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Extracellular calcium sensed by a novel cation channel in hippocampal neurons

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ABSTRACT **Extracellular concentrations of Ca^{2+} change rapidly and transiently in the brain during excitatory synaptic activity. To test whether such changes in Ca^{2+} can play a signaling role we examined the effects of rapidly lowering Ca^{2+} on the excitability of acutely isolated CA1 and cultured hippocampal neurons. Reducing Ca^{2+} excited and depolarized neurons by activating a previously undescribed nonselective cation channel. This channel had a single-channel conductance of 36 pS, and its frequency of opening was inversely proportional to the concentration of Ca^{2+}. The inhibition of gating of this channel was sensitive to ionic strength but independent of membrane potential. The ability of this channel to sense Ca^{2+} provides a novel mechanism whereby neurons can respond to alterations in the extracellular concentration of this key signaling ion.**

The entry of Ca^{2+} into neurons provides a major signal for the regulation of Ca^{2+}-dependent channels and enzymes and contributes to the induction of various forms of synaptic plasticity including long-term potentiation (1). In contrast, little attention has been paid to the possibility that changes in extracellular Ca^{2+} might themselves play a specific signaling role in controlling postsynaptic excitability. Excitatory activity causes a transient decrease in the extracellular concentration of Ca^{2+} due to activation of both postsynaptic glutamate receptors of the *N*-methyl-D-aspartate (NMDA) subtype and voltage-dependent Ca^{2+} channels (2–6). Decreases in extracellular Ca^{2+} are most pronounced in the pyramidal cell layers of the hippocampus, where it can fall by as much as 0.6 mM during trains of stimulation. These changes in Ca^{2+} are further enhanced during seizure activity (2–6).

Lowering Ca^{2+} reduces its charge-shielding effects on groups located at the membrane surface (7, 8). By this mechanism, Ca^{2+} nonselectively influences the activation of voltage-dependent currents (8). Calcium also alters the gating and permeation properties of ion channels (7). In some cases, the selectivity of a channel can be lost when Ca^{2+} is reduced. For example, Na^+ will readily permeate Ca^{2+} channels when extracellular Ca^{2+} is lowered to nanomolar concentrations (9). A loss of selectivity may account for a rapidly inactivated inward current recorded in neurons in response to a step decrease in extracellular Ca^{2+} (10).

Ca^{2+} also blocks a variety of nonselective cation channels. For example, Ca^{2+} suppresses a nonselective cation current in *Xenopus* oocytes (11). Membrane depolarization or lowering Ca^{2+} relieves the inhibition and reveals the presence of this current. Other nonselective cation channels blocked by Ca^{2+} include nucleotide-gated (12, 13), stretch-activated cation (14), hemi-gap junction (15), and Ca^{2+}-selective channels (16). For most of these channels Ca^{2+} likely binds to a site located within the channel pore, thus accounting for the voltage dependence of the currents (11).

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Activation of NMDA receptors results in an influx of Ca^{2+} into neurons and a decrease in extracellular Ca^{2+}. NMDA responses inactivate with the influx of Ca^{2+} (17–20), and this may occur through the binding of calmodulin to the C termini of NMDA receptor subunits (21). Inactivation of these currents can be minimized if extracellular Ca^{2+} is reduced to or below 200 μM during the application of NMDA (17–20). Using similar approaches we have found that decreasing Ca^{2+} can itself activate an inward current. Therefore, we have investigated the responses of both cultured and acutely isolated CA1 hippocampal neurons to transient decreases in the extracellular concentration of Ca^{2+}.

METHODS

Cultured mouse hippocampal neurons were grown as previously described (22) and were used for recordings 12–20 days after plating. CA1 pyramidal neurons were isolated from neonatal rats according to Wang and MacDonald (23). The extracellular solution contained 140 mM NaCl/5.4 mM KCl/25 mM Hepes/33 mM glucose, with (0.0005–0.001 mM) or without tetrodotoxin, pH 7.4, using NaOH; 320–335 mosmolar. NMDA and extracellular solutions containing various concentrations of Ca^{2+} were applied using a multibarreled perfusion apparatus.

