Calcium Activates Two Types of Potassium Channels in Rat Hippocampal Neurons in Culture

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Several calcium-dependent potassium currents can contribute to the electrophysiological properties of neurons. In hippocampal pyramidal cells, 2 afterhyperpolarizations (AHPs) are mediated by different calcium-activated potassium currents. First, a rapidly activated current contributes to action-potential repolarization and the fast AHP following individual action potentials. In addition, a slowly developing current underlies the slow AHP, which occurs after a burst of action potentials and contributes substantially to the spike-frequency accommodation observed in these cells during a prolonged depolarizing current pulse. In order to investigate the single Ca^{2+}-dependent channels that might underlie these currents, we performed patch-clamp experiments on hippocampal neurons in primary culture.

When excised inside-out patches were exposed to 1 μM Ca^{2+}, 2 types of channel activity were observed. In symmetrical bathing solutions containing 140 mM K^+, the channels had conductances of 19 pS and 220 pS, and both were permeable mainly to potassium ions. The properties of these 2 channels differed in a number of ways. At negative membrane potentials, the small-conductance channels were more sensitive to Ca^{2+} than the large channels. At positive potentials, the small-conductance channels displayed a flickery block by Mg^{2+} ions on the cytoplasmic face of the membrane. Low concentrations of tetraethylammonium (TEA) on the extracellular face of the membrane specifically caused an apparent reduction of the large-channel conductance. The properties of the large- and small-conductance channels are in accord with those of the fast and slow AHP, respectively.

When calcium ions enter a cell during an action potential, one of the immediate consequences is the triggering of other membrane currents, which in turn, regulate excitability. There are several reports of 2 calcium-activated potassium currents ($I_{K_{Ca}}$) in a number of cell types: *Aplysia* neurons (Deitmer and Eckert, 1985), amphibian ganglia (Pennefather et al., 1985), muscle (Blatz and Magleby, 1986), a pituitary cell line (Lang and Ritchie, 1988), and central neurons (Lancaster and Adams, 1986). One $I_{K_{Ca}}$ that is relatively invariant among different cells is a tetraethylammonium- (TEA) and voltage-sensitive current involved in repolarization of the action potential (Lancaster and Nicoll, 1987; Lancaster and Pennefather, 1987). This current is derived from the activation of large-conductance K^+ channels (Lang and Ritchie, 1988). A second $I_{K_{Ca}}$ exists that has functional similarity among different preparations; it is poorly voltage sensitive and underlies a slow afterhyperpolarization (AHP) that is responsible for action-potential frequency adaptation. The pharmacological properties of this current vary such that, depending on the cell type under investigation, it may be apamin sensitive (e.g., bullfrog or rat sympathetic ganglion) or apamin insensitive (e.g., hippocampus). Some properties of apamin-sensitive channels have been reported (Blatz and Magleby, 1986; Lang and Ritchie, 1988). This paper reports on the properties of 2 calcium-activated potassium channels in cultured hippocampal neurons.

Some of this work has appeared in abstract form (Lancaster et al., 1987).

Materials and Methods

Dissociated cell culture. Hippocampi were removed from rat pups at embryonic day 17–18. The tissue was then minced with iridectomy scissors and triturated with a Pasteur pipette fire-polished to a fine bore. The cell suspension was plated onto polylysine-coated coverslips (5 per dish) to give a cell density of approximately 60,000 per 35-mm culture dish. The plating medium consisted of Modified Eagle's Medium (MEM) with Earle's basic salt solution (UCSF Cell Culture Facility) supplemented with glucose (3.6 mM), rat serum (3%) and horse serum (4%), both sera from GIBCO. Neither enzymes nor antibiotics were used in the preparation or maintenance of the cultures. As the glia approached confluence, usually at about 5–6 d in culture, the mitotic inhibitor cytosine arabinoside was added at a concentration of 1 μM; this was removed after 24 hr.

Electrophysiology. Gigaseal patch recordings using the inside-out patch configuration (Hamill et al., 1981) were performed at room temperature on pyramidally shaped neurons between 4 and 21 d in culture. The bath solution had the following composition (in mM): NaCl, 124; $MgCl_2$, 10; KCl, 1.3; HEPES, 10; EGTA, 0.5. Calcium in the bath solution was buffered to low levels to eliminate possible Ca^{2+} contamination of the flow system when using an inside-out patch configuration. In the absence of $[Ca^{2+}]_o$, the high $[Mg^{2+}]_o$ appeared to enhance viability during the recording period. Inside-out membrane patches were positioned in a flow system controlled by a latching valve (General Valve Co.), which provided a brisk and consistent means of changing the solution at the cytoplasmic face of the patch (Brett et al., 1986). The composition of the flow solution that bathed the exposed (i.e., intracellular) face of the patch was (in mM) KCl, 140; NaCl, 10; HEPES, 10; and EGTA, 0.5. $CaCl_2$ was added as appropriate to give the desired free-Ca^{2+} concentrations (Magleby and Pallotta, 1983). In some experiments, $MgCl_2$ was added. Pipette solutions consisted of either the bath solution or, more usually, the high-K^+ flow solution, to give symmetrical charge-carrier distribution when in the flow system. $MgCl_2$ at either 0.5 or 1 mM was routinely added to the pipette solution purely for the convenience of easier seal formation in the absence of other divalent ions. All experimental solutions were made using deionized water (Nanopure). Voltages are expressed as conventional membrane potentials.

