The Journal of Neuroscience, October 1992, 12(10): 4122–4132

Postsynaptic Spike Firing Reduces Synaptic $GABA_A$ Responses in Hippocampal Pyramidal Cells

T. A. Pitler and B. E. Alger

Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

Using intracellular recording techniques in CA1 cells in the hippocampal slice, we studied the responses of cells to synaptically released and iontophoretically applied GABA. With high-resistance, Cl^--filled electrodes, which inverted and enlarged the responses at normal resting potentials, we examined spontaneous GABA-mediated IPSPs. Usually we recorded the spontaneous events in the presence of carbachol (10–25 μM), which significantly increased IPSP frequency and blocked potentially confounding K^+ conductances. Following a train of action potentials, spontaneous IPSPs were transiently suppressed. This suppression could not be accounted for by membrane conductance changes following the train or activation of a recurrent circuit. Whole-cell voltage-clamp recordings in the slice indicated that the amplitudes of the spontaneous $GABA_A$ inhibitory postsynaptic currents (IPSCs) were also diminished following the action potential train. In some cases BAY K 8644, a Ca^{2+} channel agonist, enhanced the suppression of IPSPs, while buffering changes in $[Ca^{2+}]_i$ with EGTA or BAPTA prevented it. The monosynaptically evoked IPSC in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and *dl*-2-amino-5-phosphonovaleric acid (APN) was also diminished following a train of action potentials; however, iontophoretically applied GABA responses did not change significantly. These studies suggest that localized physiological changes in postsynaptic $[Ca^{2+}]_i$ potently modulate synaptic $GABA_A$ inputs and that this modulation may be an important regulatory mechanism in mammalian brain.

GABA is a major inhibitory neurotransmitter in the mammalian CNS. In the hippocampus, GABAergic neurotransmission is largely carried out by feedback and feedforward local circuit interneurons. A recurrent GABAergic circuit synapses on the pyramidal cell somata and mediates a simple monophasic IPSP. A feedforward GABAergic circuit probably involves at least two other neurons activated by excitatory afferent fibers and produces a complex IPSP with both early and late phases (Alger and Nicoll, 1982; Sivilotti and Nistri, 1991). Two general classes of GABA receptors, $GABA_A$ and $GABA_B$, mediate these phases. In this article, we will be primarily concerned with the $GABA_A$ response, which mediates both the simple monophasic IPSP of the recurrent circuit and the early phase of the complex IPSP generated by feedforward activation. $GABA_A$ responses are Cl^- dependent and sensitive to bicuculline and picrotoxin (Kandel et al., 1961; Eccles et al., 1977; Segal and Barker, 1984; Gray and Johnston, 1985).

There is a growing body of evidence for the regulation of $GABA_A$ conductance by the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) (Inoue et al., 1986; Taleb et al., 1987; Chen et al., 1990; Llano et al., 1991; Marchenko, 1991). The affinity of the $GABA_A$ receptor for GABA decreases with increasing $[Ca^{2+}]_i$ (Inoue et al., 1986; Marchenko, 1991). Chen et al. (1990), using an intracellular perfusion technique in acutely isolated cells, demonstrated that elevated $[Ca^{2+}]_i$ depressed iontophoretic GABA responses, and intracellular constituents that enhanced dephosphorylation depressed GABA responses (but cf. Marchenko, 1991). The conclusion of these studies was that $GABA_A$ receptor function was jointly controlled by an interplay between Ca^{2+} and phosphorylation–dephosphorylation mechanisms. Llano et al. (1991) found that Ca^{2+} entry induced by 100 msec depolarizing voltage steps increased responses to exogenous GABA but reduced synaptic $GABA_A$ responses. However, there is little evidence that increases in $[Ca^{2+}]_i$ can regulate $GABA_A$ responses under more physiological conditions.

We have undertaken the present study to examine whether elevations of $[Ca^{2+}]_i$ induced by trains of action potentials could affect $GABA_A$ responsiveness. Our results indicate that synaptic $GABA_A$ responses are very sensitive to changes in $[Ca^{2+}]_i$ and that this may represent an important disinhibitory mechanism in hippocampal neurons.

A preliminary report of these results has been published previously (Pitler and Alger, 1990).

Materials and Methods

Male Sprague–Dawley rats (200–300 gm) were deeply anesthetized with ether or halothane and decapitated. The brain was removed rapidly and the hippocampus dissected free. Hippocampal slices, 400 μm thick, were cut on a tissue chopper for conventional sharp electrode recordings or on a vibratome for patch-slice recordings. Slices were kept in a holding chamber at room temperature at the interface of physiological medium and a humidified 95% O_2, 5% CO_2 atmosphere for >1 hr until needed. In the recording chamber, slices were held submerged between two nylon nets and perfused with warm (31°C) bubbled (95% O_2, 5% CO_2) saline comprised of (in mM) NaCl, 124; KCl, 3.5; NaH_2PO_4, 1.25; $NaHCO_3$, 26; $CaCl_2$, 2.5; $MgCl_2$, 3.5; and glucose, 10.

Pyramidal cells from the CA1 region of thick hippocampal slices were recorded from using both conventional intracellular and whole-cell patch recording techniques (Blanton et al., 1989; Otis et al., 1991). For conventional recordings, we used 50–120 MΩ electrodes filled with 3 M KCl (unless otherwise noted). Whole-cell recordings were obtained with 2–6 MΩ electrodes filled with 130–150 mM KCl, KCH_3SO_3, or $CsCH_3SO_3$, 1–5 mM Mg-ATP, 10 mM HEPES (pH 7.3), and a Ca^{2+} buffering system (EGTA or BAPTA plus $CaCl_2$) that was varied according to the experiment. To obtain low levels of free Ca^{2+} buffering, we used either 100 μM EGTA plus 50 μM added $CaCl_2$, 350 μM EGTA

Received Feb. 13, 1992; revised Apr. 15, 1992; accepted May 27, 1992.

This work was supported by U.S. Public Health Service Grants NS27968 (T.A.P.) and NS22010 (B.E.A.) and an award from the UMAB Directed Research Initiative Fund.

Correspondence should be addressed to Bradley E. Alger, Ph.D., Department of Physiology, University of Maryland School of Medicine, 655 West Baltimore Street, Baltimore, MD 21201.

with no added $CaCl_2$, or 2 mM BAPTA plus 0.2 mM $CaCl_2$. To preserve Ca^{2+}-dependent processes when recording in the whole-cell mode, it was necessary to reach a compromise between a buffering system weak enough to allow $[Ca^{2+}]$ to fluctuate and induce Ca^{2+}-dependent phenomena and a system strong enough to prevent rapid "sealing up" of the patch with a consequent increase in series resistance. In these cases, "low-Ca^{2+} buffering" capacity was assessed by the presence of a Ca^{2+}-dependent afterhyperpolarization (AHP) following a burst of action potentials elicited by a 200 msec depolarizing current pulse. "High-Ca^{2+} buffering" capacity was obtained by using 10 mM BAPTA plus 1 mM $CaCl_2$ in the recording electrode solution. In cells recorded under these conditions, it was generally impossible to elicit Ca^{2+}-dependent AHPs.

For the majority of experiments, Cl^- was the predominant anion in the recording electrode to facilitate the observance of $GABA_A$-mediated spontaneous events. Diffusion of Cl^- from the recording electrode reversed the Cl^- gradient for the cell, causing spontaneous Cl^--dependent events to be large (1–10 mV, 20–350 pA) and depolarizing at normal resting potentials.

Action potentials were elicited by 20 Hz trains of depolarizing constant current pulses 20 msec in duration. The amplitude was adjusted to trigger a single action potential per current pulse. Duration of the train ranged from 0.1 to 5 sec (2–100 action potentials). When examining responses under voltage clamp, we used a hybrid clamp configuration in which the amplifier was switched into current-clamp mode in order to elicit the train of action potentials and then electronically switched back into voltage-clamp mode at the end of the train (Lancaster and Adams, 1986). For stimulation of inhibitory synaptic pathways, bipolar stimulating electrodes were made from insulated stainless steel and placed within 400 μm of the cell under study. The slice was bathed in medium containing 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 20 μM *dl*-2-amino-5-phosphonovaleric acid (APV) to block excitatory synaptic transmission in these cases.

For iontophoretic application of GABA, 4–20 MΩ pipettes were filled with 1 M GABA at pH 5–5.5. A backing current of 20 pA was always on. GABA was ejected by 100–500 msec pulses of current ranging from 50 to 400 pA. The iontophoretic electrode was positioned close to the cell soma to produce a rapid effect with relatively low currents.