Patch electrodes contained 140 mM CsCl or CsF/35 mM CsOH/10 mM Hepes/2 mM $MgCl_2$/11 mM EGTA/2 mM TEA/1 mM $CaCl_2$/4 mM MgATP, pH 7.3, using CsOH; 300 mosmolar. In some experiments no $CaCl_2$ was added and EGTA was replaced with 11 mM 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA). The relative Na^+ and K^+ permeability ratios P_{Na}/P_{Cs} and P_K/P_{Cs} were determined from the reversal potentials in Na^+-rich (150 mM NaCl/25 mM Hepes/33 mM glucose) or K^+-rich (20 mM KCl/130 mM *N*-methyl-D-glucamine/25 mM Hepes/33 mM glucose) solutions using the constant field equation in the following form:

$$P_{Na}/P_{Cs} = \frac{[Cs^+]_i \cdot e^{(EF/RT)} \cdot [e^{(EF/RT)} + 1]}{2[Na^+]_o}, \qquad [1]$$

where E is the reversal potential and F, R, and T have their usual meanings.

The single-channel open probability was determined from the ratio of the time spent in the open state to the duration of recording: $P_o = (t_1 + t_2 + \ldots + t_n)/Nt_{tot}$, where t is the amount of time that n channels are open and N is the maximum number of levels observed in the patch. Data are expressed as the mean ± SEM, and a paired Student's t test was employed.

RESULTS

In the presence of physiological concentrations of both Ca^{2+} and Mg^{2+} a rapid reduction in Ca^{2+} from 1.5 to 0.5 mM strongly depolarized and excited neurons (Fig. 1*A*). This

Abbreviation: NMDA, *N*-methyl-D-aspartate.

graded depolarization was readily reversible and it was insensitive to tetrodotoxin (Fig. 1*B*). In such recordings (Fig. 1 *C* and *D*) a threshold for excitation was detected with as little as a 100 μM decrease in Ca^{2+}.

To examine the mechanism of this excitation, cells were bathed in a solution lacking Mg^{2+} and voltage clamped to a holding potential of −60 mV. Reducing Ca^{2+} from 2 to 0 mM (nominally free) induced a slowly activating (τ = 992 ± 85 ms, n = 28) sustained inward current that rapidly recovered (Fig. 2 *A* and *B*). The amplitude of this current ranged from 150 to 1,500 pA (mean 803 ± 120 pA, n = 8, acutely isolated CA1 neurons; 745 ± 30 pA, n = 76, cultured hippocampal neurons). The reversal potential for the response to low Ca^{2+} was approximately 0 mV (Fig. 2*B*).

In some neurons we examined NMDA-induced currents as well as responses to lowered concentrations of Ca^{2+}. NMDA currents demonstrated various degrees of inactivation (Fig. 2*C*, a and b). When the concentration of Ca^{2+} was simultaneously reduced to 200 μM, inactivation of the currents was reduced (Fig. 2*C*). However, lowering Ca^{2+} itself generated a current of a magnitude, that, with appropriate kinetics, was sufficient to account for the apparent loss of inactivation in some cases (Fig. 2*C*, a) and contribute to the apparent reduction of inactivation in others (Fig. 2*C*, b). The responses to low Ca^{2+} were insensitive to competitive antagonists of NMDA (AP5, 100 μM) and AMPA receptors (CNQX, 100 μM), demonstrating that these responses were not the consequence of activation of glutamate receptors. We also examined the response of human epithelial kidney (HEK293) cells to step reductions in Ca^{2+} because recombinant NMDA receptors are most often expressed in these cells. Reductions of Ca^{2+} in the absence of NMDA also evoked large inward currents in some of these cells (Fig. 2*C*, c).