Data were obtained using an Axopatch amplifier (Axon Instruments) filtered at 1 kHz (4-pole Bessel filter) and stored on magnetic tape or

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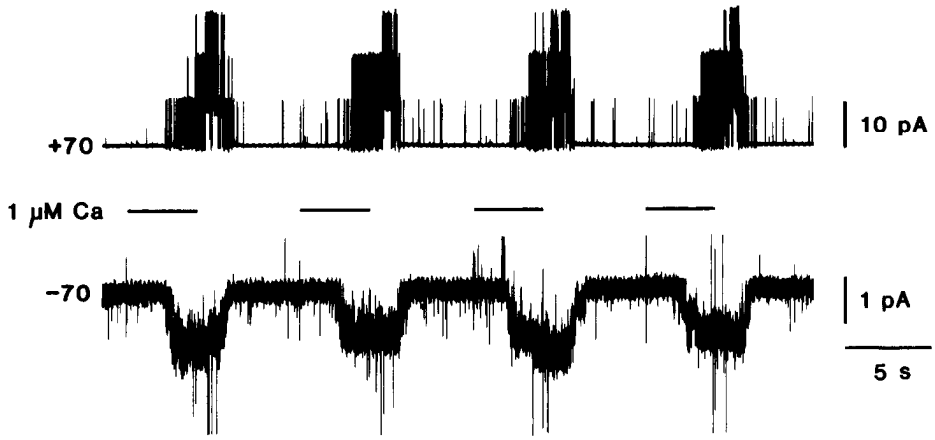

Figure 1. Large- and small-conductance Ca^{2+}-activated K^+ channels in inside-out membrane patches. Successive 4-sec applications of 1 μM Ca^{2+} were made to the cytoplasmic face of a patch held at +70 mV (*top trace*) and at −70 mV (*bottom trace*). Note the difference in gain of the 2 traces.

digitized and stored on computer disk, in the latter case with pCLAMP IV data acquisition and analysis software run on a personal computer (PC's Limited). Data were analyzed using either pCLAMP IV with appropriate modifications or an Indec system run on a PDP 11/73 (courtesy of J. B. Lansman).

Results

All data were obtained from inside-out membrane patches in a self-contained flow system, which allowed not only rapid addition, but also rapid removal of Ca^{2+} from the exposed (intracellular) surface of the patch. This protocol allowed us to identify 2 channel types that could be activated consistently and clearly by intracellular Ca^{2+}. In the example in Figure 1, both types of Ca^{2+}-activated channels were present in the same patch. Ca^{2+} was applied for 4 sec at a frequency of 0.1 Hz, indicated by the solid lines. After a short delay due to the dead space in the flow system, Ca^{2+}-activated patch currents were observed at both depolarized (+70 mV; Fig. 1) and hyperpolarized (−70 mV; Fig. 1) membrane potentials. At +70 mV, 1 μM Ca^{2+} activated 3 large channels with a chord conductance of 220 pS. These undoubtedly represent the large-conductance Ca^{2+}-activated K^+ channel, which is a ubiquitous feature of many cell types (Barrett et al., 1982). At −70 mV, the large channels are not active (note increase in gain) because their voltage sensitivity prevents opening even in the presence of 1 μM Ca^{2+}. At the hyperpolarized potential, a second effect of Ca^{2+} was observed as a clear inward patch current that was time locked to the Ca^{2+} application. This inward patch current was due to activation of a second channel type. No apparent difference in the delay between the application of Ca^{2+} and the onset of the 2 types of channel activity was observed. The records of this small channel in subsequent figures were all taken from such episodes of Ca^{2+} application.

Figure 2*A* shows the single channel events responsible for the type of inward patch currents of Figure 1. In this patch, either 1 or 2 channels were open in the sample records used for the current–voltage (*I/V*) plot (Fig. 2*B*); records were chosen to illustrate discrete open/closed transitions rather than to be representative of the open probability, which is high at 1 μM Ca^{2+}. The *I/V* plot in Figure 2*B* is linear in the hyperpolarized region, with a slope conductance of 20 pS and an extrapolated reversal potential of −4 mV with symmetrical (140 mM) KCl as the main charge carrier.

One of the consistent features of this channel is illustrated in Figure 3*A*, 1. A 2-sec application of 1 μM Ca^{2+} activates a number of channels to produce an inward patch current at −70 mV or outward current at +70 mV. However, the Ca^{2+} induces a larger inward current than outward current. There are 2 possible explanations for this rectification: either fewer channels are open at +70 mV, or rectification occurs at the single-channel level. The records in Figure 3*B* show a similar phenomenon at the single-channel level in a different patch. The ratio of total inward to outward patch current in Figure 3*A* is 1.7 and, for the single-channel conductance in Figure 3*B*, is 1.9 (see below). Mean single-channel conductance from a number of patches at hyperpolarized levels is 18.2 ± 2.4 pS (mean ± SD; $n = 6$) and, at depolarized potentials, is 10.9 ± 1.4 pS ($n = 6$). This gives a current ratio of 1.6, which indicates that rectification at the single-channel level is sufficient to account for the rectification of the pseudomacroscopic records. The rectification further implies that the same number of channels are opened by 1 μM Ca^{2+} at +70 mV as at −70 mV, but that the unit current is smaller at depolarized potentials. This behavior is in contrast to that of the large-conductance channel at these Ca^{2+} concentrations, in which openings occur only while the membrane is depolarized (Fig. 1). A further point to be made from Figure 3*A* is that the channels remain open as long as Ca^{2+} is present; there does not appear to be any process of inactivation or desensitization even with very long Ca^{2+} applications (>20 sec). Figure 3*A*, 2, shows an expanded record of the response recorded at −70 mV to illustrate the time course of the action of Ca^{2+}, where activity rises from 0 to maximum in 70 msec.

