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Intracellular Ca^{2+} Suppressed a Transient Potassium Current in Hippocampal Neurons

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The effects of intracellular Ca^{2+} (Ca^{2+}_i) on K^+ currents in hippocampal cells were examined using acutely isolated cells obtained from adult guinea pigs. Whole-cell voltage-clamp recordings were carried out in a configuration that allowed a continuous perfusion of the intracellular medium. Recording media were made to block inward currents and allowed selective activation of K^+-dependent outward currents. Voltage-dependent outward currents consisted of an initial rapidly decaying component followed by a sustained component. The time constant of decay of the transient current was about 25 msec, and previous studies (Numann et al., 1987) showed that the kinetic and pharmacological properties of this current closely resembled the A current recorded in invertebrate neurons (Connor and Stevens, 1971; Thompson, 1982).

Intracellular perfusion of hippocampal cells with a solution containing elevated Ca^{2+} (about 4.5×10^{-4} M) elicited outward currents at the holding potential (−45 to −55 mV) and produced changes in voltage-dependent K^+ currents. The transient outward current (I_A) activated by depolarization was suppressed with increases in Ca^{2+}_i. Delayed, sustained K^+ currents were greatly potentiated. Data also showed that, among the 3 effects elicited by Ca^{2+}_i, suppression of I_A was most sensitive to Ca^{2+}_i elevation. Previous results (Numann et al., 1987) showed that I_A had a lower threshold (about −45 mV) than sustained currents (about −40 mV). By using low levels of depolarization (−40 mV), I_A can be selectively activated, and the suppressive effect of Ca^{2+}_i on I_A was confirmed on the kinetically isolated I_A. Finally, a series of experiments was carried out using extracellular Cs (10 mM) and tetraethylammonium chloride (TEA; 10 mM) to block voltage-dependent sustained currents. Depolarization still elicited I_A in this extracellular medium. Intracellular perfusion of a "high"-Ca^{2+} solution no longer activated outward currents at the holding potential, nor were the sustained currents potentiated. However, the action of Ca^{2+}_i on I_A was retained; elevation of Ca^{2+}_i suppressed I_A at all depolarizing voltage levels.

The results directly confirmed that elevation of Ca^{2+}_i activates sustained K^+-dependent outward currents. In addition, direct evidence is provided demonstrating that increases in Ca^{2+}_i suppressed and, when it occurred in sufficient quantity, blocked I_A in hippocampal cells. The effect of Ca^{2+} on I_A was confirmed under conditions in which separation of transient and sustained currents was achieved based on differences in their kinetic and pharmacological properties.

Recent studies emphasize the role of intracellular Ca^{2+} (Ca^{2+}_i) as an ionic messenger in the hippocampus, involved in processes such as long-term potentiation of excitatory synaptic transmission (Lynch et al., 1983; Malenka et al., 1988; Manilow et al., 1989) and induction of cell death (Rothman and Olney, 1987; Choi, 1988; Scharfman and Schwartzkroin, 1989; Sick and Rosenthal, 1989; Siman, 1989). Ca^{2+}_i may convey these functions through modulation of ligand- (Lynch and Baudry, 1984; Stelzer et al., 1988; Chen et al., 1990) or voltage-gated channels (Alger and Nicoll, 1980; Hotson and Prince, 1980; Lancaster and Adams, 1986; Leblond and Krnjevic, 1989). Ca^{2+}_i is known to activate K^+ channels in a variety of nerve and muscle cells (Meech, 1978; Blatz and Magleby, 1987). In the hippocampus, at least 2 types of K^+ channels underlying afterhyperpolarizations (Lancaster and Adams, 1986; Numann et al., 1987) were activated with elevations of Ca^{2+}_i. Voltage-clamp studies show that membrane depolarization also activates a transient K^+ current with a decay time constant of about 25 msec (Gustafsson et al., 1982; Segal and Barker, 1984; Numann et al., 1987). As a prominent component of outward current in hippocampal cells, this current controls action-potential threshold, pattern, and duration (Segal et al., 1984; Nakajima et al., 1986; Storm, 1987; Gean and Schinnick-Gallagher, 1989).