An Axoclamp-2 amplifier was used for all experiments. Data were stored on a VCR-based tape recorder system (Neurocorder) and played back on a rectilinear chart recorder (Gould) or into a microcomputer-based analysis program (pCLAMP, Axon Instruments) for subsequent analysis. Data are expressed as mean ± SEM.

dl-2-amino-5-phosphonovaleric acid (APV), picrotoxin, carbamylcholine chloride (carbachol), atropine, and ethylene glycol bis(β-aminoethyl)ether-*N,N'*-tetra-acetic acid (EGTA) were obtained from Sigma Chemical Corp. (St. Louis, MO). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) was obtained from Research Biochemicals, Inc. (Natick, MA). BAPTA was received from Molecular Probes (Eugene, OR). BAY K 8644 was the gift of Miles Inc. (West Haven, CT).

Results

In cells recorded with Cl^--filled electrodes, numerous spontaneous depolarizing membrane fluctuations can be observed. These events are sensitive to the $GABA_A$ antagonists picrotoxin and bicuculline, and the vast majority of events, in particular all the larger events, are blocked by TTX. These spontaneous events are due to the spontaneous activity of GABAergic interneurons and subsequent inhibitory synaptic input to pyramidal cells (Alger and Nicoll, 1980a) [small potentials resistant to TTX presumably reflect spontaneous synaptic quantal release (Edwards et al., 1990; Ropert et al., 1990)]. Although most of the inhibitory events are the result of firing of GABAergic interneurons, we will refer to them as "spontaneous IPSP(C)s" [to indicate they are recorded in current clamp as IPSPs and in voltage clamp as inhibitory postsynaptic currents (IPSCs)] since they occur without external stimulation.

Trains of action potentials reduce spontaneous IPSPs

The primary finding of this report is that, when recorded under conditions that optimize the observance of spontaneous $GABA_A$ potentials, a train of action potentials is followed by a transient period of membrane quiescence (0.5–10 sec) and subsequent gradual recovery of spontaneous IPSPs. This effect is illustrated in Figure 1; positive voltage deflections on the baseline are spontaneous IPSPs. In the first experiment (Fig. 1*A*), a brief train of action potentials (20 action potentials) was elicited by a 20 Hz train of 20 msec depolarizing current pulses. Following the train, the membrane hyperpolarized due to the activation of a Ca^{2+}-dependent K^+ conductance (Alger and Nicoll, 1980b; Hotson and Prince, 1980), and concomitantly the spontaneous IPSPs were diminished. Gradually the membrane potential and appearance of IPSPs recovered to the levels seen prior to the train. Because this protocol involved only the activation of the postsynaptic cell, it appeared that IPSP depression was due to a postsynaptic mechanism.

Membrane conductance is increased during the AHP following a train of action potentials, and the shunt might be expected to reduce the amplitude of spontaneous IPSPs under current-clamp recording conditions. Additionally, a technical difficulty with these experiments was that, under our recording conditions, the frequency of spontaneous IPSPs was not typically as high as the cell represented in Figure 1*A* (top trace), and often it was difficult to be certain that an effect had occurred because of the low resting level of spontaneous events.

We therefore applied the muscarinic agonist carbachol (10–25 μM) to the slice for two reasons. (1) Carbachol effectively blocks the Ca^{2+}-dependent K conductance underlying the AHP in these cells, thus eliminating a confounding factor (Benardo and Prince, 1982; Cole and Nicoll, 1984; Madison et al., 1987). (2) We have recently shown that carbachol greatly increases the spontaneous excitability of interneurons. This direct effect on interneurons leads to a virtually constant barrage of large spontaneous $GABA_A$ IPSPs on pyramidal cells (Pitler and Alger, 1991). This provides a high background of synaptic GABA release, enabling us to study changes in GABA responsiveness more easily.

The lower trace in Figure 1*A* shows the same cell as above bathed in 10 μM carbachol, which resulted in a significant increase in the baseline level of spontaneous IPSPs. Following the same length train of action potentials, there was a striking reduction in spontaneous IPSPs even though the AHP in this case was completely blocked. This basic effect was observed in all cells recorded under these conditions ($n = 32$). Figure 1*B* shows another cell in carbachol with a high level of spontaneous synaptic activity, which again was significantly reduced directly following the train. In this experiment, input conductance was assessed every 2 sec by monitoring the magnitude of membrane potential deflections in response to 0.2 nA, 200 msec hyperpolarizing constant current pulses. No change in conductance was detectable following the train of action potentials. The membrane potential following the train was not completely quiet, however; there were still numerous small events that tended to merge with the membrane noise, particularly just following the train.

As a further test to determine whether changes in postsynaptic K^+ conductance contributed to the observed effect, we recorded from three cells with CsCl-filled electrodes. The Cs^+ that leaked from the electrode into the cell further attenuated K^+ currents that might be activated following a train of action potentials (Puil and Werman, 1981) and not completely blocked by carbachol. As illustrated in Figure 1*C*, the effect of the action po-

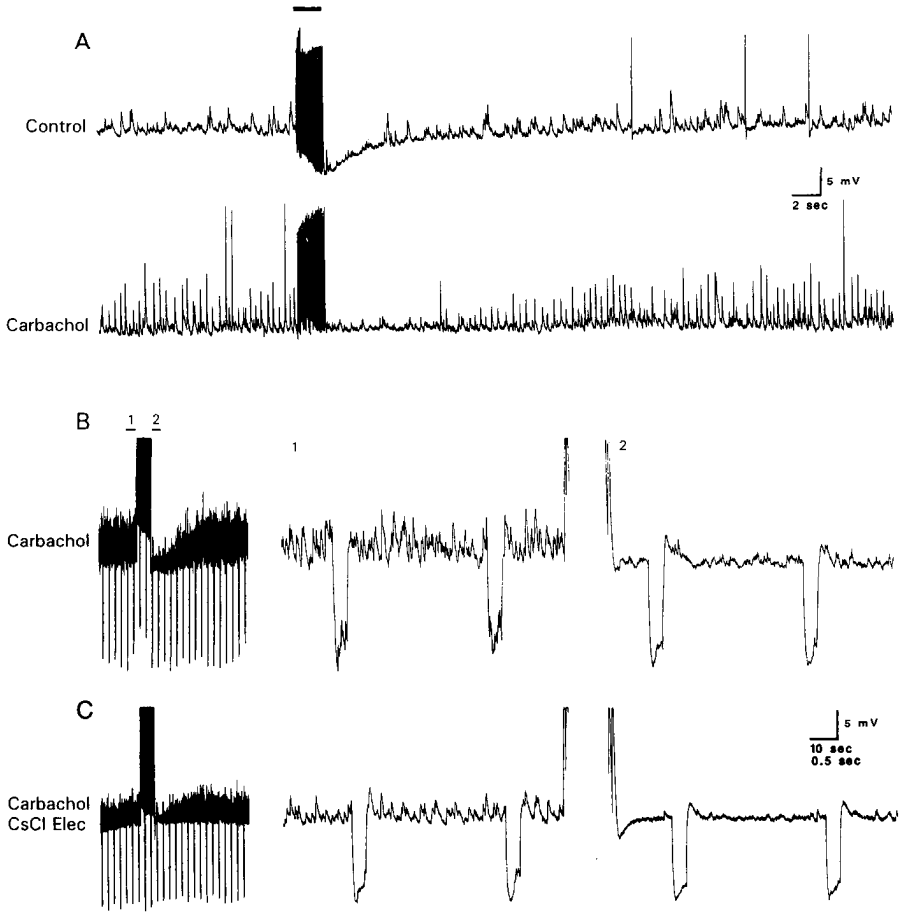

Figure 1. High-frequency trains of action potentials transiently reduce spontaneous IPSPs in hippocampal CA1 pyramidal cells. *A*, Spontaneous IPSPs appear as positive voltage deflection on the baseline when recorded with KCl-filled electrodes. A 2 sec, 20 Hz train of action potentials (*bar over trace* denotes train) elicited by repetitive 20 msec depolarizing current pulses resulted in an AHP and concomitant reduction in spontaneous IPSPs. Addition of 10 μM carbachol caused a sustained depolarization of the cell, which was repolarized to its original resting potential (−66 mV) by continuous hyperpolarizing current injection. Carbachol also caused an increase in the resting amplitude and frequency of spontaneous IPSPs, and blocked the AHP. Under these conditions there was an obvious reduction in IPSPs following the action potential train. *B*, In another cell (resting potential, −68 mV), input conductance during the experiment was assessed by 0.2 nA, 200 msec hyperpolarizing current pulses at 0.5 Hz. There was no change in conductance following the train that could account for the reduction of IPSPs. Data under *bars 1* and *2* are shown to the right at an expanded time base. *C*, This cell was recorded with an electrode containing 2 M CsCl to reduce further any outward currents following the train of action potentials (cell held at −70 mV). The reduction of IPSPs following an action potential train was similar under these conditions.

tential train on spontaneous IPSPs was similar to that observed previously, while no change in conductance was observed.