Ionic dependence

Three types of Ca^{2+}-activated channels have been described: nonselective cation channels, K^+ channels, and Cl^- channels (Colquhoun et al., 1981; Barrett et al., 1982; Owen et al., 1986). To evaluate the ionic selectivity of the small-conductance channels, experiments were performed with an Na^+ pipette solution (140 mM NaCl/10 mM KCl). Under these conditions, the extrapolated reversal potential for current through the channel shifted from about 0 mV to −50 mV, as expected for a channel that is primarily selective for K^+. Furthermore, the reversal potential does not shift if K-methylsulfate is used instead of KCl. These observations indicate that this small conductance channel is a Ca^{2+}-activated K^+ channel. Channel activity can still be observed if Na^+ is omitted from the intracellular face of the patch (see Fig. 5*B*); therefore, Ca^{2+} is not only necessary, but also sufficient to activate the channel, and there is no Na^+ dependence (Bader et al., 1985; Constanti and Sim, 1987).

Detailed kinetic analysis was usually precluded by the pres-

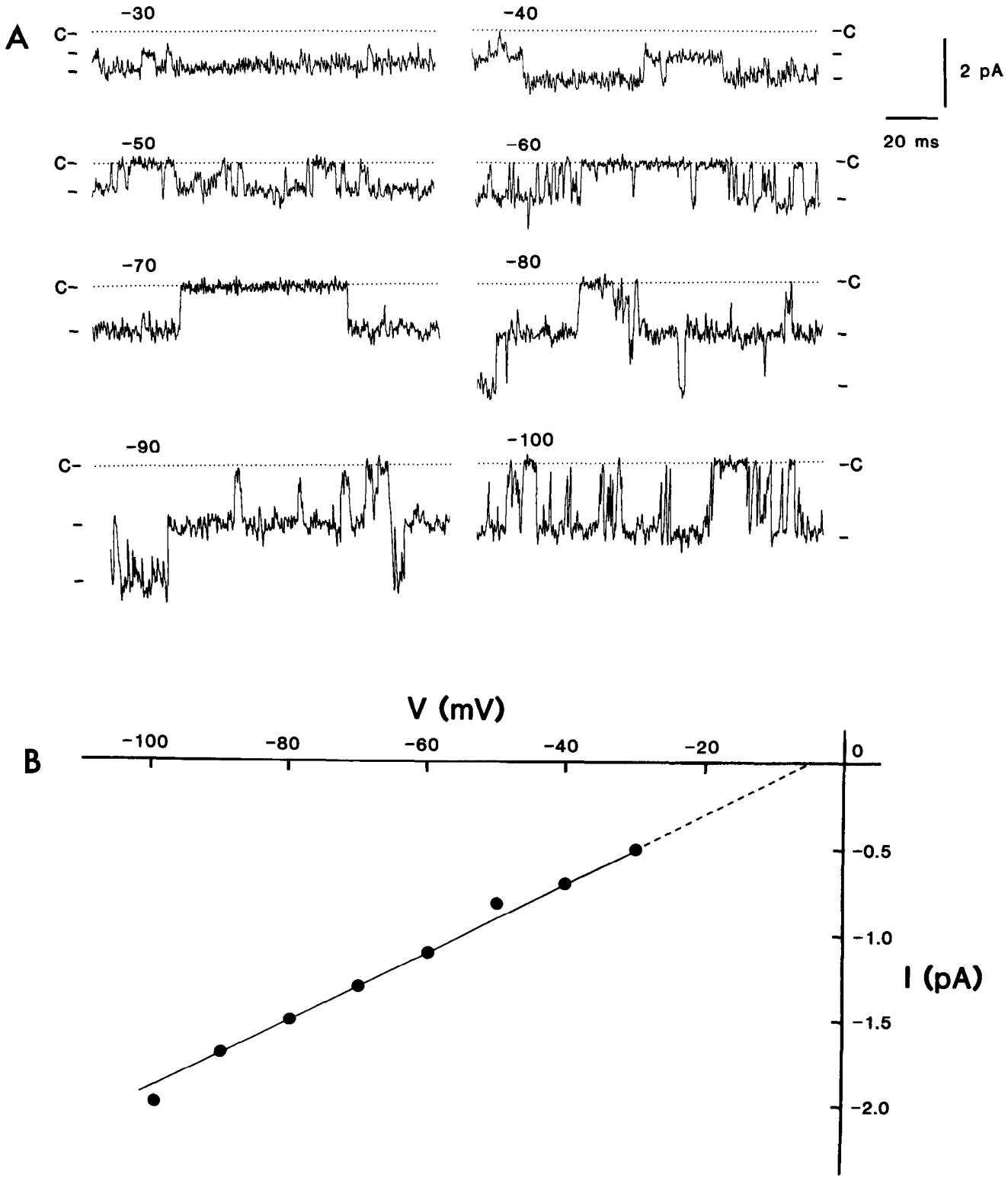

Figure 2. Linear *I/V* relation of single small-conductance Ca^{2+}-activated K^+ channels at hyperpolarized potentials. *A*, Sample traces of channel openings and closures at several potentials. In this and subsequent figures, the *dotted line* indicates the current level at which all channels were closed; *dashes* on the sides of the records denote unitary current amplitudes. *B*, *I/V* relation for the small-conductance Ca^{2+}-activated K^+ channel. Values plotted are fit by eye and indicate a slope conductance of 20 pS and a reversal potential of −4 mV.