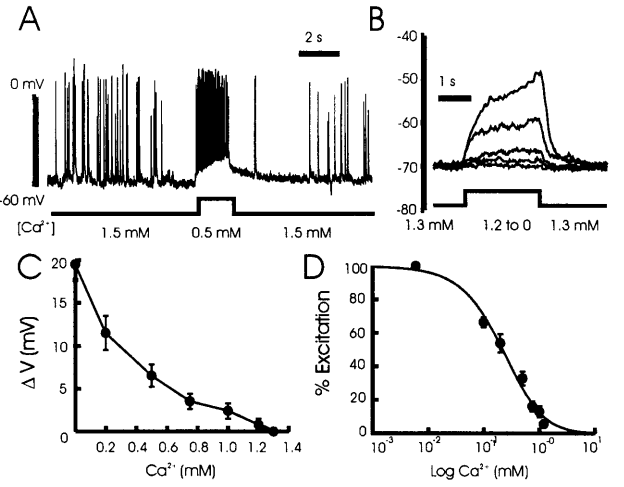

FIG. 1. The effect of lowering Ca^{2+} on the excitability of hippocampal neurons. (*A*) The membrane potential of a cultured neuron was recorded using the whole-cell current-clamp configuration (KCl instead of CsF in the recording pipette). Decreasing Ca^{2+} from 1.5 to 0.5 mM for a period of 2 s depolarized and strongly excited the cell. (*B*) In the presence of 0.5 μM tetrodotoxin and 1.5 mM Mg^{2+}, reductions of Ca^{2+} from 1.3 to 1.2, 1.0, 0.5, 0.2, and 0 mM respectively, evoked graded depolarizations. (*C*) In a series of six recordings, the amplitude of the depolarization was recorded and values plotted versus the concentration of Ca^{2+}. The control solution contained 1.3 mM Ca^{2+}, and all of the solutions contained 1.5 mM Mg^{2+}. (*D*) The normalized dose–response curve for the depolarization induced by lowering Ca^{2+}. The depolarization was normalized to that induced by the solution containing no added Ca^{2+}. The nominal concentration of Ca^{2+} in this solution was estimated to be about 20 μM (24). The logistic equation in the form $R = R_{max}/[1 + (D/EC_{50})^{n_H}]$ was used to fit the points. R represents the normalized depolarization at any given Ca^{2+} concentration, R_{max} is the response with no added Ca^{2+}, the EC_{50} value is the concentration of Ca^{2+} that produced 50% of the maximal response, and n_H is the estimated Hill coefficient (EC_{50} = 0.39 ± 0.11 mM; n_H = 1.4 ± 0.3; n = 6).