The magnitude of the effect of a train of action potentials on spontaneous IPSPs was clearly dependent upon the number of action potentials elicited. A reduction in IPSPs was often observed with very short trains (Fig. 2*A*, top trace; only five spikes), but became more pronounced and longer in duration with longer trains (Fig. 2*A*). We attempted to quantify this effect crudely as shown in Figure 2*B*. Events were counted if they exceeded 2 mV and had a characteristically fast rise time of >250 μV/msec. If the train decreased the amplitude of the IPSPs, they fell below the cutoff amplitude and an apparent decreased IPSP frequency resulted. As demonstrated by this example, longer action potential trains produced longer and more complete depression of IPSPs.

Voltage-clamp recordings show IPSC amplitude is diminished following the train

As a final test of the hypothesis that changes in passive properties of the postsynaptic cell were responsible for the synaptic GABA response decrease, we voltage clamped the cells and recorded the IPSCs. We used a "hybrid clamp" protocol in which the cell was suddenly switched to voltage clamp after a brief period in current clamp when a series of action potentials was triggered. The change in spontaneous $GABA_A$ events following a train of action potentials could also be seen under these voltage-clamp recording conditions (Fig. 3), further supporting the conclusion that the effect was not due to indirect postsynaptic conductance changes. Notice in Figure 3 that there is an absence of large events while numerous small events persist. Gradually there was a recovery of IPSCs to full amplitude.

We constructed an IPSC amplitude histogram for intervals before, immediately following, and again after a 10 sec recovery poststimulus (e.g., Fig. 4). Cells were held hyperpolarized to −80 mV in order to maximize the amplitude of IPSCs. Note that because of the difficulty of determining if small fluctuations in baseline current were IPSCs, the smallest-amplitude bin has a more narrowly restricted range and is therefore smaller than the rest. Events were chosen by their shape and amplitude (fast rise, slow decay, minimum 30 pA), as all events that met these criteria were picrotoxin sensitive. In Figure 4*A*, we demonstrate a reduction and gradual recovery of the IPSCs following a train of action potentials. The amplitude histogram in Figure 4*B* shows a shift toward smaller amplitudes of events directly following the train. The median amplitude of the events decreased in this case from between 150 and 200 pA in control to between 50 and 100 pA for the 4 sec period following the train. The apparent frequency of events is also diminished, probably because the smaller events merge with the membrane noise and cannot be counted any longer. We cannot, therefore, rule out a presynaptic contribution to the reduction in IPSCs. Following 10 sec of recovery, the number and amplitude of IPSCs returned to control levels.

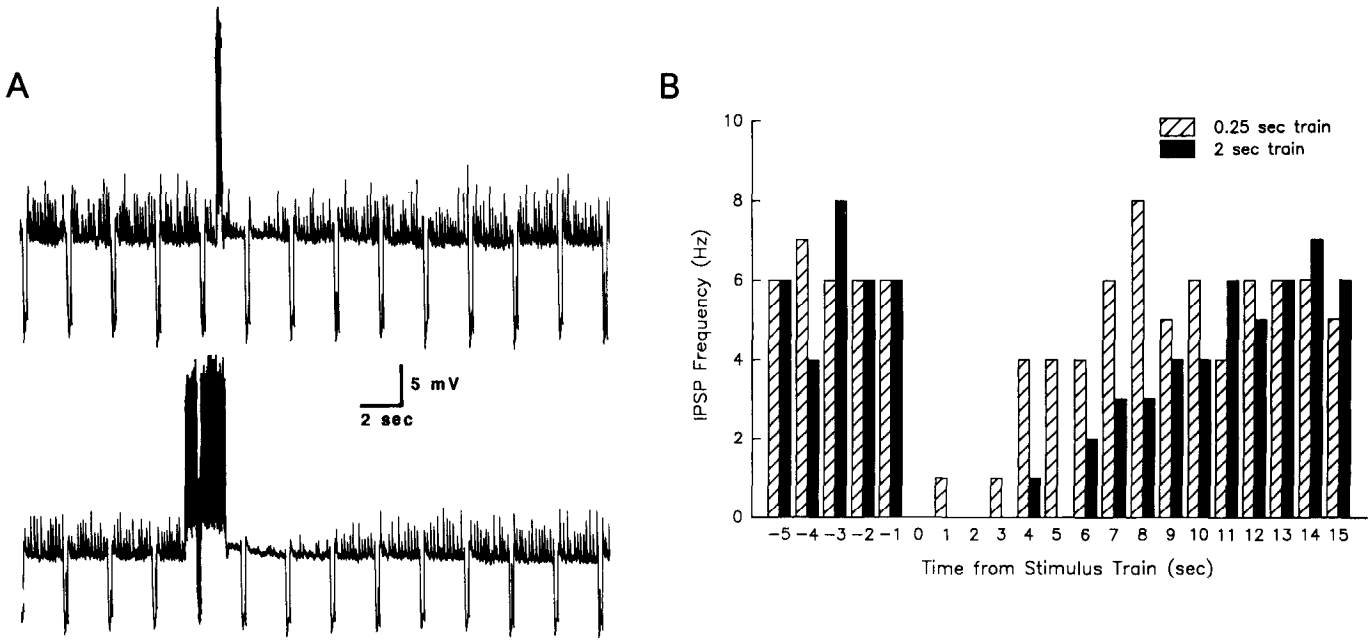

Figure 2. Duration of the action potential train affected the magnitude of spontaneous IPSP suppression. The cell was bathed in 25 μM carbachol and held at its original resting potential (−64 mV) by continuous hyperpolarizing current injection. *A*, Traces from the same cell are shown with a 0.25 sec, 20 Hz train of action potentials (*top trace*) and a 2 sec, 20 Hz train of action potentials (*bottom trace*). The longer train produced a more complete and prolonged depression of IPSPs. *B*, A frequency histogram with 1 sec bins was constructed. IPSPs were included in the analysis if they exceeded 2 mV and had rise times of >250 μV/msec.

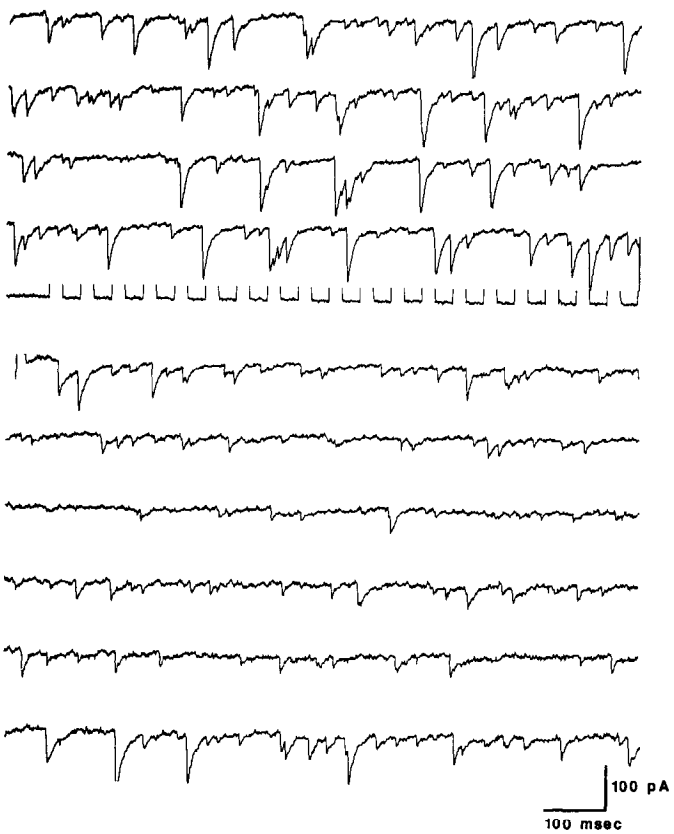

Figure 3. Spontaneous IPSCs recorded under whole-cell voltage-clamp conditions were reduced following a train of action potentials. The cell was clamped at its original resting potential before the addition of 25 μM carbachol (−63 mV). The traces are from a continuous record showing IPSCs recorded under voltage clamp and, *fifth row from the top*, the injection of depolarizing current pulses to induce a train of action potentials during the switch into current clamp. Switching back into voltage clamp showed that IPSC amplitudes were reduced following the train. Consecutive 1 sec records are shown in the *top 10 traces*; the *bottom trace* was obtained following a 22 sec recovery period from the end of the action potential train.

The reduction of IPSP(C)s is not mediated through a polysynaptically activated pathway

The dramatic alteration of IPSP(C)s caused by firing of the postsynaptic cell strongly suggests that a postsynaptic factor is responsible. However, activation of the pyramidal cell could in principle influence the firing of GABAergic interneurons through a recurrent pathway. In general, we would expect firing of pyramidal cells to increase the excitability of interneurons, but a more complex, possibly polysynaptic, pathway could depress the interneurons. To rule out this possibility, we employed the glutamate antagonists CNQX (10 μM) and APV (20 μM), which block the synaptic output of the pyramidal cell (Davies et al., 1990). As illustrated by the example in Figure 5, we saw no qualitative differences between cells bathed in normal medium and those in the presence of glutamate antagonists (n = 4).