ence of multiple channels in the patches. Figure 4 shows kinetic data from a patch in which only a single channel was observed. In general, the open time distribution could be fit satisfactorily by a single exponential function. This is shown in Figure 4*A*, in which the open time histogram is plotted for 1 μM Ca^{2+}. The histogram was fitted with a single exponential with a time constant of 7.8 msec. The closed time distribution required at least a double exponential for a reasonable fit (Fig. 4*B*). Examination

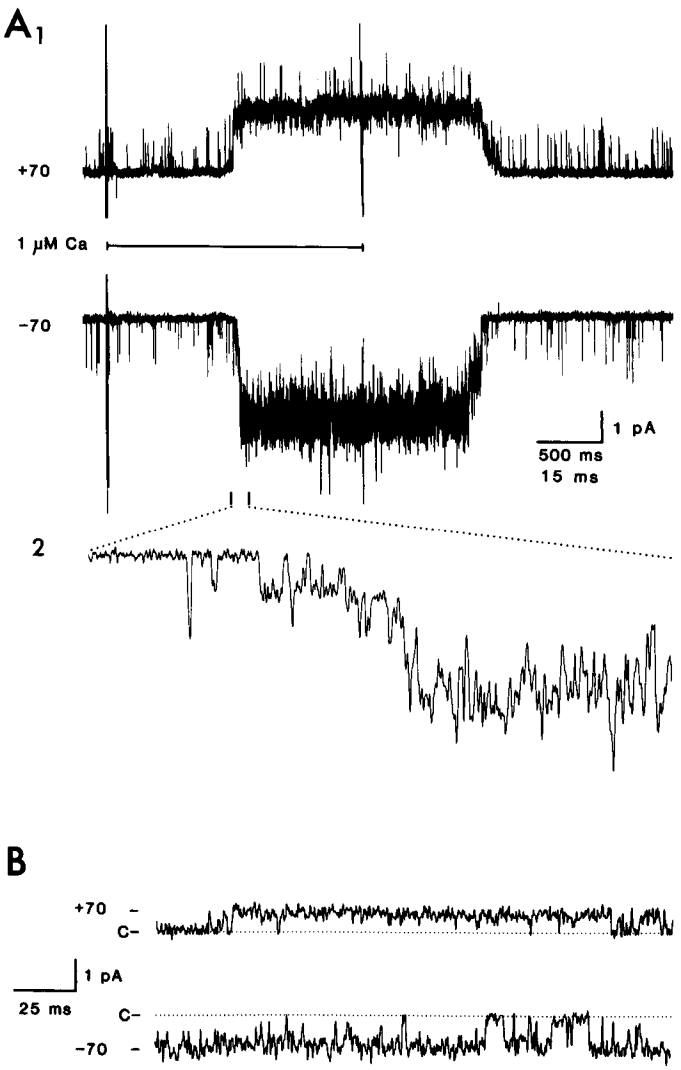

Figure 3. Rectification of the small Ca^{2+}-activated K^+ conductance. *A*, 1, Two-second applications of Ca^{2+} (1 μM) to a patch containing several small-conductance channels. The shift in baseline current associated with Ca^{2+} application is smaller at depolarized potentials (*top trace*; +70 mV) than at hyperpolarized potentials (*bottom trace*; −70 mV). *A*, 2, Expanded view of the onset of channel activity at −70 mV to illustrate single-channel openings. The *dotted line* indicates that portion of the lower trace of *A*, 1, that is illustrated here. *B*, Records from another patch containing only 1 small Ca^{2+}-activated K^+ channel. Single-channel rectification is apparent as different unit current amplitudes at +70 mV and −70 mV. All traces were obtained in the absence of Mg^{2+} at the intracellular face.

of channel activity in a number of patches at positive and negative potentials suggested little voltage sensitivity. For example, in a patch that had only one channel the open probability for 1 μM Ca^{2+} was 0.51 at −70 mV and, at +70 mV, was 0.50.

All experiments described so far were performed without Mg^{2+} at the intracellular surface. However, physiological levels of intracellular free Mg^{2+} are in the 1–2-mM range (Alvarez-Leefmans et al., 1987). When Mg^{2+} (1 mM) is present at the intracellular surface, the behavior of the small $I_{K_{Ca}}$ changes drastically. In the presence of physiological concentrations of Mg^{2+} at the cytoplasmic face, pseudomacroscopic outward patch currents were much reduced; that is, Mg^{2+} caused inward rectification. Figure 5*A* illustrates the inward and outward patch currents evoked by 1 μM Ca^{2+} in the absence of Mg^{2+} and, subsequently, in the presence of 0.5 mM Mg^{2+}. The outward current was reduced, but the inward current was unaffected by Mg^{2+}. A different patch is shown in Figure 5*B* to demonstrate the single-channel correlate of this rectification. This patch was recorded with 1 mM Mg^{2+} present at the intracellular face, and Na^+ had been omitted. The behavior of the channel was normal at −70 mV and −100 mV, but at the depolarized potentials, channel opening was followed rapidly by the conductance turning off so that no reliable measure of the unit current amplitude could be made.