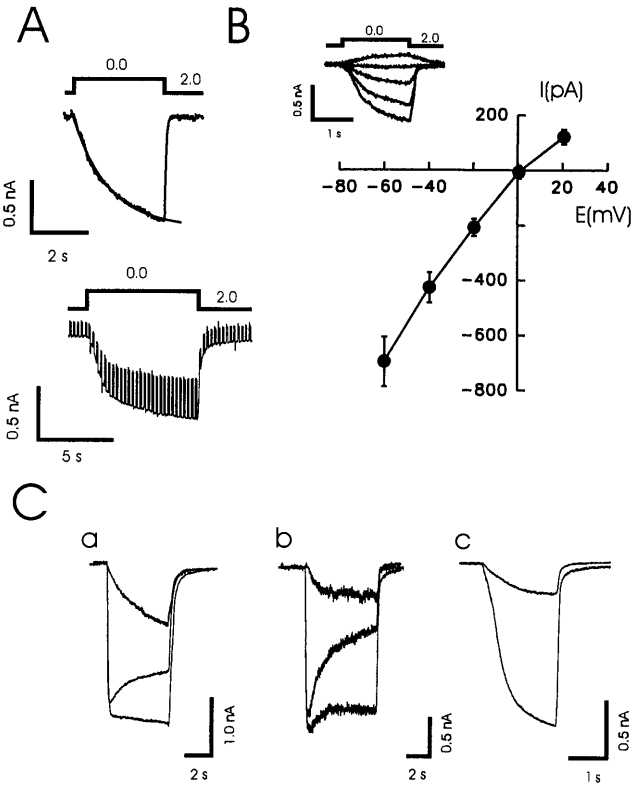

FIG. 2. Lowering Ca^{2+} induces a nonspecific cation current in cultured and isolated hippocampal neurons. (*A*) A step reduction in the concentration of Ca^{2+} evoked a substantial inward current in a cultured hippocampal neuron. This inward current was associated with an increase in membrane conductance as assessed by repeating a depolarizing voltage step. (*B*) In a group of 15 neurons the current–voltage relationships (I-V) were determined for the response to a change from 2 to 0 mM Ca^{2+} while holding at values from −60 to +20 mM. The currents demonstrated a near-linear I-V curve and reversed at +0.3 ± 1.8 mV. (*C*) Superimposed responses to NMDA (100 μM, 3 μM glycine) with and without a simultaneous decrease in Ca^{2+} (to 200 μM) are shown for two cultured neurons (a and b). The smallest current in each case is the superimposed response to a decrease in Ca^{2+} alone. A response of a HEK293 cell to a decrease in Ca^{2+} (2 mM to 200 μM) alone (NMDA was not applied) is shown before and following a partial block with Gd^{3+} (2 μM) (c). These currents ranged from 50 to 1500 pA in different HEK cells.

The current evoked by lowering Ca^{2+} was dependent on the presence of either extracellular Na^+ or K^+ (Fig. 3*A*). Reducing the concentrations of both NaCl and KCl in the extracellular solution by half shifted the reversal potential toward more negative values (a 15.3 ± 2.8 mV shift, n = 4; Fig. 3 *B* and *C*) as anticipated for a nonselective cation current. A more detailed analysis showed the following rank order of relative permeability ratios: Na^+ (1.3) ≥ K^+ (1.28) > Cs^+ (1). In contrast, no anion permeability was detected and there was no shift in the reversal potential when all extracellular Cl^- was substituted with gluconate (Fig. 3 *D* and *E*). In addition, no currents were detected in response to a decrease of Ca^{2+} from 2.0 to 0.5 mM when all monovalent cations were replaced with the nonpermeant ion *N*-methyl-D-glucamine (not shown), suggesting that the Ca^{2+} permeability of these channels is unlikely to be substantial. Suction or pressure applied to the patch electrode did not alter the responses to lowered Ca^{2+}.

A number of drugs, reported to block nonselective cation channels, including flufenamic acid (25), spermine (26, 27), and amiloride (28), reversibly reduced the amplitude of this inward current (−60 mV, 100 μM flufenamic acid, 39.7 ±