Involvement of intracellular calcium in the suppression of IPSP(C)s

Although the magnitude of the effect of a train of action potentials on IPSP(C)s varied, we saw evidence of reductions in all cells recorded under standard conditions. Since our data were consistent with the hypothesis that voltage-dependent Ca^{2+} entry during the train of action potentials diminished the IPSPs, we attempted to buffer postsynaptic changes in $[Ca^{2+}]_i$ in order to block this effect. For high-resistance electrode recordings, 100 mM EGTA was included in the filling solution. Given sufficient

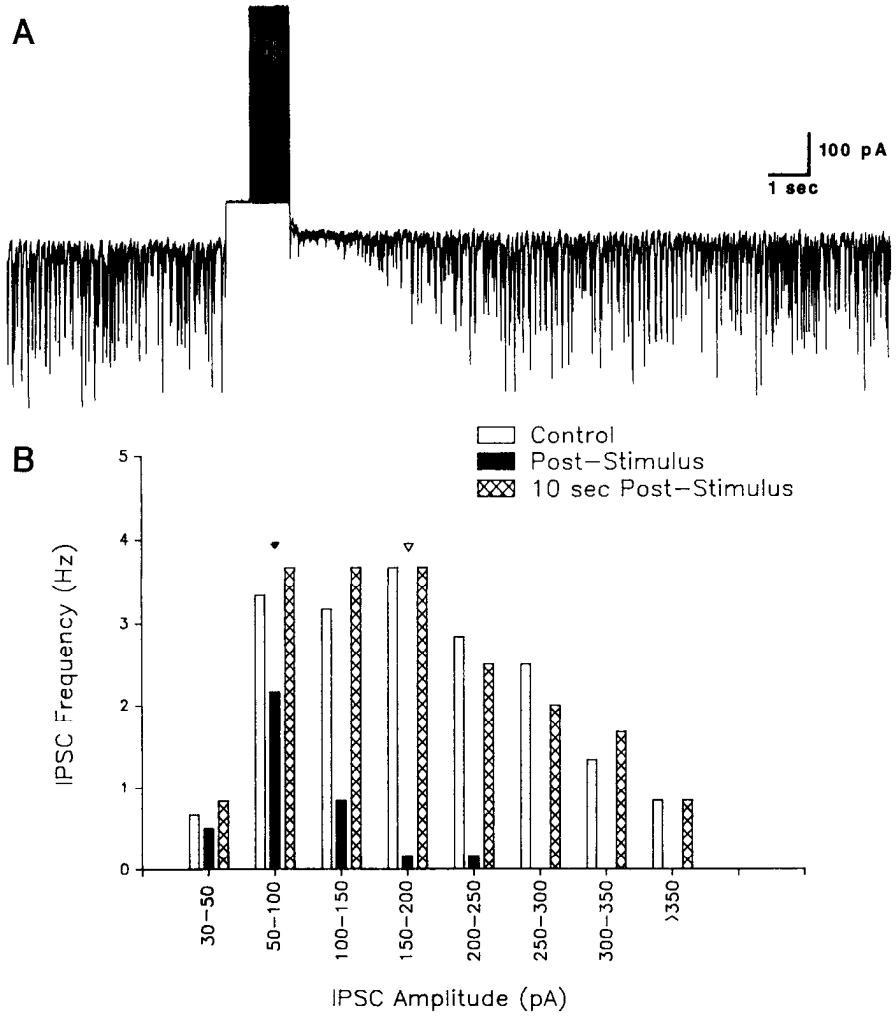

Figure 4. Current record and amplitude histogram of IPSCs before and following a train of action potentials. *A*, Current record for the cell is shown; this cell was held hyperpolarized to −80 mV to increase IPSC amplitudes. The action potential train nearly completely blocked spontaneous IPSCs for a short time; IPSCs then gradually recovered to baseline. *B*, A 4 sec interval directly following the train and 3 sec intervals preceding and 10 sec following the train were used to construct the amplitude histogram. Two replicates were used for the amplitude histogram; a slightly longer period following the train was analyzed because of the lower frequency at that time. Fewer events were counted directly following the train, and their median amplitude, shown by the position of *arrowheads* over the appropriate bin, was reduced (▼, poststimulus; ▽, control and following 10 sec recovery).

time for EGTA to leak into the cell (>15 min), the effect of a train of action potentials on spontaneous IPSPs was blocked (n = 6) (Fig. 6). Ca^{2+}-dependent AHPs and accommodation were blocked in these cells as well (Krnjević et al., 1975; Schwartzkroin and Stafstrom, 1980; Hablitz, 1981; Madison and Nicoll, 1984), demonstrating the effectiveness of EGTA in these experiments.

Whole-cell patch-clamp recording provides a more effective way to control $[Ca^{2+}]_i$ levels. The low access resistance allows rapid exchange of the electrode contents with the cell cytoplasm. There was a block of IPSP(C)s (see Figs. 3, 4, 7*A*) following the action potential train under a variety of low-Ca^{2+} buffering conditions (see Materials and Methods) using this technique (n = 10). Under high-Ca^{2+} buffering conditions, however, the suppression of IPSP(C)s by a train of action potentials was effectively prevented (n = 6). In the example shown in Figure 7*B*, both current-clamp and voltage-clamp records for the same cell are shown. A quantitative analysis of these cells is shown below. IPSCs were included in the histograms if they exceeded 50 pA and had a characteristic appearance. Under low-buffer conditions, there was a decrease in IPSCs during a 5 sec bin directly following the train, while under high-Ca^{2+} buffering conditions, there was no reduction in IPSCs directly following the train.

The results thus far are consistent with a hypothesis that a train of action potentials leads to an elevation in $[Ca^{2+}]_i$, which in turn mediates the observed effects on the spontaneous IPSPs.

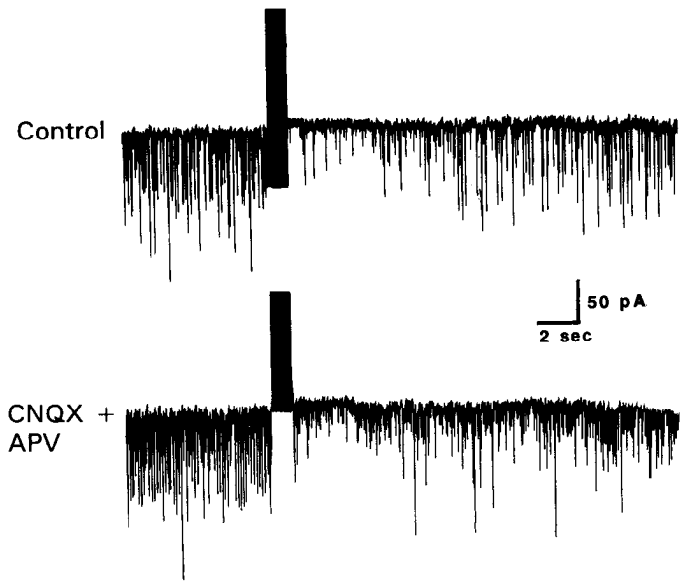

Figure 5. Inclusion of glutamate antagonists to block recurrent pathways does not prevent the suppression of IPSCs following the action potential train. The cell was voltage clamped at −63 mV throughout the experiment. The *top trace* shows the reduction of IPSCs following an action potential train. The same cell is shown in the *bottom trace* following the addition of 10 μM CNQX and 20 μM APV to the bathing medium. Qualitatively no differences were observed.