The Mg^{2+} block was readily reversible as shown in Figure 6*A*. All records are from a single patch at a potential of +85 mV. In the absence of Ca^{2+} (Fig. 6, top trace), the patch was silent. After addition of 1 μM Ca^{2+}, activity of the small channel appeared; subsequent addition of 1 mM Mg^{2+} to the intracellular flow reduced the channel open time to give the flickering block also seen in Figure 5*B*. When Mg^{2+} was flushed from the intracellular face of the patch, the channel resumed normal activity. At negative potentials (in symmetrical 1 mM Mg^{2+}), the electrochemical gradient would tend to drive Mg^{2+} from the extracellular to the intracellular side of the patch. However, extracellular Mg^{2+} (1 mM) had no obvious effect on channel kinetics because it could be omitted from the pipette without altering activity.

On several occasions (as in Fig. 6*A*), the small channel could be recorded with apamin (25 nM) in the pipette. Unlike a small Ca^{2+}-activated K^+ channel in muscle cells (Blatz and Magleby, 1984) or GH_3 pituitary cells (Lang and Ritchie, 1988), the small channel in cultured hippocampal neurons is not demonstrably sensitive to apamin.

The 2 Ca^{2+}-activated K^+ channels in hippocampal neurons display different sensitivities to the K^+ channel blocker TEA. The large channel was quite sensitive to external TEA, that is, TEA included in the pipette solution (Fig. 7*A*). The 2 recordings in Figure 7*A* are from different patches, but the apparent conductance of the channel in the presence of TEA was below the normal range of conductances by about a factor of 2. An EC_{50} in the submillimolar range is the same as reported for this channel in other cells (Smart, 1987). The most likely explanation for the reduction in conductance by TEA is a rapid, unresolved block (note the increased noise during channel openings), which caused an apparent decrease in the unit current amplitude. TEA also has a similar effect on delayed-rectifier K^+ channels (Spruce et al., 1987). The small channel in hippocampal neurons was not affected by submillimolar concentrations of TEA at either depolarized or hyperpolarized potentials.

Discussion

This paper provides evidence that hippocampal neurons in culture have 2 types of K^+ channels that are activated by intracellular Ca^{2+}. In symmetrical (140 mM) K^+, the conductances are about 220 pS and 19 pS; the smaller conductance channel inwardly rectifies so that the conductance at depolarized potentials is about 11 pS.

The large Ca^{2+}-activated channel in hippocampal neurons (Franciolini, 1988; present results) has similar properties to that which has been described in numerous cell types, namely, large conductance (~200 pS), voltage dependence, and Ca^{2+} and TEA sensitivities (Marty, 1981; Pallotta et al., 1981; Adams et al.,

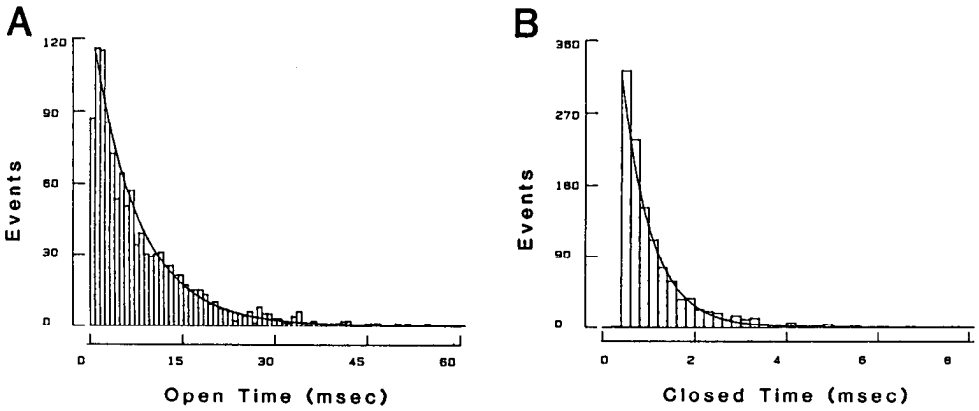

Figure 4. Open and closed time histograms for patch containing single Ca^{2+}-activated K^+ channel during prolonged (40 sec) application of 1 μM Ca^{2+} at −70 mV. *A*, Distribution of open times was fit by a single exponential of time constant 7.8 msec. *B*, Distribution of closed times was fit with the sum of 2 exponentials having time constants of 0.49 and 1.49 msec.

1982; Barrett et al., 1982; Wong et al., 1982; Iwatsuki and Petersen, 1985; Smart, 1987).

The small-conductance Ca^{2+}-activated channel appears to be a K^+ channel because the reversal potential is 0 mV in symmetrical K^+ and shifts in a negative direction with a physiological ionic gradient, indicating a selectivity for K^+ over Na^+. A Ca^{2+}-activated nonspecific cation channel has been reported (Colquhoun et al., 1981), and though it shares a lack of strong voltage dependence with the channel described here, the cation channel is permeable to both Na^+ and K^+. In addition, the nonspecific channel appears to require higher Ca^{2+} concentrations for activation and does not rectify in the presence of intracellular Mg^{2+} (Colquhoun et al., 1981), further indicating that it differs from the small channel described here. Calcium-activated Cl^- currents have been observed in central neurons (Owen et al., 1986), but the small channel reported here is unlikely to be a Cl^- channel because activity was still observed when Cl^- was replaced by the impermeant anion methylsulfate.