The possibility that Ca^{2+}_i may control transient K^+ current activation has received an increasing amount of attention. At present, data relevant to this issue have been derived from experiments involving manipulation of extracellular Ca^{2+} or addition of Ca^{2+} channel blockers. Evidence obtained by these approaches is indirect and inconclusive. In addition, while some studies show that the transient K^+ current was suppressed by removal of extracellular Ca^{2+} or addition of Ca^{2+} channel blockers (Thompson, 1977; MacDermott and Weight, 1982; Salkoff, 1983; Galvan and Sedlmeir, 1984; Zbicz and Weight, 1985; Bourque, 1988), others suggest a lack of effect of such a manipulation (Gustafsson et al., 1982; Segal et al., 1984; Halliwell et al., 1986; Surmeier et al., 1989) or that the effects can be explained by changes in charge screening on the cell membrane surface due to alterations in the concentration and composition of extracellular divalent cations (Numann et al., 1987; Mayer

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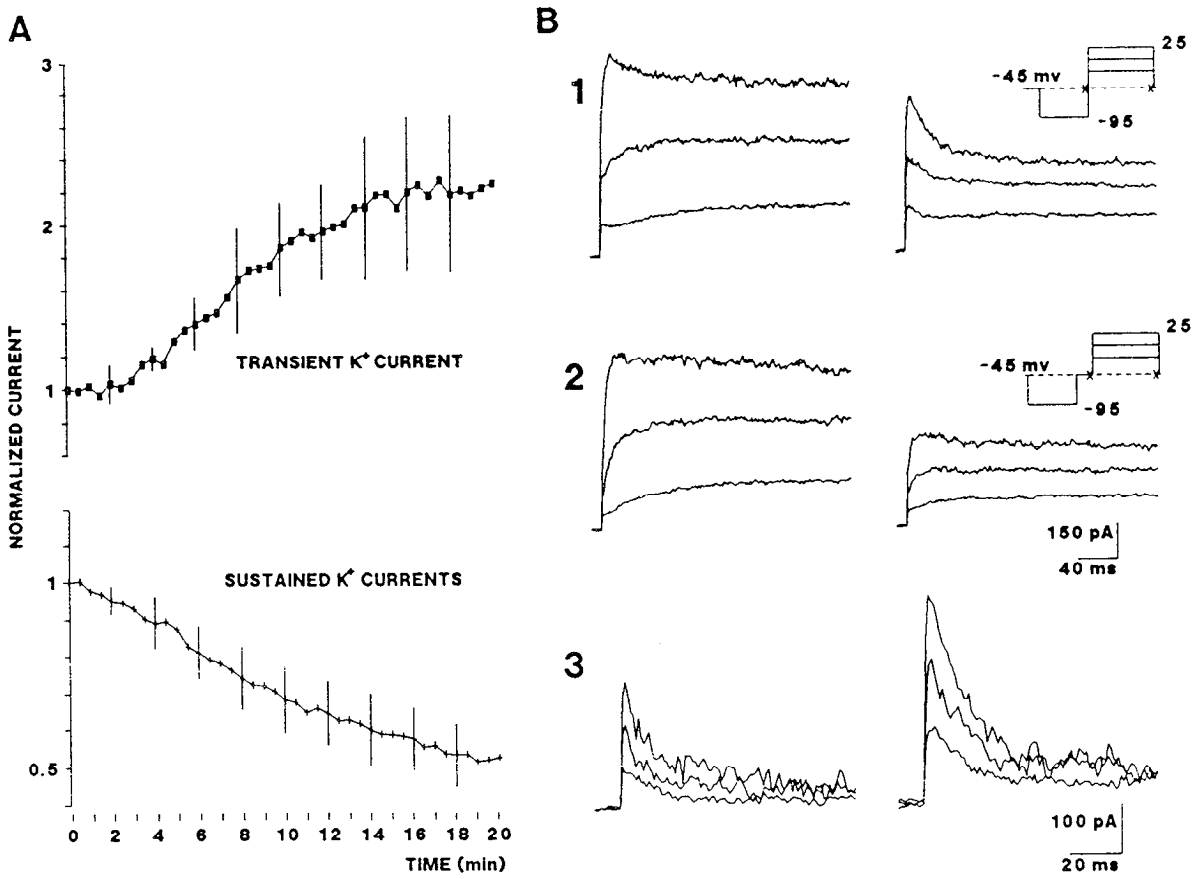