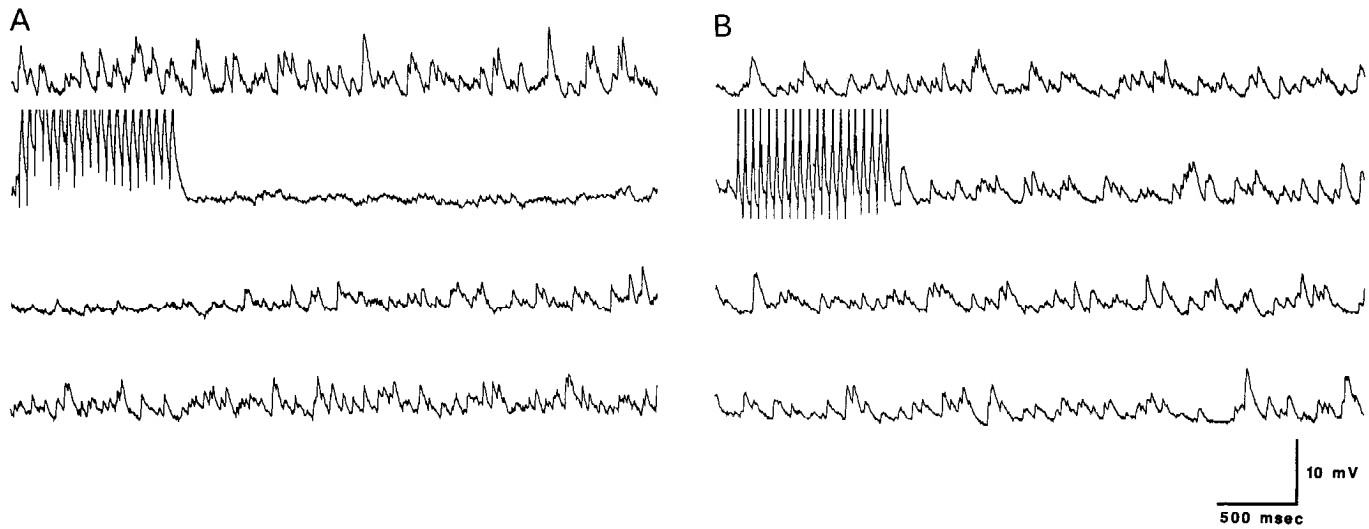

Figure 6. Continuous voltage records of IPSPs recorded with a 3 M KCl electrode (*A*; resting potential, −67 mV) and a 3 M KCl plus 100 mM EGTA electrode (*B*; resting potential, −64 mV). The *beginning of the second trace from the top* in both panels shows a train of action potentials elicited by depolarizing current pulses. In *A*, spontaneous IPSPs were reduced following the train, while in *B*, the reduction of IPSPs was blocked by the inclusion of EGTA in the recording electrode.

Unfortunately, it is difficult to modulate postsynaptic Ca^{2+} influx without affecting synaptic transmission and thereby altering the level of spontaneous IPSPs. One method of doing this is with the Ca^{2+} channel agonist BAY K 8644. BAY K 8644 increases the open time of L-type Ca^{2+} channels (Brown et al., 1984; Nowycky et al., 1985), but since L-type channels do not appear to be involved with normal synaptic transmission in the hippocampus (Jones and Heinemann, 1987; Aicardi and

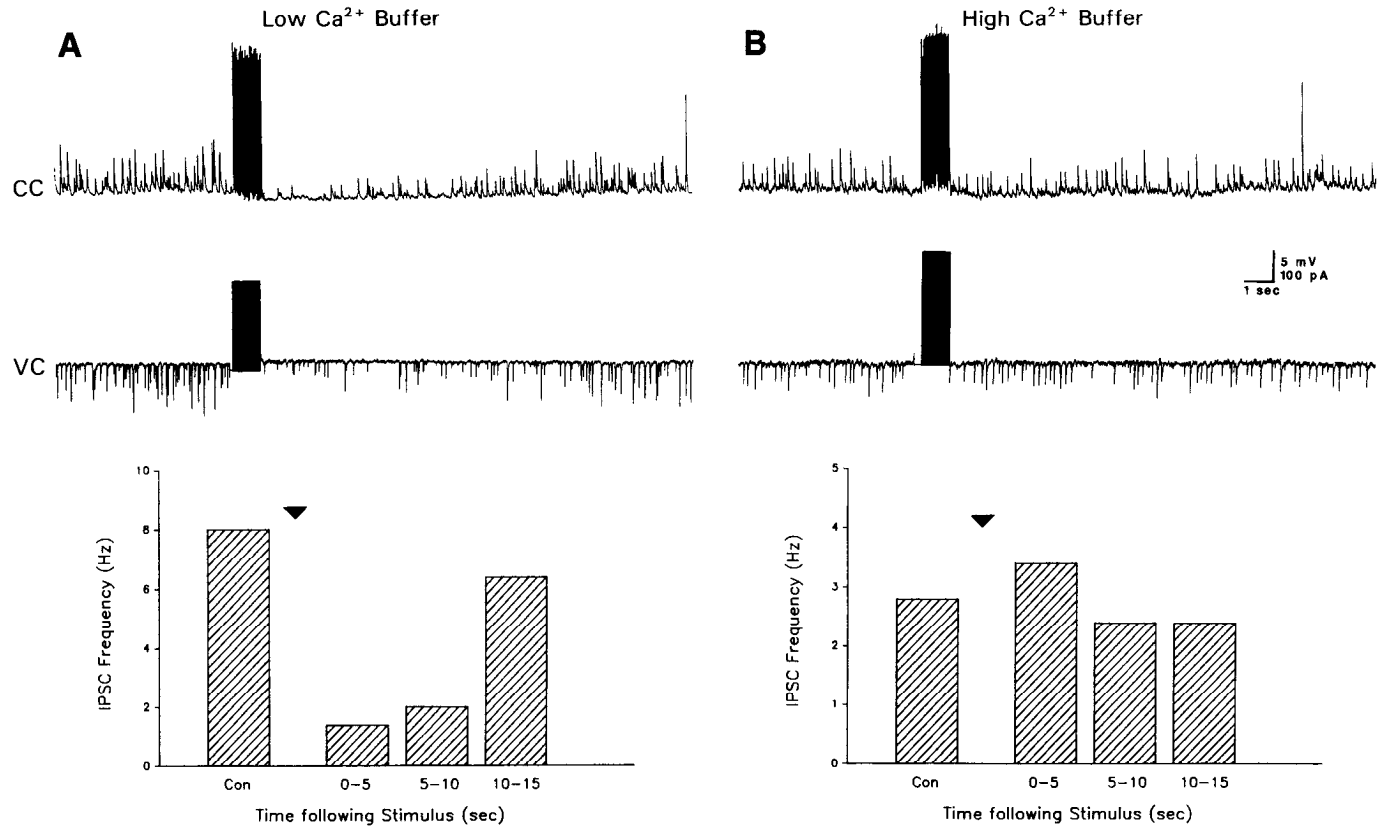

Figure 7. Whole-cell patch-clamp recordings of IPSP(C)s with electrodes containing either low-Ca^{2+} or high-Ca^{2+} buffering conditions. *A*, Current clamp (*CC, top trace*) and voltage clamp (*VC, bottom trace*) are shown for the same cell recorded with an electrode including 100 μM EGTA and 50 μM $CaCl_2$ (membrane potential, −65 mV). Spontaneous IPSP(C)s were reduced following the action potential train. *B*, Cell recorded with an electrode containing 10 mM BAPTA and 1 mM $CaCl_2$ (membrane potential, −56 mV). The reduction of spontaneous IPSP(C)s was prevented in this case. IPSC frequency histograms were constructed for the records above. Data were binned in 5 sec intervals before and following the stimulus train (*arrowheads*); all events that exceeded 50 pA were included in the analysis.

Schwartzkroin, 1990; O'Regan et al., 1990), BAY K 8644 does not overtly affect the occurrence of spontaneous IPSPs. In about half the cells we tested, BAY K 8644 increased the Ca^{2+}-dependent AHP following a burst of action potentials and increased the width of action potentials; both effects would be consistent with the reported Ca^{2+}-channel agonist properties of BAY K 8644. In four of eight cells, BAY K 8644 prolonged the depression of spontaneous IPSPs following a train of action potentials (see Fig. 8). The effect of BAY K 8644 became more pronounced with longer trains. The dihydropyridine antagonist nifedipine generally had no effect by itself but, when present in the wash solution, aided in recovery from the BAY K 8644 effect (Fig. 8). An enhanced reduction of IPSPs in BAY K 8644 was well correlated with an effect on action potential width in these cells. It was not possible to correlate a BAY K 8644 effect on the reduction of IPSPs with Ca^{2+}-dependent AHPs since the AHPs were blocked in these cells by carbachol.

Monosynaptically evoked IPSCs are diminished directly following a train of action potentials

We also examined the evoked GABAergic IPSC following action potential trains. We included CNQX and APV in the bathing medium in order to block the EPSP and prevent polysynaptic activation of GABAergic interneurons (Davies et al., 1990). Carbachol was not present in these experiments. In most cases the patch pipette contained KCH_3SO_3 rather than KCl so that $GABA_A$-mediated IPSCs would be outward and easily separated from any residual inward excitatory current. When a low-Ca^{2+} buffering solution was used, the response elicited 400 msec following a 1 sec train of action potentials was 81.6 ± 4.7% of control ($n = 5$; Fig. $9A_1$). This is as opposed to 100.0 ± 5.7% when high-Ca^{2+} buffering was used in the recording electrode ($n = 5$; Fig. $9A_2$). These values were significantly different ($p < 0.05$). If we added tetraethylammonium (TEA) to the recording medium, we often elicited Ca^{2+} spikes during the train, which were followed by unusually large outward Ca^{2+}-activated K^+ currents. In these cases ($n = 3$), the monosynaptic IPSC showed a much larger suppression than in control medium (compare Fig. $9B_1$, control, with Fig. $9B_2$, after TEA was added). It is possible that in these cases the cell was not adequately voltage clamped and the response was smaller owing to an underlying unobserved hyperpolarization. In four cells, we recorded with $CsCH_3SO_3$ electrodes. Cs in the electrode tended to cause Ca^{2+} spikes during the train, but blocked much of the outward current following the train. In these cases, the IPSC was reduced to 67.5 ± 10.7% of control (e.g., Fig. $9C_1$). High-Ca^{2+} buffering combined with $CsCH_3SO_3$ electrodes resulted in an uncontrollably large depolarization during the train and unusually large Ca^{2+}-dependent K^+ current following the train. The persistence of the outward current indicated that this buffering method was unable to clamp $[Ca^{2+}]_i$ when a massive Ca^{2+} influx was initiated. The evoked IPSC was nearly completely blocked in these cases ($n = 2$), much as it was in the case of TEA in the recording medium.