A last point to be made regarding the ionic dependence of the small channel is that there does not appear to be any dependence on the presence of Na^+ at the intracellular surface. It has been reported that a Ca^{2+}-activated K^+ current in neurons of the olfactory cortex has some dependence on Na^+ entry (Constanti and Sim, 1987); however, in hippocampal neurons, it is clear that Na^+ is neither necessary nor sufficient to activate the small channel (Fig. 6*B*).

As yet, we have no firm explanation for the rectification displayed by the small channel in the absence of internal Mg^{2+}, but one possibility is that there is a degree of blockade by the 10 mM Na^+ present in the solutions. Blockade by intracellular Na^+ (Yellen, 1984) may be temporally unresolved and appear as a decrease in the amplitude of outward currents; in this case, it could be similar to the Mg^{2+} block that we observed. However, it seems unlikely that sodium and magnesium would interact with the same binding site because of their different valencies and ionic radii (Na^+, 0.95 Å; Mg^{2+}, 0.65 Å). A second possibility would be some channel asymmetry such that negative charges on one side of the channel would cause local accumulation of K^+ ions over and above the concentration present in the bulk solution, analogous to the mechanism proposed to account for the large conductance yet high selectivity of some K^+ channels (Jordan, 1987).

At negative membrane potentials, the small channel has a higher Ca^{2+} sensitivity than the large channel. This quality is

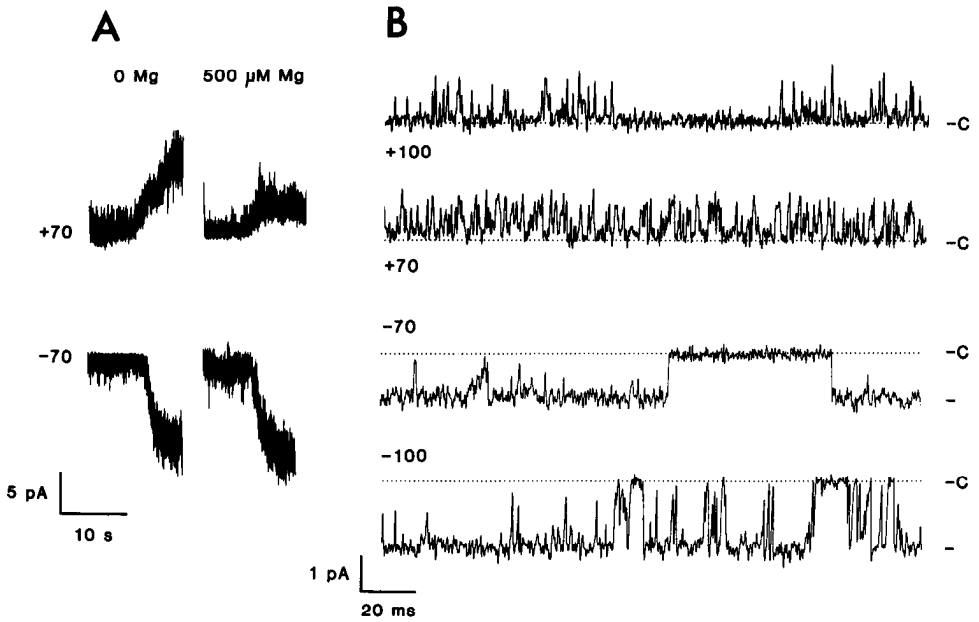

Figure 5. Voltage-dependent blockade of small channels by cytoplasmic Mg^{2+}. *A*, Onset of Ca^{2+}-induced channel activity in a single patch at +70 mV and −70 mV in the presence of nominally 0 mM Mg^{2+} and 500 μM Mg^{2+}. The current is substantially reduced by Mg^{2+} at the depolarized potential. *B*, Single-channel correlate of the Mg^{2+} blockade. Channel activity at depolarized potentials is flickery and has no clearly defined unit current amplitude. Na^+ was omitted from the cytoplasmic solution.