Figure 1. Effects of intracellular perfusion of low-Ca^{2+} solution on K^+ currents. *A*, The *top curve* shows the increase in I_A peak amplitude during such perfusion. The amplitude of the sustained current, measured at the end of the depolarizing pulse (when the A current would be completely inactivated), shows gradual reduction (*bottom curve*). *B*, Outward current activated immediately following cell penetration (*left records*) and 20 min after low-Ca^{2+} perfusion (*right records*). The currents illustrated were elicited during the period indicated between the *X*s on the voltage protocols (*right*). *B, 2,* An intercalating pulse (70 msec, −45 mV) was applied to inactivate I_A following the conditioning in hyperpolarization. Subtraction of records obtained in *B, 2,* from corresponding ones in *B, 1,* yielded the time course of I_A (*B, 3*).

and Sugiyama, 1988). Furthermore, evidence suggests that divalent cations in extracellular solutions may directly affect potassium channels by binding to a specific domain of the channel protein (Begenisich, 1989). In this paper, we demonstrate directly that elevation of Ca^{2+}_i suppressed a prominent transient K^+ current in hippocampal cells.

Materials and Methods

Experiments were carried out on acutely dissociated hippocampal CA1 pyramidal cells from adult guinea pigs (Kay and Wong, 1986). To examine potassium currents, cells were perfused extracellularly with (in mM) Trizma base, 140; KCl, 5; HEPES, 10; $CaCl_2$, 0.2; $MgCl_2$, 1; $CoCl_2$, 2; and D-glucose, 24; with pH adjusted to 7.4 by adding methanesulfonic acid. Substitution of Na^+ by Tris in the extracellular solution prevented the activation of Na^+ current. The presence of $CoCl_2$ blocked Ca^{2+} currents. Whole-cell currents were recorded with a patch-clamp amplifier (List), following a procedure described by Hamill et al. (1981).

For intracellular perfusion, inlet and outlet plastic tubes were inserted into the recording electrode down to the tip; solutions were exchanged and circulated through the pipette under pressure during recording sessions. Details of the recording arrangement were presented previously (Chen et al., 1990). Intracellular perfusing solutions used to fill the recording pipette consisted of (in mM) ATP, 0.5; $MgCl_2$, 1; BAPTA, 10; leupeptin, 0.1; HEPES, 10; and K^+-methane-sulfonic, 120. The pH of intracellular solutions was adjusted to 7.2 by adding KOH. The above control ("low"-Ca^{2+}) solution yielded $[Ca^{2+}]$ levels of less than 10^{-8} M measured by Ca^{2+} electrode (Radiometer). For the "high"-Ca^{2+} solution, BAPTA was reduced to 0.5 mM, and 1 mM $CaCl_2$ was added. Measured $[Ca^{2+}]$ was 4.5×10^{-4} M. In experiments where the high-Ca^{2+} solution was used, perfusion of high Ca^{2+} was terminated when the membrane effects of elevated Ca^{2+} became apparent and before a full exchange of the high-Ca^{2+} solution within the cell had occurred. Thus, the effect of intracellular Ca^{2+} reported here was observed at intracellular Ca^{2+} levels below 4.5×10^{-4} M. We used a higher level of Ca^{2+} for the perfusion so that we could observe Ca^{2+} effects more quickly following the solution switch.

Results

Effects of low-Ca^{2+} intracellular perfusion on voltage-activated K^+ currents

Step depolarizations to above −45 mV from −95 mV activated outward currents consisting of an initial transient outward current followed by delayed sustained currents. The transient outward current decayed with a time constant of about 25 msec. Previous studies showed that this current shares similar properties with the transient outward current (I_A) described in molluscan cells and is blocked by 4-aminopyridine (4-AP) in a voltage-dependent manner (Numann et al., 1987). The delayed currents consisted of at least 3 components, including 2 that are activated by Ca^{2+}_i and 1 that is not affected by Ca^{2+}_i and inactivates slowly (Numann et al., 1987).