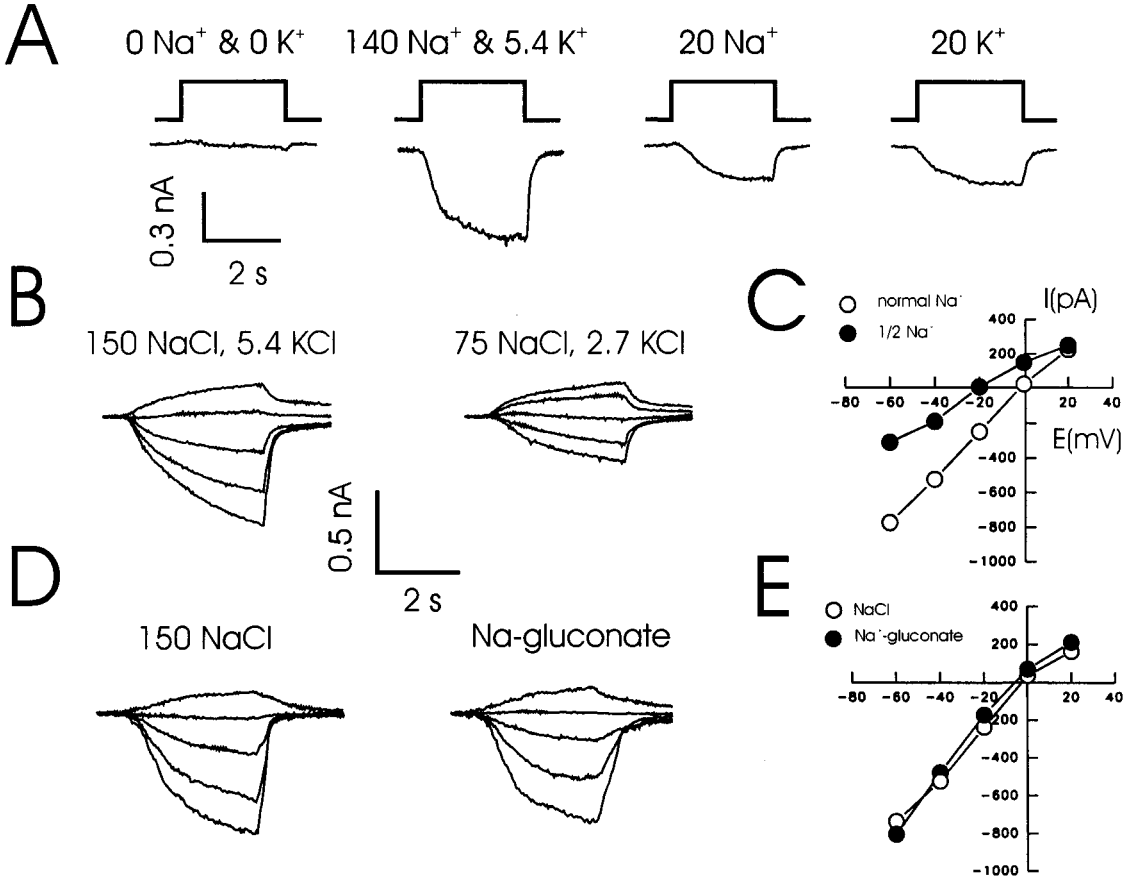

FIG. 3. The inward current evoked by decreasing Ca^{2+} is carried by Na^+ and K^+ but not by Cl^-. (*A*) Example traces showing the responses of cultured hippocampal neurons to a decrease in Ca^{2+}. Ca^{2+} was reduced from 2.0 to 0 mM in four different extracellular solutions: (*i*) No Na^+ or K^+, replacement with 150 mM NMDG; (*ii*) in the presence of 140 NaCl and 5.4 mM KCl; (*iii*) in 20 mM NaCl; and (*iv*) in 20 mM KCl. In the absence of monovalent cations, step reductions of Ca^{2+} evoked little or no inward current, demonstrating that the response was not due to some kind of nonspecific breakdown of the membrane. However, when the usual extracellular solution (140 NaCl, 5.4 KCl) was used, an inward current with an amplitude of about 500 pA was recorded from the same cell. A smaller current was recorded when the extracellular solution contained only 20 mM Na^+ or only K^+. The holding potential was −60 mV. (*B*) A step reduction in the concentration of Ca^{2+} was used to evoke responses in the presence of a solution containing 150 mM NaCl and 5.4 mM KCl or a second solution with half the concentration of Na^+ (75 mM) and K^+ (2.7 mM). In each case the holding potential of the cell was varied between −60 and +20 mV. (*C*) Such data ($n = 3$) was then used to construct I-V curves. When the concentration of monovalent ions was reduced by one-half, the reversal potential shifted toward more hyperpolarized values (see text). (*D*) Substitution of all of the extracellular Cl^- with gluconate had little influence on the responses to lowered Ca^{2+} and did not alter the I-V curve ($n = 3$) for this response (*E*).

5.2%, $n = 6$, $P < 0.01$; 1 mM amiloride, 63.7 ± 8.4%, $n = 7$, $P < 0.01$; 1 mM spermine, 49.0 ± 10.4%, $n = 3$, $P < 0.05$). On the other hand, the inward current evoked by lowering Ca^{2+} was unaltered by the inclusion of nucleotides (e.g., GTP[γS], 1.5 mM; GDP[βS], 0.5 mM) in the recording pipette, suggesting that it was not mediated by a G protein-coupled channel or by a nucleotide-gated cation channel (29), nor was it eliminated by the addition of high concentrations of either EGTA or BAPTA (Ca^{2+} chelators) to the recording pipette.

Hablitz *et al.* (10) reported that reductions in Ca^{2+} evoked a rapidly inactivating inward current in avian dorsal root ganglia neurons that was blocked by verapamil and Cd^{2+}. They attributed this current to voltage-dependent Ca^{2+} channels that had lost their ionic selectivity. In contrast, the current we observed in central neurons was noninactivating and it was not blocked (not shown) by the Ca^{2+} channel antagonists verapamil (50 μM) or nitrendipine (50 μM).