In four cells, we performed a similar experiment on evoked monosynaptic IPSPs, but with KCl electrodes so that if there were an unclamped hyperpolarization following the train, it would be expected to increase the inward $GABA_A$ current recorded with Cl^--containing electrodes, as opposed to reducing the amplitude of an outward IPSC recorded with KCH_3SO_3 electrodes. The inward IPSC in these cases decreased to 77.8 ± 11.1 of control (Fig. $9C_2$).

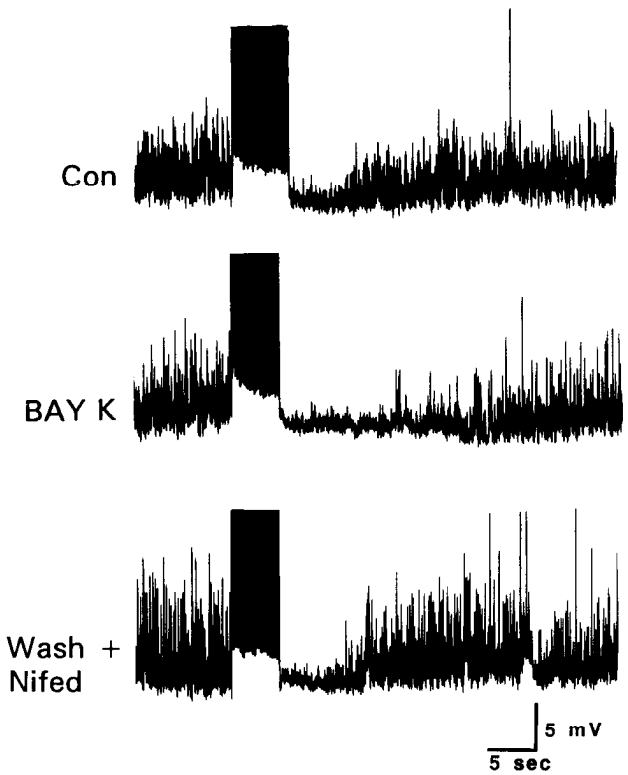

Figure 8. IPSP reduction following the action potential train was prolonged by 5 μM BAY K 8644. The cell was continually bathed in medium containing 25 μM carbachol and held at its original resting potential of −67 mV (*Con*). BAY K 8644 prolonged the suppression of IPSPs following the 5 sec, 20 Hz train of action potentials. Addition of 5 μM nifedipine while washing out BAY K 8644 reversed this effect.

Voltage-clamped responses to iontophoretic GABA application were not reduced by trains of action potentials

In order to determine whether the depression of synaptic $GABA_A$ responses was paralleled by a depression of exogenously activated GABA responses, we iontophoresed GABA onto the cell. We used primarily whole-cell recording techniques with electrodes containing KCl and low-Ca^{2+} buffering, although KCH_3SO_3-filled pipettes were used in three cases. In these experiments, no change or a small reduction with little recovery was seen in the iontophoretic response following a train of action potentials (Fig. 10). To ensure that the Ca^{2+} buffering in our whole-cell electrodes, although low, did not interfere with our ability to produce an observable effect, we repeated this experiment in five cells using high-resistance single-electrode voltage-clamp techniques. Electrodes of 50–90 MΩ were filled with either 3 M KCl or 2 M CsCl. Switching frequencies ranged from 3 to 5 kHz. The headstage output was continually monitored to ensure adequate settling time for the microelectrode. We found no difference between results obtained with switched single-electrode voltage clamp and whole-cell voltage clamp. Possible explanations for the lack of a reduced iontophoretic $GABA_A$ response following an action potential train are discussed below.

Discussion

Evidence is accumulating from studies on a variety of cell types that increases in $[Ca^{2+}]_i$ depress $GABA_A$ function (Inoue et al., 1986, 1987; Taleb et al., 1987; Chen et al., 1990; Maruyama et

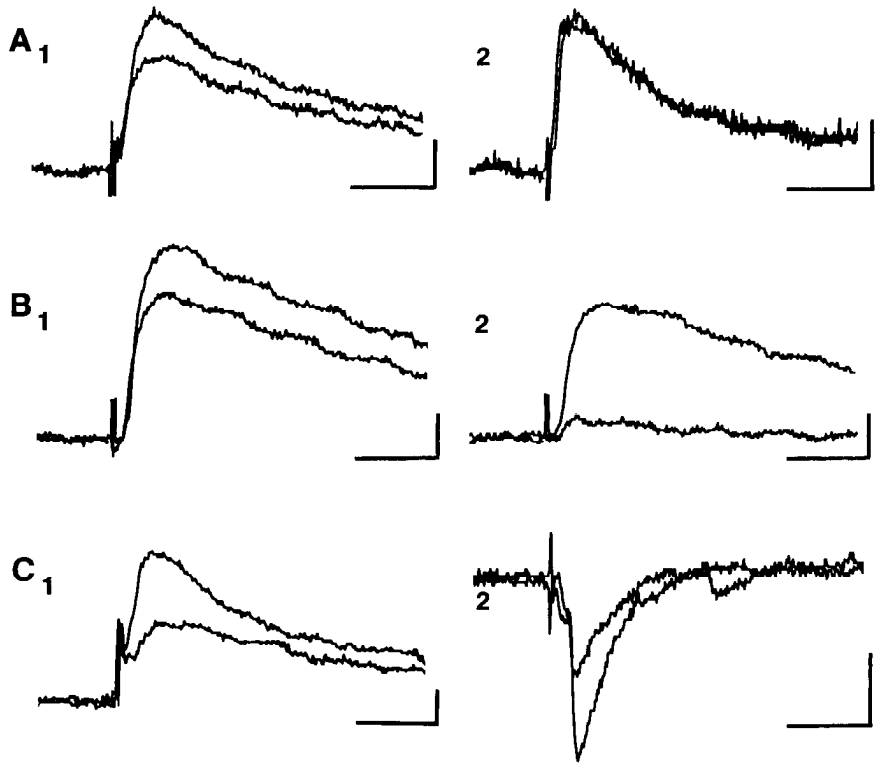

Figure 9. Evoked monosynaptic IPSCs are reduced following an action potential train. The cell was bathed in medium containing 10 μM CNQX and 20 μM APV, and stimulating electrodes were placed within 400 μm of the cell in order to evoke a monosynaptic IPSC. A_1, Cell was recorded with a KCH_3SO_3-filled whole-cell patch-clamp electrode containing 2 mM BAPTA and 0.2 mM $CaCl_2$. Traces preceding and 400 msec following a 1 sec, 20 Hz train of action are superimposed; the *larger trace* is the control trace. Outward currents following the train were subtracted in this and subsequent traces. A_2, This record is similar to that in A_1 except that high-Ca^{2+} buffering was employed in the recording electrode (10 mM BAPTA, 1 mM $CaCl_2$) for this cell. No change in the evoked IPSC following the action potential train was observed. *B*, Superimposed records showing the reduction of the evoked IPSC under typical recording conditions (*1*) following application of 5 mM TEA to the same cell (*2*). C_1, Cell recorded with a CsCl-filled whole-cell electrode in order to block K^+ currents. A similar reduction of the evoked IPSC following the action potential train was found in these cells. C_2, Cell recorded with a KCl-filled whole-cell recording electrode also showed a reduction of the evoked IPSC. A large unclamped outward current could not explain this result. Calibration (all traces), 100 pA, 400 msec.

al., 1990; Marchenko, 1991); however, there has been very little confirmation that physiological activation of cells can suppress synaptic $GABA_A$ responses. Our data demonstrate that a Ca-dependent process, initiated by a train of action potentials, does so, thus supporting the hypothesis of a physiological role for this effect. $GABA_A$ response reduction facilitates the development of long-term potentiation (Wigström and Gustafsson, 1983), kindling (Stelzer et al., 1987), and the epileptic burst discharge (Wong and Prince, 1979). The results of this article provide evidence that physiological increases in $[Ca^{2+}]_i$, apparently by voltage-dependent Ca^{2+} influx, can reduce synaptic $GABA_A$ responses in hippocampal CA1 pyramidal cells.