Continuous intracellular perfusion with the low-Ca^{2+} (control) solution caused a gradual increase of the I_A and a decrease of sustained K^+ currents (Fig. 1*A*). After 20 min of perfusion, I_A

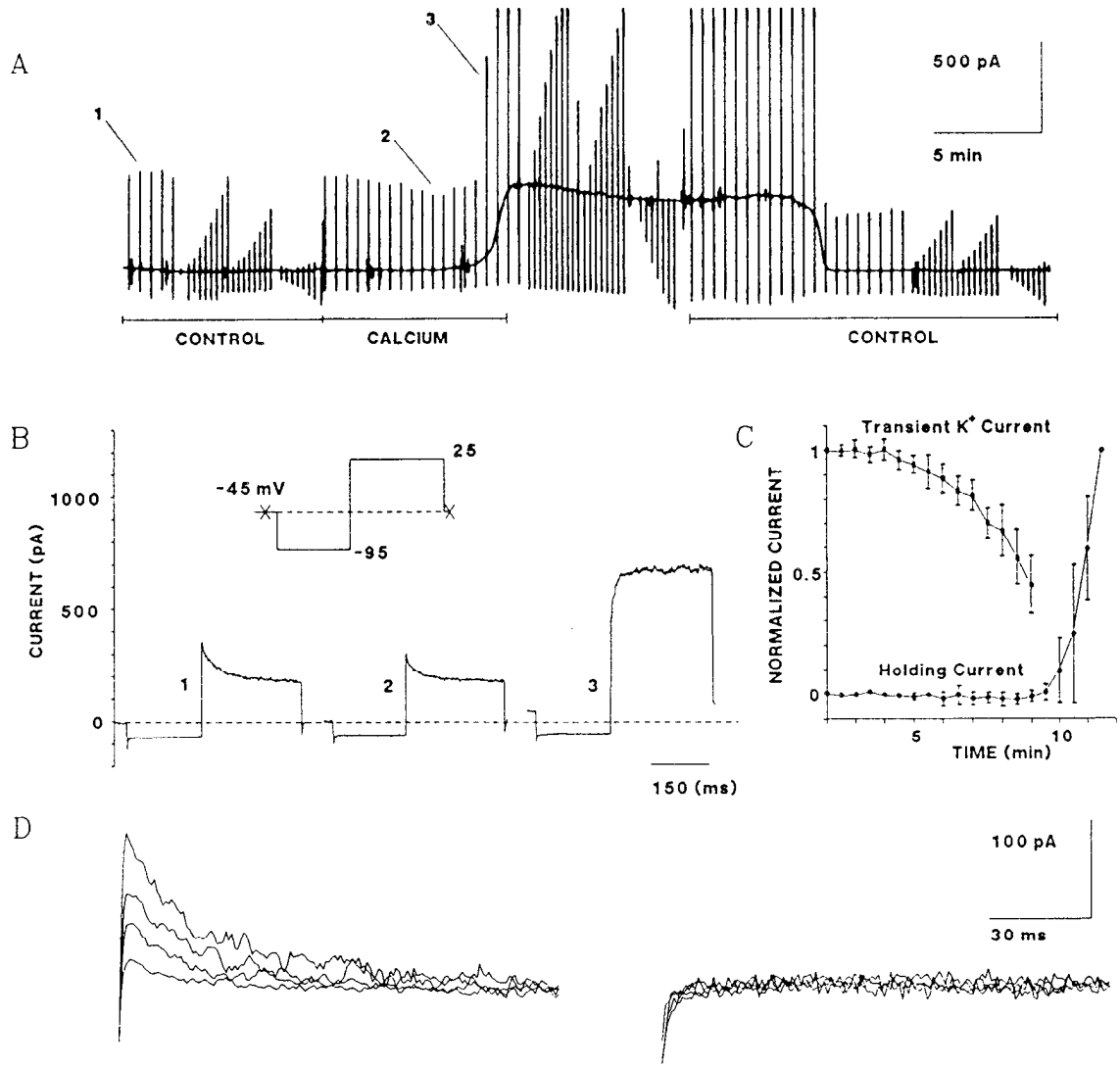

Figure 2. Effects of intracellular perfusion of high Ca^{2+} on holding and K^+ currents. *A,* The continuous chart record of the experiment. The recordings began with control solution perfusion. High-Ca^{2+} solution was introduced for the period indicated by the *bar* labeled calcium below the record. As the effects of high Ca^{2+} became apparent, the positive pressure used to force the high-Ca^{2+} solution into the pipette was stopped (period indicated by the *gap* between the bar labeled calcium and the *second bar* labeled control). The *continuous curve* in the graph records the holding current (at −45 mV). Vertical deflections register voltage-dependent K^+ currents elicited by voltage-clamp pulses. Peak current is clipped off in the record. Three of these current traces (marked *1, 2,* and *3*) were expanded and are shown in *B. B,* Current records activated at control (*1*) and during high-Ca^{2+} (*2, 3*) perfusion conditions. *Zero* on the *current axis* indicates absolute 0. The currents illustrated were elicited during the period indicated between the *X*s on the voltage protocol. *C,* Plots of time courses of the change in peak amplitudes of the transient K^+ current (I_A) and holding current in response to high-Ca^{2+} perfusion. Data were obtained by averaging results from 5 cells. The *bars* represent the SD of the data. *Zero* on the *time axis* of the graph represents 5 min before reduction in I_A