We next examined the relative sensitivity of this current to various divalent cations (Fig. 4). A change in Ca^{2+} from 2 to 0 mM was used to activate the current, and differing concentrations of test cations were then added to construct dose-inhibition relationships. The concentration of Ca^{2+} that inhibited 50% of the current (IC_{50}) was 0.15 mM (±0.04 mM; $n = 7$) (Fig. 4*B*). Magnesium was less potent with an IC_{50} of 0.34 mM (± 0.10 mM, $n = 5$) as were Ba^{2+} ($IC_{50} = 0.32 \pm 0.10$ mM, $n = 5$) and Cd^{2+} ($IC_{50} = 0.37 \pm 0.05$ mM, $n = 4$).

Gadolinium and neomycin are potent activators of a recently cloned calcium-sensing receptor (30). Furthermore, mechanosensitive, nonselective cation channels are highly sensitive to Gd^{3+} (31). Therefore, we tested the effects of Gd^{3+} and neomycin on the current generated by reducing Ca^{2+} (Fig. 4 *A* and *B*). Gadolinium was the most potent blocker of this current, with an IC_{50} of 1.4 μM (±0.49, $n = 5$), followed by neomycin (4.3 ± 1.1 μM, $n = 6$).

Calcium functions to stabilize membrane potential by shielding negatively charged groups on the plasma membrane. By this means, Ca^{2+} can influence the gating properties of a number of ion channels (7, 8). To further explore the possibility that this mechanism accounted for the activation of the nonselective cation current, we reduced the ionic strength of the extracellular solution by 25%. This would reduce the shielding effects of monovalent cations and thus reduce their potential competition with divalent cations at these sites. Consistent with this hypothesis, reducing the ionic strength increased the apparent affinity for Ca^{2+} and Mg^{2+} by a factor of 3 to 4 (Fig. 4*C*).

Many of the previously described nonselective cation channels are voltage-dependent or at least demonstrate a voltage-

