA is a marked shortening of the duration of the AHP_s. This effect is evident in comparisons made two ways. (i) There is a difference between the AHP_s of cells held for at least 5 minutes each with $KMeSO_4$ electrodes (half-time of decay of AHP_s is 0.85 ± 0.36 second; $N = 19$) and those recorded with EGTA electrodes (0.33 ± 0.45 second; $N = 16$). A *t*-test shows that this difference is significant at $P < .001$ and holds even when cells from the same slice are compared. (ii) We examined the effects of EGTA in nine cells by comparing the half-time of decay of the AHP_s produced within 1 minute of entering the cell (0.66 ± 0.31 second) and after recording from the cell for at least 5 minutes (0.32 ± 0.08 second). The difference is again significant at $P < .001$. In nine cells recorded with $KMeSO_4$ electrodes, the same measuring procedure revealed no shortening of the AHP_s. Our findings with EGTA support our suggestion that the AHP_s in CA1 is a calcium-dependent potassium potential. Thus the differences between our results and those of Schwartzkroin and Stafstrom in CA3 neurons are probably due to differences (for example, size) between the cell types themselves. In preliminary experiments with cells from the CA2 and CA3 region, we have not seen marked effects of EGTA on the AHP_s.

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9. It was recently reported that penicillin blocks hippocampal IPSP's [(*2, 6*); R. Dingledine and L. Gjerstad, *Brain Res.* **168**, 205 (1979)] and hence may itself be a GABA antagonist [R. A Davidoff, *ibid.* **36**, 218 (1972); D. R. Curtis C. J. A. Game, G. A. R. Johnston, R. M. McCulloch, R. M. MacLachlan, *ibid.* **43**, 242 (1972)], although this has not yet been demonstrated in the hippocampus.
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12. The normal perfusion medium consisted of 116.4 m*M* NaCl, 5.4 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.3 m*M* $MgSO_4$, 26.2 m*M* $NaHCO_3$, 0.92 m*M* Na_2PO_4, and 11 m*M* glucose. When $CoCl_2$ or $BaCl_2$ was present, $MgCl_2$ replaced $MgSO_4$ and Na_2PO_4 was omitted to avoid precipitating insoluble salts. Responsiveness was not altered. Pentobarbital ($10^{-4}M$) was added to the bathing medium in some experiments to enhance IPSP's and to reveal the action of a feedforward inhibitory system recently described in hippocampus (*14*). Since pentobarbital enhances synaptic inhibition, this drug provides a "worst-case" test for a nonsynaptic form of inhibition. We have observed all of the phenomena described here in the absence of pentobarbital, however. Lower than normal temperatures are necessary to prolong viability of the slice when it is submerged [J. A. Harvey, C. N. Scholfield, D. A. Brown, *Brain Res.* **76**, 235 (1974); C. N. Scholfield, *J. Physiol. (London)* **275**, 535 (1978)]. Potentials similar to the ones studied here occur (*6*) in hippocampal slices maintained in a different experimental configuration at 37°C, so temperature is unlikely to be a complicating factor.
13. In many cells fine increments (0.1 V) in stimulus strength near the threshold produced partial responses. However, the AHP_s size was invariably correlated with the size of the preceding depolarization, and AHP_s's became maximal with increases in stimulus strength in an abrupt fashion compared to synaptic potentials.
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16. When recording from a pyramidal cell with a KCl-filled electrode even for long periods of time (more than 1 hour) in normal medium, there typically remains a late, slow AHP in response to synaptic stimulation. It might be objected that this apparent AHP is a dendritic IPSP whose action is not reversed because the cell is incompletely filled with Cl^-. However, when IPSP's are potentiated with pentobarbital, a large, purely depolarizing response quickly appears (Fig. 2A). This indicates that the Cl^- gradient is reversed throughout the cell and suggests that the late hyperpolarization seen with KCl electrodes in normal saline is a calcium-dependent potassium potential [compare C. Yamamoto, *Exp. Neurol.* **35**, 154 (1972)].
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21. Although Ba^{2+} is a poor calcium substitute for "synchronous" evoked release [R. Miledi, *Nature (London)* **212**, 1233 (1966); E. M. Silinksy, *J. Physiol. (London)* **274**, 157 (1978)], evoked release may be preserved when calcium is completely washed from the tissue [E. M. McLachlan, *ibid.* **267**, 497 (1977); F. J. Alvarez-Leefmans, A. DeSantis, R. Miledi, *ibid.* **294**, 387 (1979)]. Barium does cause a marked prolongation in the duration of the preceding burst (Fig. 2B) and can directly release intracellular calcium [R. Meech and R. Thomas, *ibid.* **298**, 111 (1980)]. Since either of these effects would increase intracellular calcium concentrations, antagonism of the AHP_s by barium is especially significant.
22. While the AHP_s and AHP_d are very similar (Fig. 2C), we have noted subtle differences. For example, the former may reverse at a slightly less polarized level in a given cell than the latter. In four cells in which reversal potentials were determined for both AHP_s and AHP_d, the mean difference was 3.0 ± 1.8 mV. This may be explained by assuming that an inward calcium current continues to flow during the initial part of the AHP_s and that the AHP_s more efficiently activates dendritic as well as somatic calcium channels.
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24. It has recently been demonstrated that large inward currents underlying a burst can be reversed under voltage clamp conditions in these cells (D. Johnston and T. H. Brown, *Science*, in press; personal communication). There is thus the possibility that a component of the true epileptic interictal state is a "giant" excitatory postsynaptic potential (EPSP) rather than an all-or-none burst (*3*). However, even in this instance it may be that there is a calcium influx that could mediate an AHP_s, consistent with our hypothesis. A graded calcium entry would account for the partial responses we have seen (*13*).
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27. Supported by PHS grant GM-23478, NSF postdoctoral fellowship SM-177-12315, and NIH postdoctoral fellowship 9 F32 NS05744-02 (B.E.A.) and research career development award NS-00287 (R.A.N.).

31 March 1980; revised 21 August 1980