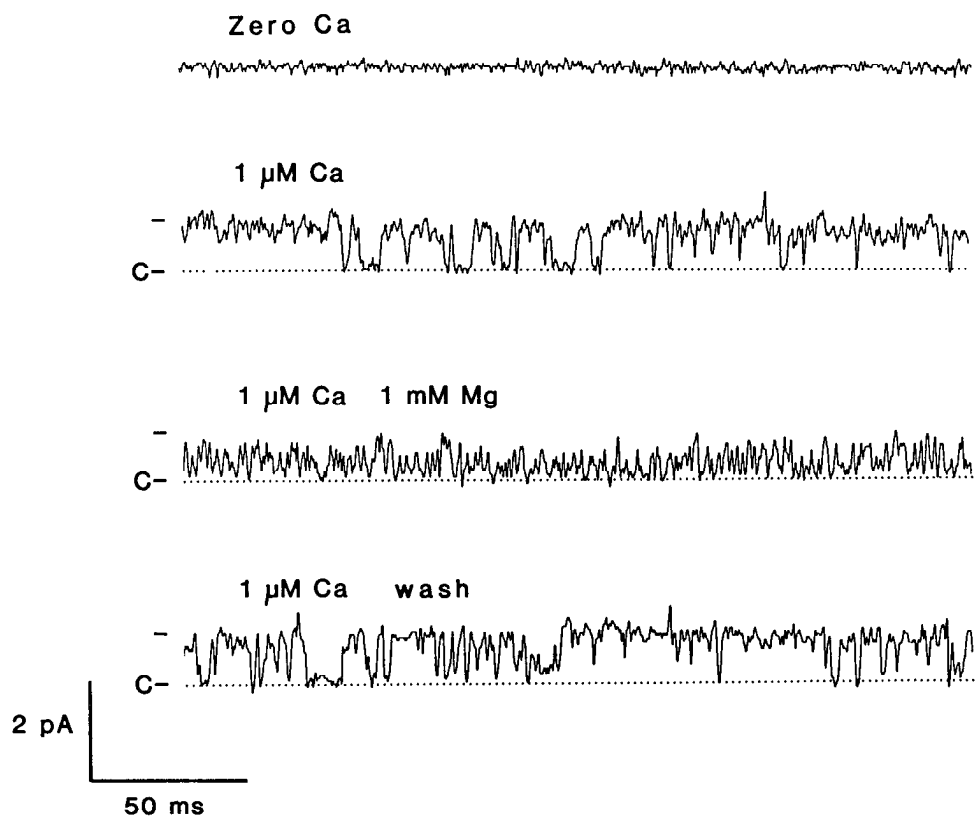

Figure 6. Reversible effects of 1 mM Mg^{2+} on Ca^{2+}-activated channel openings. These records are from a single patch showing the blocking effect of 1 mM Mg^{2+} on the cytoplasmic face. The membrane potential was +85 mV. Records were digitally filtered at 625 Hz.

also found for small versus large channels in muscle (Blatz and Magleby, 1986) and GH_3 cells (Lang and Ritchie, 1988). In 1 μM Ca^{2+}, the small channel is active at least 50% of the time regardless of membrane potential. In contrast, 1 μM Ca^{2+} rarely opens the large channel at negative potentials and only during considerable depolarization is activity observed. A further contrast between the large and small channels is provided by the differential effects of low concentrations of TEA, which cause significant blockade of the large channel without obvious effect on the small channel.

The small Ca^{2+}-activated K^+ channels (but not the large conductance channels) are blocked when Mg^{2+} is present at the intracellular face of the patch at depolarized potentials. This behavior is reminiscent of local anesthetic block of the ACh receptor (Neher and Steinbach, 1978). As the channel opens at depolarized potentials, the magnesium ion is driven into the channel by the positive intracellular potential; hence, the apparent closures are really blocking events. At large potentials, the driving force on the magnesium ion is greater, and the block is more pronounced. This block causes a profound inward rectification and is a recently uncovered mechanism that accounts for the inwardly rectifying potassium current (Matsuda et al., 1987; Vandenberg, 1987) and is seen in the ATP-sensitive K^+ channel (Horie et al., 1987), both of cardiac ventricular cells. As in these previous studies, Mg^{2+} block of the small Ca^{2+}-activated K^+ channel seems to be voltage dependent, which indicates that the binding site lies within the voltage drop across the membrane. Because external Mg^{2+} does not have the blocking effect on the channel that internal Mg^{2+} has, the channel must present asymmetric access to the blocking site. The slight rectification observed in the absence of Mg^{2+}, with symmetrical charge carrier, also implies an asymmetric channel. It is unclear whether the Mg^{2+} block plays any role under normal physiological conditions.

The results in this paper allow us to make some simple comparisons between the channels we have observed and macroscopic currents that have been reported. Ca^{2+}-activated K^+ currents of at least 2 distinct types have been observed in a number of preparations, including muscle (Blatz and Magleby, 1984; Romey and Lazdunski, 1984); *Aplysia* (Deitmer and Eckert, 1985), bullfrog sympathetic neurons (Goh and Pennefather, 1987), GH_3 pituitary cells (Ritchie, 1987), rat sympathetic neurons (Kawai and Watanabe, 1986; Smart, 1987), and rat hippocampal neurons (Brown and Griffith, 1983; Lancaster and Adams, 1986). A voltage- and TEA-sensitive current comparable to the large channel appears to be involved in action-potential repolarization (Adams et al., 1982; Gola et al., 1986; Lancaster and Nicoll, 1987; Lancaster and Pennefather, 1987; Storm, 1987), and activation of this channel during action potentials has been demonstrated directly (Lang and Ritchie, 1988). While the involvement of this channel in repolarization has been challenged (Ikemoto et al., 1989), the pharmacological characteristics of repolarization closely match those of the large channel.