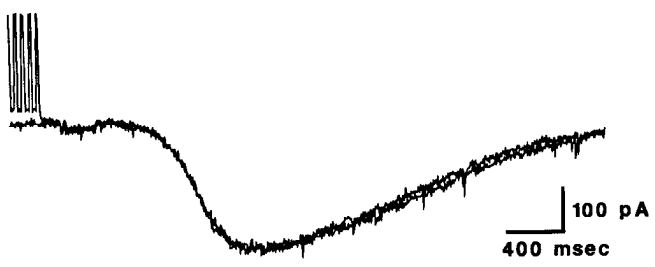

Figure 10. Voltage-clamped responses to iontophoretic GABA application were not reduced following trains of action potentials. An iontophoretic GABA response was monitored from a cell recorded with a KCl-filled whole-cell electrode. The response closely following a 2 sec train of action potentials is superimposed upon a control response obtained before the action potential train. No consistent change was found for iontophoretic responses following action potential trains.

CA1 cells were recorded with Cl^--filled electrodes in the presence of carbachol to produce a continuous barrage of spontaneous depolarizing IPSP(C)s (Pitler and Alger, 1991). In these cells, a train of action potentials consistently produced a transient reduction in spontaneous IPSPs. The results cannot be attributed to indirect effects of a postsynaptic conductance change following a train of action potentials. The increase in conductance following a train of action potentials was nearly completely blocked by carbachol or by CsCl-filled electrodes. Voltage-clamp recordings of synaptic currents will not be affected by changes in postsynaptic membrane properties, and these also showed reductions in IPSCs following trains of action potentials. Inadequate voltage-clamp control cannot explain these results since the unclamped membrane would be hyperpolarized following the train and hyperpolarization would only increase the amplitude of inward spontaneous IPSCs as opposed to the decrease that was observed.

While carbachol was used to enhance the frequency of IPSPs

in most experiments, we did see clear examples of a reduction in spontaneous IPSPs without addition of carbachol; therefore, this effect did not require cholinergic receptor activation. Moreover, experiments showing the reduction of monosynaptic IPSCs were done in the absence of carbachol. However, in a few cells there was no change in spontaneous IPSPs following the train of action potentials before carbachol, and yet a reduction was induced following carbachol application. This can probably be attributed to the large depolarization and increase in Ca^{2+} influx induced by carbachol (Reynolds and Miller, 1989; Boess et al., 1990).

The possibility that the reduction of IPSP(C)s was due to a recurrent polysynaptic pathway appears improbable since it was not eliminated by the glutamate antagonists CNQX and APV. These antagonists block the synaptic output of pyramidal cells (Davies et al., 1990), thereby preventing the activation of a feedback circuit.

While a change in the reversal potential for Cl^- has been implicated in the reduction of IPSPs during repetitive synaptic activation (McCarren and Alger, 1985; Huguenard and Alger, 1986; Thompson and Gähwiler, 1989a,b), it is unlikely that this could be the basis of the results reported here since a series of action potentials activated directly by postsynaptic current injection will not alter the transmembrane concentrations of Cl^- in these cells. With high $[Cl^-]_i$, E_{Cl} is substantially depolarized with respect to the resting potential. Action potential depolarizations would, by reducing the driving force on Cl^-, actually reduce any Cl^- efflux from the cells. There is no evidence for Ca^{2+}-dependent Cl^- conductance in adult CA1 pyramidal cells (Madison et al., 1986).

We were impressed by how few spikes were required to produce an observable effect. In many cases, as few as two closely spaced action potentials caused a noticeable reduction in spontaneous IPSPs. Clearly, longer trains of action potentials had more complete and longer-lasting effects, closely paralleling the effect of varying the duration of a train of action potentials on activation of the Ca^{2+}-dependent AHP (Gustafsson and Wigström, 1981; Madison and Nicoll, 1984). Indeed, the duration of the reduction in IPSPs following the action potential trains was similar to the duration of the Ca^{2+}-dependent AHP that follows a series of action potentials in normal medium.

The clearest link of IPSP(C) reduction to Ca^{2+} was the ability of $[Ca^{2+}]_i$ chelators to block it. For whole-cell recordings in this study, the concentrations of BAPTA and Ca^{2+} had to be titrated such that, with low-Ca^{2+} buffering, we were able to generate a Ca^{2+}-dependent AHP following a burst of action potentials and with high-Ca^{2+} buffering the AHP was blocked. The suppression of IPSPs following action potentials was well correlated with Ca^{2+} buffering in this way. Interestingly, IPSCs were not blocked immediately upon termination of the train of action potentials when $[Ca^{2+}]_i$ per se would probably be highest (Knöpfel et al., 1990), but rather declined until becoming minimal at about 200 msec after the train. This time course is strongly reminiscent of the time course of the slow Ca^{2+}-dependent K AHP in hippocampal CA1 cells. Lancaster et al. (1991) have shown that the delay to peak of the AHP cannot be explained by slowed kinetics of Ca^{2+}–K^+ channel interaction, but rather must be due to delayed diffusion of Ca^{2+} from its entry point to the channels. Alternatively, slow kinetics of ionic channel behavior are often attributed to the involvement of second messenger systems. It will be important to learn the cause of the delay in onset of the IPSC depression after an action potential train.

Our hypothesis is that Ca^{2+} enters cells through voltage-dependent Ca^{2+} channels. Indeed, in half of the cells tested, BAY K 8644 caused a further reduction in IPSPs following a train of action potentials. It is not clear why BAY K 8644 does not work more consistently; however, Ca^{2+} currents are quite variable and may have greater or lesser dihydropyridine-sensitive components. Alternatively, BAY K 8644 has antagonistic properties (Sanguinetti et al., 1986; Kamp et al., 1989; Jones and Jacobs, 1990), which may have prevailed over its agonist properties in some cases. Nifedipine had no effect by itself on the magnitude of reduction in IPSPs following the train of action potentials; however, nifedipine did allow a rapid reversal of the BAY K 8644 effect (Fig. 8), possibly by counteracting the agonist properties of BAY K 8644.

Although a detailed quantitative analysis was not performed, it appeared that the control level of spontaneous IPSP(C)s was higher when 100 mM EGTA was included in our sharp-electrode recordings. A similar result was often noted when high-Ca^{2+} buffering was used with whole-cell recordings; however, under these conditions the IPSCs ran down slowly over the course of the experiment, unlike cells recorded with low-Ca^{2+} buffering conditions, possibly supporting the hypothesis of a requirement of GABA responses for a minimal amount of Ca^{2+} (Taleb et al., 1987; Mody et al., 1990).

It is interesting to contrast our results with those of Llano et al. (1991), who also found a decrease in synaptic GABA responses following activation of the postsynaptic cell. The biggest difference is that Llano et al. invariably found that responses to exogenous GABA were potentiated by a Ca-dependent process, whereas we were unable to detect any change in exogenous GABA response. Llano et al. also found that, in TTX, there was an increase in the mean amplitude of spontaneous IPSPs, with quite large responses continuing to occur in TTX. We found a consistent decrease in mean amplitude, with no large events ever occurring, even without TTX. BAPTA blocked the decrease in synaptic events in our cells, but not in the Purkinje cells, although this could have been due to the large size of the Purkinje cell and remote location of certain inhibitory synapses. Besides the obvious possibility of tissue-specific, and receptor-specific, differences, there were some others: Llano et al. used prolonged, strong depolarizing voltage-clamp steps (to +20 mV) to activate the Purkinje cells. We used trains of brief, just suprathreshold, current pulses to trigger action potentials. Their protocol may have produced larger and more prolonged increases in $[Ca^{2+}]_i$.

Our data suggest that a postsynaptic rise in $[Ca^{2+}]_i$ is responsible for the decrease in the synaptic GABA responses. We suspect that the mechanism is the Ca^{2+}-induced dephosphorylation that affects GABA receptors (Chen et al., 1990). Nevertheless, the direct experiment to test part of the hypothesis, involving GABA iontophoresis, has yielded negative results in our hands so far. It is possible that receptors activated by our iontophoretic technique, in which the GABA pipette is aimed at the cell soma, were not exposed to sufficiently high $[Ca^{2+}]_i$ to be affected. An alternative possibility, similar to the one suggested by Llano et al. (1991), is that the rise in postsynaptic $[Ca^{2+}]_i$ generates a diffusible second messenger that escapes from the postsynaptic cell and prevents transmitter release from presynaptic terminals. This would explain why only the synaptic responses were affected in our experiments. In either case, the data emphasize the role of the postsynaptic neuron in controlling its own activity.

References

Aicardi G, Schwartzkroin PA (1990) Suppression of epileptiform burst discharges in CA3 neurons of rat hippocampal slices by the organic calcium channel blocker, verapamil. Exp Brain Res 81:288–296.

Alger BE, Nicoll RA (1980a) Spontaneous inhibitory post-synaptic potentials in hippocampus: mechanism for tonic inhibition. Brain Res 200:195–200.

Alger BE, Nicoll RA (1980b) Epileptiform burst afterhyperpolarization: calcium-dependent potassium potential in hippocampal CA1 pyramidal cells. Science 210:1122–1124.