amplitude was detectable following the introduction of high-Ca^{2+} solution. *D,* I_A activated by depolarizing pulses to −10 mV under the control (*left*) and high-Ca^{2+} (*right*) conditions, obtained by the subtraction procedure (Numann et al., 1987).

increased by 125% (+5% SD, $n = 5$), and delayed current decreased by 48% (±8% SD, $n = 5$). The isolated time course of I_A (Fig. 1*B,* 3) was obtained by subtracting the delayed current activated by a protocol (Fig. 1*B,* 2) from total outward current activated by another (Fig. 1*B,* 1).

Increases in the amplitude of I_A occurred without alterations in the kinetic properties of the current. The rate of decay was 26.6 ± 7.7 msec (mean ± SD; $n = 5$) before control (low-Ca^{2+}) perfusion and 28.6 ± 7.2 msec ($n = 5$) after 20 min of perfusion. The decrease in sustained K^+ currents was expected because Ca^{2+}-dependent components would be suppressed in the control solution. The results also suggest that the reduction in $Ca^{2+}{}_i$ produces an unexpected increase in the amplitude of I_A. We internally perfused the hippocampal cell with the high-Ca^{2+} solution to test this hypothesis.

Effects of high-Ca^{2+} intracellular perfusion on K^+-currents

Figure 2 shows the effect of elevated $Ca^{2+}{}_i$ on outward currents activated by depolarization and on the holding current at −45 mV. Figure 2*A* shows a chart record of the membrane current in response to high-Ca^{2+} perfusion and recovery from such an effect. Membrane currents were first recorded with control intracellular perfusate. Introduction of the high-Ca^{2+} solution resulted in the development of an outward current at the holding