The TEA- and voltage-insensitive conductance (I_{AHP}; Pennefather et al., 1985) forms only a minor fraction of the total Ca^{2+}-activated conductance, at least in the bullfrog (Lancaster and Pennefather, 1987) and GH_3 cells (Ritchie, 1987) and thus is unlikely to contribute greatly to action-potential repolarization. However, this current seems to remain active as long as intracellular Ca^{2+} is raised after action potentials/depolarization, which is of the order of hundreds of milliseconds (Smith et al., 1983). Functionally, this slow $I_{K_{Ca}}$ acts to curtail repetitive firing (Madison and Nicoll, 1984; Goh and Pennefather, 1987). It

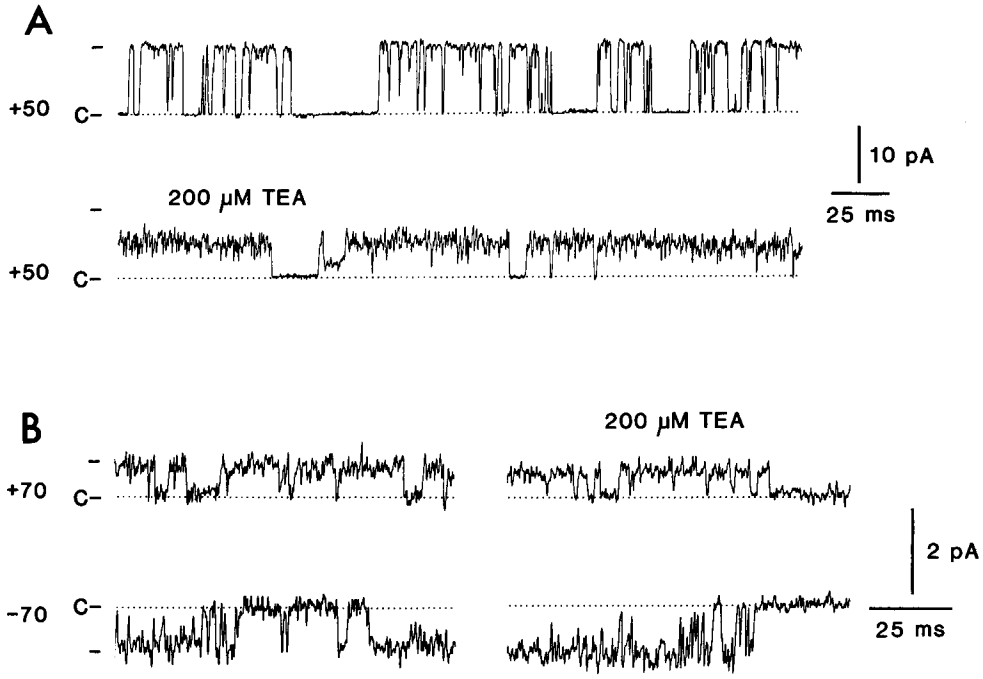

Figure 7. Effects of TEA on Ca^{2+}-activated K^+ channels. *A*, With 200 μM TEA in the pipette, the large channel's apparent unit current amplitude is reduced by rapid blocking events that could not be resolved. *B*, In contrast, the small channel is not obviously affected by extracellular TEA.

should be noted that the voltage-insensitive $I_{K_{Ca}}$ in GH_3 cells and bullfrog neurons is blocked by apamin (Goh and Pennefather, 1987; Ritchie, 1987), whereas the functionally equivalent current in hippocampal neurons is apamin insensitive (Lancaster and Nicoll, 1987). The small conductance channel we have described is a likely candidate to underlie the Ca^{2+}-activated K^+ current (I_{AHP}), which generates the slow AHP and spike frequency adaptation in hippocampal neurons. A similar conclusion has been reached for apamin-sensitive channels in GH_3 cells (Lang and Ritchie, 1988). It remains to be determined whether the small channel reported here displays the transmitter sensitivity that has been demonstrated for I_{AHP}.

An interesting difference between I_{AHP} in bullfrog ganglion neurons and rat hippocampal neurons is the time course. In ganglion cells, I_{AHP} is maximal immediately after a spike or burst of spikes (Pennefather et al., 1985), whereas in hippocampal neurons, there is a pronounced delay of a few hundred milliseconds before the peak AHP conductance is reached (Lancaster and Adams, 1986). Although it is not entirely clear why this delay occurs, there are a number of constraining facts that rule out possible mechanisms. Because $I_{K_{Ca}}$ is involved in spike repolarization, it is clear that Ca^{2+} can enter the cell during an action potential. Why, then, does I_{AHP} take so long to reach a peak? One possibility is that the AHP channel has extremely slow kinetics, but this explanation is unlikely because activation can be quite rapid (Fig. 3*A*, 2). An alternate explanation for the delay could be that there is involvement of a further messenger subsequent to Ca^{2+} entry; however, Ca^{2+} activation of the small channels does not require soluble second messengers because the small channels can be observed in an inside-out patch in a flow system. The fairly rapid activation of these channels also tends to argue against the involvement of membrane-bound second messengers; however, it is conceivable that, in intact cells, channel opening is delayed by some cytoplasmic constituent that is lost during patch excision. A third remote possibility for the delay could be Ca^{2+}-induced Ca^{2+} release as described in muscle (Endo et al., 1970). There is no evidence for this process in hippocampal cells, and furthermore, dantrolene does not affect the slow AHP (B. Lancaster and R. A. Nicoll, unpublished observations). Experiments in hippocampal slices were carried out at a higher temperature than the patch-clamp experiments, but this would only act to obscure any differences. Given what is known about the small Ca^{2+}-activated K^+ channel and the slow development of I_{AHP} in the intact cell, these facts suggest that the majority of small channels may simply not have immediate access to the raised intracellular Ca^{2+} that follows a burst of action potentials.

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