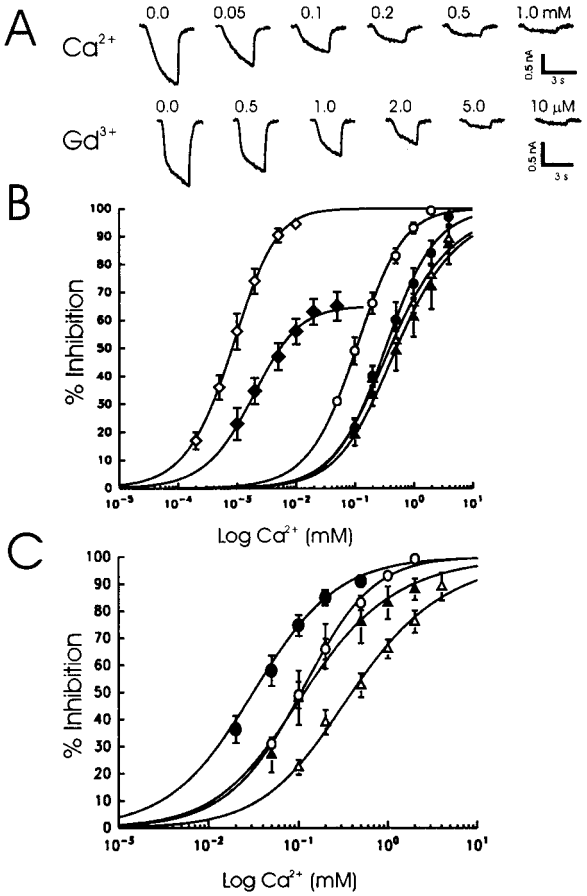

FIG. 4. Divalent and polyvalent cations block the inward currents induced by lowering Ca^{2+}. (*A*) Dose-dependent block of inward currents. The inward current was induced by a reduction of Ca^{2+} from 2 to 0 mM at a holding potential of −60 mV. Different concentrations of cations were added to the low Ca^{2+} solution, and the normalized inhibition of this current was plotted against the concentration of a given cation (*B*). The dose-inhibition curves were fitted using the logistic equation. The doses that produced 50% block of the current were: Gd^{3+} (◇), 0.0014 ± 0.00049 mM (n = 5); neomycin (◆), 0.0043 ± 0.0011 mM (n = 6); Ca^{2+} (○), 0.15 ± 0.04 mM (n = 7); Ba^{2+} (●), 0.32 ± 0.10 mM (n = 4); Mg^{2+} (△), 0.34 ± 0.14 mM (n = 5); and Cd^{2+} (▲), 0.37 ± 0.05 mM (n = 4). Higher concentrations of neomycin activated an additional inward current, thus apparently limiting the inhibition of the nonspecific cation current. (*C*) The apparent affinity of divalent cations was increased when the ionic strength in the extracellular solution was reduced. The open symbols represent Ca^{2+} (○) and Mg^{2+} (△) dose-inhibition curves in the presence of solutions of normal ionic strength (140 mM NaCl, 5.4 mM KCl), and the filled symbols represent Ca^{2+} (●) and Mg^{2+} (▲) in the presence of solution of reduced ionic strength (35 mM NaCl, 1.4 mM KCl, supplemented with sucrose). The recording pipettes contained either CsCl (or CsF) and 2 mM TEA.

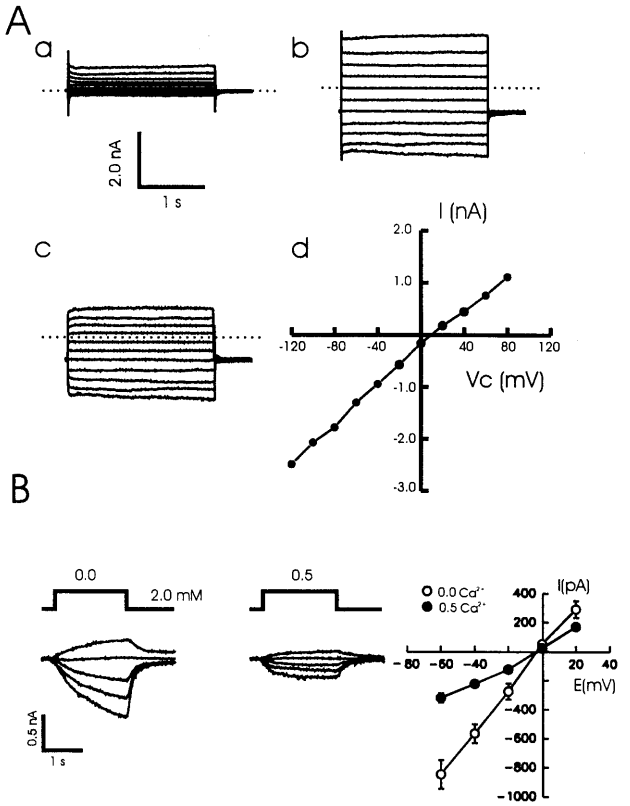

FIG. 5. The nonselective cation current demonstrates little or no voltage dependence. (*A*) An example recording from a cultured hippocampal neuron (holding, −40 mV). In (a) the response to a series of voltage steps from −120 to +80 mV in the presence of Ca^{2+} (2 mM) is shown. The same series was then repeated following a decrease of Ca^{2+} to nominally free values (b), and the difference responses (b-a) were calculated (c). The dotted line marks the initial holding current. No current relaxations were observed, and the I-V curve (d) was linear. Identical results were observed in another four cells. The reversal potential was slightly more positive than 0 mV because of the pipette solution that had been diluted (120 mM CsF). (*B*) Steady-state currents in response to two different changes in Ca^{2+} concentration (0 and 0.5 mM) were also examined. The I-V curves (n = 3) for each were approximately linear over the range tested, and both reversed at 0 mV, demonstrating that Ca^{2+} did not cause an obvious voltage-dependent block.

dependent blockade by Ca^{2+}. We therefore examined whether or not the nonselective cation current in neurons showed any voltage dependency. A series of voltage steps was made in the presence and absence of Ca^{2+} (Fig. 5*A*). No voltage relaxations were observed during these steps (Fig. 5*A*, c) and the current-voltage relationships were linear, demonstrating that this current is not voltage-dependent. In another series of cells we activated this current by decreasing Ca^{2+} to one of two different values (0 or 0.5 mM) while the holding potential was set at values between −60 and +20 mV (Fig. 5*B*). The steady-state relationships between holding potential and current amplitude were about linear for both Ca^{2+} concentrations.