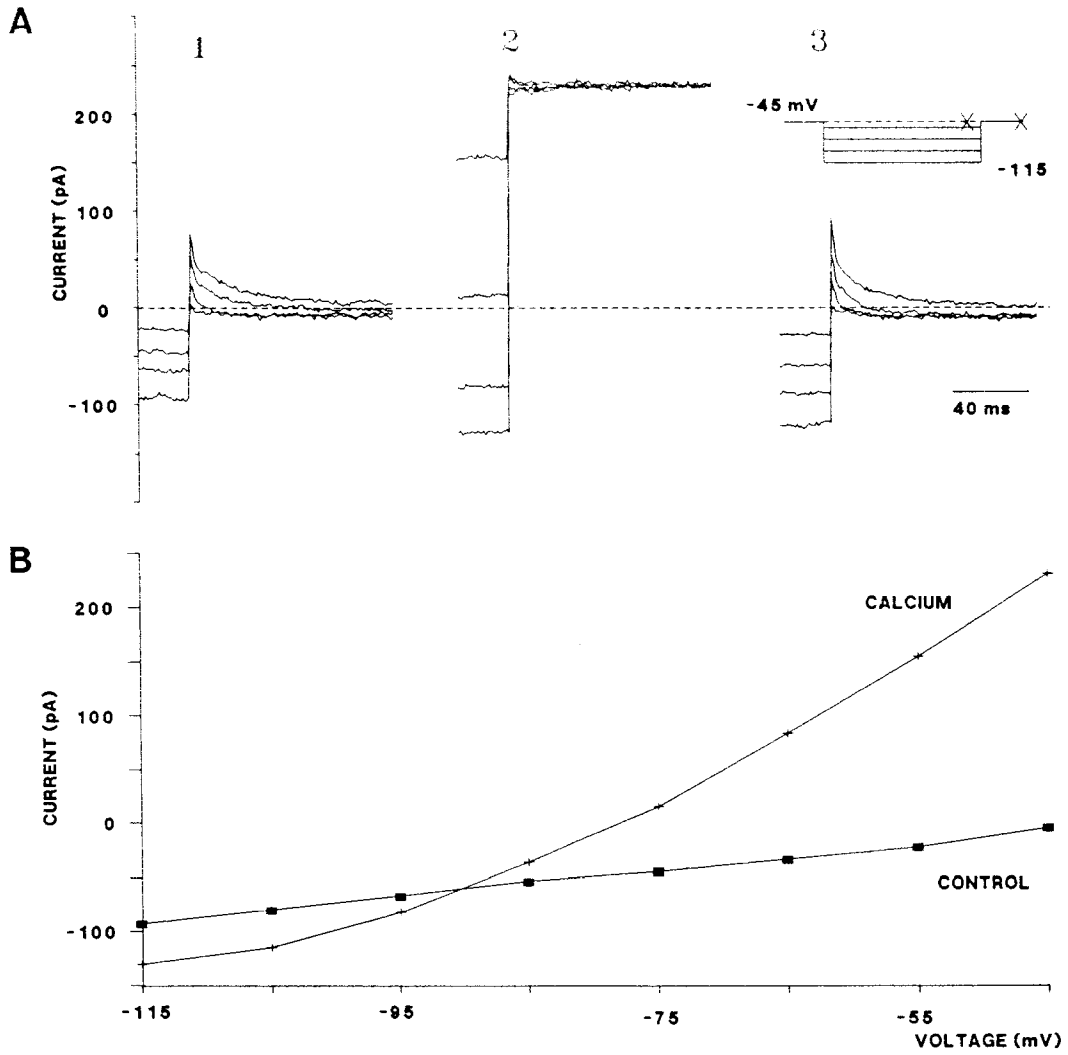

Figure 3. *A*, The current responses to hyperpolarizing voltage steps to −55, −75, −95, and −115 mV at a holding potential of −45 mV. *A, 1*, is obtained in the control condition. During intracellular perfusion with the high-Ca^{2+} solution, current activated by hyperpolarization increased (*A, 2*), and transient current activated upon repolarization to −45 from various hyperpolarizations is no longer detectable. The effects of high Ca^{2+} are reversible (*A, 3*). The currents illustrated were elicited during the period indicated between the *X*s on the voltage protocol (*upper right*). *B*, A plot of the current–voltage relationship at the hyperpolarizing range. The 2 curves crossed over at about −90 mV, close to the estimated K^+ reversal potential.

potential. The outward holding current was readily reversed upon washout with control perfusate. Figure 2*B* shows outward currents activated by depolarization at different times following introduction of the high-Ca^{2+} solution. Elevation of $Ca^{2+}{}_i$ produced an expected increase in sustained K^+ currents. On the other hand, I_A, observed in isolation by the subtraction method (Fig. 2*D*), appeared to be blocked by the high-Ca^{2+} intracellular solution. Data in Figure 2*B* also show that suppression of the transient K^+ current occurred before $Ca^{2+}{}_i$ reached a sufficient level to induce changes in holding current and sustained K^+ currents (cf. Fig. 2*B*, 1 with *B*, 2). Similar results were observed in 4 other cells tested. Figure 2*C* shows that I_A decreased, on the average, by 55% (±12% SD, $n = 5$) before changes in the holding current were observed.

Selective activation of I_A *by low-voltage depolarization*

To avoid possible errors introduced by the subtraction procedure, we carried out another set of experiments taking advantage of the differences in the threshold of I_A and sustained K^+ currents. Previously, we showed that the threshold of I_A is below −45 mV, whereas sustained K^+ currents have thresholds more depolarized than −45 mV (Numann et al., 1987). Depolarization of hippocampal cells to −45 mV should primarily activate I_A (Fig. 3*A*, 1). Following introduction of the high-Ca^{2+} solution (Fig. 3*A*, 2), outward current developed at the holding potential (−45 mV), and hyperpolarizing voltage steps were accompanied by larger inward currents because of the increase in conductance. Figure 3*B* plots inward currents accompanying hyperpolarizing steps in the control and high-Ca^{2+} conditions. Reversal potential for the current activated by the high-Ca^{2+} solution, assumed to be the point where the 2 curves intersect, is about −90 mV, suggesting that $Ca^{2+}{}_i$ activated a potassium conductance at the holding potential. Figure 3*A*, 2, shows that, after elevation of $Ca^{2+}{}_i$, repolarization to −45 mV from various hyperpolarizing steps no longer activated I_A. The suppression of I_A by $Ca^{2+}{}_i$ was reversed upon washout with control solution (Fig. 3*A*, 3).