Alger BE, Nicoll RA (1982) Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied *in vitro*. J Physiol (Lond) 328: 105–123.

Benardo LS, Prince DA (1982) Ionic mechanisms of cholinergic excitation in mammalian hippocampal pyramidal cells. Brain Res 249: 333–344.

Blanton MG, Lo Turco JJ, Kriegstein AR (1989) Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. J Neurosci Methods 30:203–210.

Boess FG, Balasubramanian MK, Brammer MJ, Campbell IC (1990) Stimulation of muscarinic acetylcholine receptors increases synaptosomal free calcium concentration by protein kinase-dependent opening of L-type calcium channels. J Neurochem 55:230–236.

Brown AM, Kunze DL, Yatani A (1984) The agonist effect of dihydropyridines on Ca channels. Nature 311:570–572.

Chen QX, Stelzer A, Kay AR, Wong RKS (1990) $GABA_A$ receptor function is regulated by phosphorylation in acutely dissociated guinea-pig hippocampal neurones. J Physiol (Lond) 420:207–221.

Cole AE, Nicoll RA (1984) Characterization of a slow cholinergic postsynaptic potential recorded *in vitro* from rat hippocampal pyramidal cells. J Physiol (Lond) 352:173–188.

Davies CH, Davies SN, Collingridge GL (1990) Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J Physiol (Lond) 424:513–531.

Eccles J, Nicoll RA, Oshima T, Rubia FJ (1977) The anionic permeability of the inhibitory postsynaptic membrane of hippocampal pyramidal cells. Proc R Soc Lond [Biol] 198:345–361.

Edwards FA, Konnerth A, Sakmann B (1990) Quantal analysis of inhibitory synaptic transmission in the dentate gyrus of rat hippocampal slices: a patch-clamp study. J Physiol (Lond) 430:213–249.

Gray R, Johnston D (1985) Rectification of single GABA-gated chloride channels in adult hippocampal neurons. J Neurophysiol 54:134–142.

Gustafsson B, Wigström H (1981) Evidence for two types of afterhyperpolarization in CA1 pyramidal cells in the hippocampus. Brain Res 206:462–468.

Hablitz JJ (1981) Altered burst responses in hippocampal CA3 neurons injected with EGTA. Exp Brain Res 42:483–485.

Hotson JR, Prince DA (1980) A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. J Neurophysiol 43: 409–419.

Huguenard JR, Alger BE (1986) Whole-cell voltage-clamp study of the fading of GABA-activated currents in acutely dissociated hippocampal neurons. J Neurophysiol 56:1–18.

Inoue M, Oomura Y, Yakushiji T, Akaike N (1986) Intracellular calcium ions decrease the affinity of the GABA receptor. Nature 324: 156–158.

Inoue M, Sadoshima J, Akaike N (1987) Different actions of intracellular free calcium on resting and GABA-gated chloride conductances. Brain Res 404:301–303.

Jones RSG, Heinemann UH (1987) Differential effects of calcium entry blockers on pre- and postsynaptic influx of calcium in the rat hippocampus *in vitro*. Brain Res 416:257–266.

Jones SW, Jacobs LS (1990) Dihydropyridine actions on calcium currents of frog sympathetic neurons. J Neurosci 10:2261–2267.

Kamp TJ, Sanguinetti MC, Miller RJ (1989) Voltage- and use-dependent modulation of cardiac calcium channels by the dihydropyridine (+)-202-791. Circ Res 64:338–351.

Kandel ER, Spencer WA, Brinley FJ Jr (1961) Electrophysiology of hippocampal neurons. I. Sequential invasion and synaptic organization. J Neurophysiol 24:225–242.

Knöpfel T, Vranesic I, Gähwiler BH, Brown DA (1990) Muscarinic and β-adrenergic depression of the slow Ca^{2+}-activated potassium conductance in hippocampal CA3 pyramidal cells is not mediated by a reduction of depolarization-induced cytosolic Ca^{2+} transients. Proc Natl Acad Sci USA 87:4083–4087.

Krnjević K, Puil E, Werman R (1975) Evidence for Ca^{2+}-activated K^+ conductance in cat spinal motoneurons from intracellular EGTA injections. Can J Physiol Pharmacol 53:1214–1218.

Lancaster B, Adams PR (1986) Calcium-dependent current generating the afterhyperpolarization of hippocampal neurons. J Neurophysiol 55:1268–1282.

Lancaster B, Nicoll RA, Perkel DJ (1991) Calcium activates two types of potassium channels in rat hippocampal neurons in culture. J Neurosci 11:23–30.

Llano I, Leresche N, Marty A (1991) Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. Neuron 6:565–574.

Madison DV, Nicoll RA (1984) Control of the repetitive discharge of rat CA1 pyramidal neurones *in vitro*. J Physiol (Lond) 354:319–331.

Madison DV, Malenka RC, Nicoll RA (1986) Phorbol esters block a voltage-sensitive chloride current in hippocampal pyramidal cells. Nature 321:695–697.

Madison DV, Lancaster B, Nicoll RA (1987) Voltage clamp analysis of cholinergic action in the hippocampus. J Neurosci 7:733–741.

Marchenko SM (1991) Mechanism of modulation of GABA-activated current by internal calcium in rat central neurons. Brain Res 546: 355–357.

Maruyama T, Ikemoto Y, Akaike N (1990) Effect of temperature on the inhibition of the GABA-gated response by intracellular calcium. Brain Res 507:17–22.

McCarren M, Alger BE (1985) Use-dependent depression of IPSPs in rat hippocampal pyramidal cells *in vitro*. J Neurophysiol 53:557–571.

Mody I, MacIver MB, Tanelian DL (1990) Calcium mediates the halothane-induced prolongation of spontaneous inhibitory postsynaptic currents in hippocampal neurons. Soc Neurosci Abstr 16:805.

Nowycky MC, Fox AP, Tsien RW (1985) Long-opening mode of gating of neuronal calcium channels and its promotion by the dihydropyridine calcium agonist Bay K 8644. Proc Natl Acad Sci USA 82:2178–2182.

O'Regan MH, Kocsis JD, Waxman SG (1990) Depolarization-dependent actions of dihydropyridines on synaptic transmission in the *in vitro* rat hippocampus. Brain Res 527:181–191.

Otis TS, Staley KJ, Mody I (1991) Perpetual inhibitory activity in mammalian brain slices generated by spontaneous GABA release. Brain Res 545:142–150.

Pitler TA, Alger BE (1990) Elevation of intracellular Ca inhibits GABAergic IPSPs in hippocampal neurons. Soc Neurosci Abstr 16:467.

Pitler TA, Alger BE (1991) Excitation of GABAergic interneurons by acetylcholine in rat hippocampus. Soc Neurosci Abstr 17:581.

Puil E, Werman R (1981) Internal cesium ions block various K conductances in spinal motoneurons. Can J Physiol Pharmacol 59:1280–1284.

Reynolds IJ, Miller RJ (1989) Muscarinic agonists cause calcium influx and calcium mobilization in forebrain neurons *in vitro*. J Neurochem 53:226–233.

Ropert N, Miles R, Korn H (1990) Characteristics of miniature inhibitory postsynaptic currents in CA1 pyramidal neurones of rat hippocampus. J Physiol (Lond) 428:707–722.

Sanguinetti MC, Krafte DS, Kass RS (1986) Voltage-dependent modulation of Ca channel current in heart cells by Bay K8644. J Gen Physiol 88:369–392.

Schwartzkroin PA, Stafstrom CE (1980) Effects of EGTA on the calcium-activated afterhyperpolarization in hippocampal CA3 pyramidal cells. Science 210:1125–1126.

Segal M, Barker JL (1984) Rat hippocampal neurons in culture: properties of GABA-activated Cl^- ion conductance. J Neurophysiol 51: 500–515.

Sivilotti L, Nistri A (1991) GABA receptor mechanisms in the central nervous system. Prog Neurobiol 36:35–92.

Stelzer A, Slater NT, ten Bruggencate G (1987) Activation of NMDA receptors blocks GABAergic inhibition in an *in vitro* model of epilepsy. Nature 326:698–701.

Taleb O, Trouslard J, Demeneix BA, Feltz P, Bossu J-L, Dupont J-L, Feltz A (1987) Spontaneous and GABA-evoked chloride channels on pituitary intermediate lobe cells and their internal Ca requirements. Pfluegers Arch 409:620–631.

Thompson SM, Gähwiler BH (1989a) Activity-dependent disinhibi-

tion. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus *in vitro*. J Neurophysiol 61:501–511.

Thompson SM, Gähwiler BH (1989b) Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide, and membrane potential on E_{Cl^-} in hippocampal CA3 neurons. J Neurophysiol 61: 512–523.

Wigström H, Gustafsson B (1983) Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition. Nature 301:603–604.

Wong RKS, Prince DA (1979) Dendritic mechanisms underlying penicillin-induced epileptiform activity. Science 204:1228–1231.