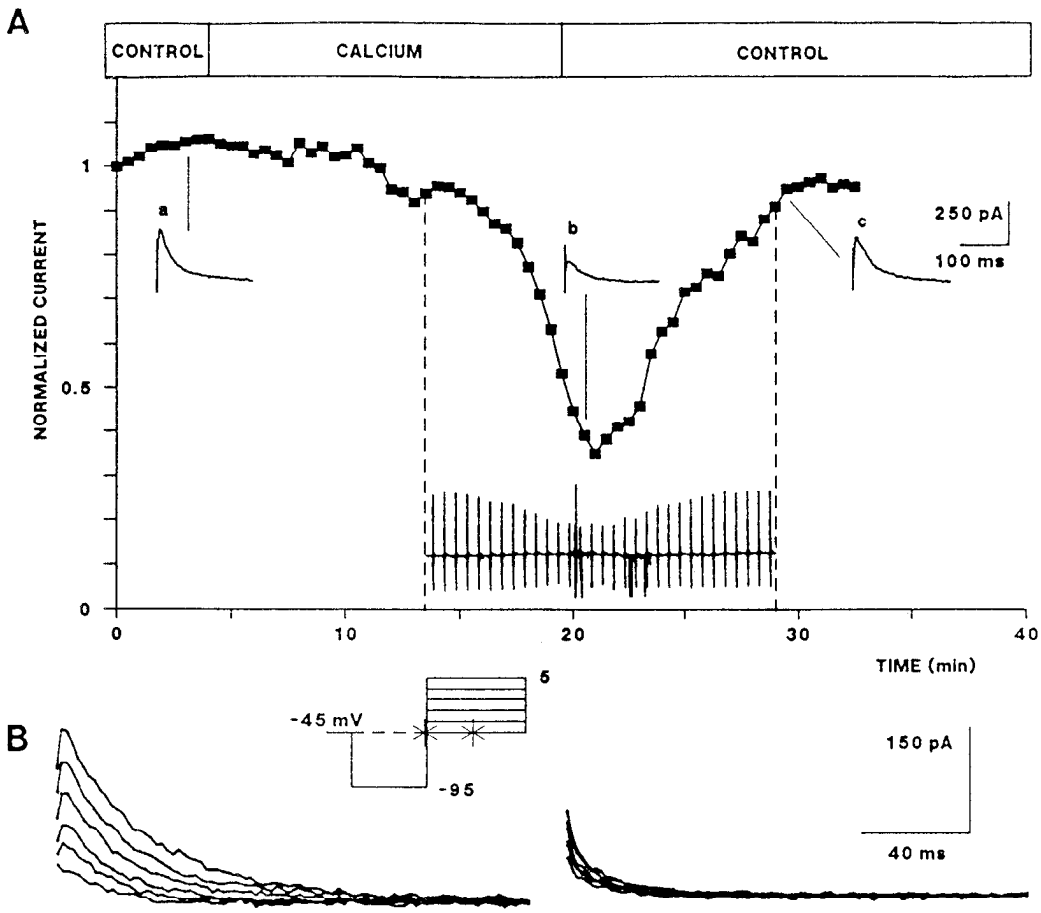

Figure 4. Membrane current responses obtained when extracellular perfusate contained Cs and TEA (both 10 mM). The *data points* plotted in *A* indicate the peak amplitude of I_A under different conditions. The introduction of high-Ca^{2+} solution reduced I_A. I_A amplitude increased when the control solution was again introduced. The *inset* shows current records activated by depolarizations to 0 mV following hyperpolarizing pulses of −100 mV for 250 msec. An original *chart record* of the experiment is also presented in *A*. The chart record shows the time course of currents recorded corresponding to the time scale provided in the x-axis of the graph. *B*, The current record obtained in control (*left*) and high-Ca^{2+} conditions (*right*). The currents illustrated were elicited during the period indicated between the *asterisks* on the voltage protocol. The records were obtained after leak subtraction. Leak conductance was determined by currents elicited by hyperpolarizing pulses. Analysis of data showed that TEA and Cs did not affect the decay rate of the A current. The inactivation time constant was 25.8 and 22.3 msec, respectively, at 0 and −20 mV (compared to 28.6 ± 7.2 msec, mean ± SD, at 0 mV in the absence of Cs and TEA). The fact that outward current, activated at all voltages tested, decayed completely to baseline (after leak subtraction) indicates that sustained currents were blocked by Cs and TEA, and that only I_A was activated by the depolarization.

Selective activation of I_A *in the presence of extracellular Cs and TEA*

Interpretation of data such as those obtained in Figures 2 and 3 is complicated by large K^+ conductances increases to K^+ currents that occurred with elevated $Ca^{2+}{}_i$. We found that addition of tetraethylammonium (TEA; 10 mM) and Cs (10 mM) to the extracellular perfusate blocked sustained K^+ currents but not I_A. Figure 4 shows the results obtained under this condition. Peak amplitude of I_A was first monitored when the cell was internally perfused with the control solution. Upon the introduction of the high-Ca^{2+} solution, peak amplitude of I_A gradually decreased (Fig. 4*A*). Chart records for the experiments are also shown in Figure 4*A*. The data showed that outward current activated at the holding potential (−45 mV) was also blocked by extracellular TEA and Cs. A noticeable difference between the chart record shown in Figure 4 and that shown in Figure 2*B* is that intracellular high Ca^{2+} no longer activated outward current at the holding potential (−45 mV). Figure 4*B* shows I_A elicited from another cell before (left record) and after high-Ca^{2+} solution perfusion. Current records were obtained after leak subtraction. The data show that I_A was blocked in the high-Ca^{2+} perfusion.

Discussion

The study directly demonstrates that $Ca^{2+}{}_i$ suppressed and, in sufficient concentration, blocked a prominent transient K^+ current, I_A, in hippocampal cells. The results do not provide precise information on the level of $Ca^{2+}{}_i$ required to exert an effect on I_A. We observed that $Ca^{2+}{}_i$ suppressed I_A just before potentiation of the sustained current, and activation of outward currents at the holding potential (−45 mV) occurred. Previous studies showed that, in rat myotube cells, $Ca^{2+}{}_i$ began to induce outward current at −45 mV when its concentration increased to about 5 μM (Barrett et al., 1982). Presumably, suppression of I_A also occurs at this level of $Ca^{2+}{}_i$ if properties of outward current channels in hippocampal cells are comparable to those in the myotube. Other studies (Hotson and Prince, 1980) have shown that single action potentials in hippocampal cells elevated $Ca^{2+}{}_i$

sufficiently to activate K^+ currents. If the rise in $Ca^{2+}{}_i$ following action potentials had equal access to all K^+ channels in the cell, we expect that suppression of I_A could also be induced by normal activities of hippocampal cells.

Our results show that $Ca^{2+}{}_i$ produced opposing effects on voltage-dependent K^+ currents in hippocampal cells. It suppressed I_A and enhanced sustained K^+ currents. The relative contribution of the 2 effects on cell excitability would depend on factors such as the sensitivity of the outward currents to $Ca^{2+}{}_i$, the time course of the Ca^{2+} rise, and the duration of effects of Ca^{2+} on the different currents. Data showing that I_A was suppressed during the high-Ca^{2+} perfusion before activation of the outward holding current and sustained K^+ currents (Fig. 2) suggest that conditions may exist such that tonic levels of $Ca^{2+}{}_i$ can selectively modulate I_A and regulate the excitability of hippocampal cells.

Suppression of I_A by 4-AP has been shown to reduce the action potential threshold and latency in hippocampal cells (Segal et al., 1984; Nakajima et al., 1986; Storm, 1987; Gean and Schinnick-Gallagher, 1989). Thus, modulation of I_A by $Ca^{2+}{}_i$ may enhance postsynaptic excitability and increase the efficacy of EPSPs to trigger action potentials. Previous studies showed that high-frequency stimulation of afferent fibers to hippocampal cells allowed initial subthreshold EPSPs to trigger action potentials afterwards without changes in amplitude (Taube and Schwartzkroin, 1988). Data here suggest that at least one way such modifications may arise is via the intracellular action of Ca^{2+} on I_A. $Ca^{2+}{}_i$ also increased neuronal excitability in the *Hermissenda* by reducing K^+ currents (including a transient K^+ current); in this preparation, suppressive effects of $Ca^{2+}{}_i$ on K^+ currents have been suggested to be a mechanism underlying associative learning (Alkon, 1984).

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