Using outside-out patches we then examined whether or not we could detect single-channel events in response to decreases in Ca^{2+}. Reducing Ca^{2+} consistently induced inward current channel openings (Fig. 6*A*). The relationship between holding potential and the amplitude of single-channel currents was near linear (Fig. 6*B*), yielding a single-channel conductance of 36 pS. In another series of patches, the open probability (P_o) of these channels was substantially reduced by 2 μM Gd^{3+} (Fig. 6*C*) without any change in the single-channel amplitude (control P_o = 0.21 ± 0.13; Gd^{3+} P_o = 0.10 ± 0.07, $P < 0.05$, n = 5). Increasing Ca^{2+} to 0.5 mM also decreased the single-channel open probability (0 mM Ca^{2+}, P_o = 0.24 ± 0.09; 0.5 mM Ca^{2+}, P_o = 0.09 ± 0.05, $P < 0.01$, n = 4) without affecting either single-channel amplitude or open times. This evidence demonstrates that both Gd^{3+} and Ca^{2+} primarily act to inhibit the gating of this nonselective cation channel.

DISCUSSION

Our results demonstrate that hippocampal neurons possess a unique nonselective cation channel whose gating is sensitive to changes in the concentration of extracellular Ca^{2+}. Even in the presence of Mg^{2+}, which can itself inhibit gating of this channel, decreases in Ca^{2+} were sufficient to activate inward currents and excite neurons. Furthermore, during intense

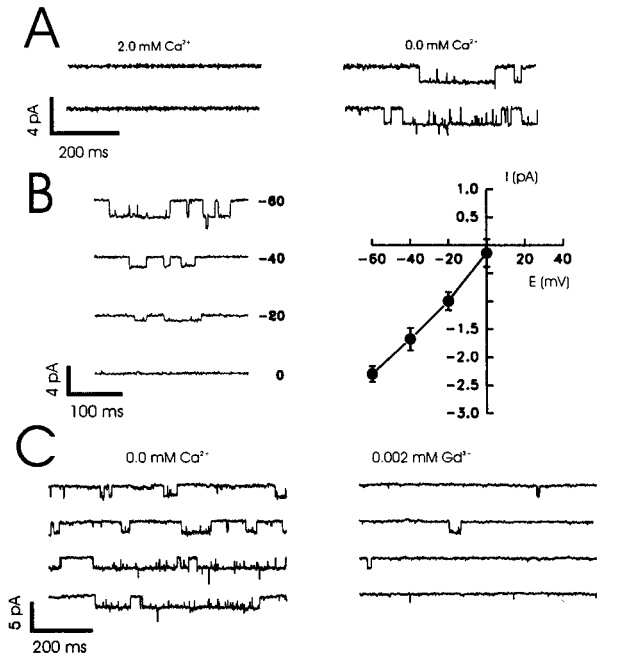

FIG. 6. Single-channel recordings in outside-out patches taken from cultured hippocampal neurons. (*A*) An example record showing the activation of single-channel openings in response to a step reduction in the concentration (2 to 0 mM) of Ca^{2+}. The holding potential was −60 mV. (*B*) I-V relationship for single-channel currents. Representative trances show the activation of these currents at holding potentials ranging from −60 to 0 mV. The I-V curve was constructed from the results of recordings from eight such patches. The channel showed a linear I-V relationship with a slope conductance of 36 ± 2.6 pS ($n = 8$). (*C*) Effect of Gd^{3+} on single-channel currents. Gd^{3+} (0.002 mM) significantly decreased the channel-open probability (control $P_o = 0.21 \pm 0.13$; Gd^{3+} $P_o = 0.10 \pm 0.07$, $n = 5$, $P < 0.05$) without having any effect on single-channel amplitude.

synaptic excitation, decreases in extracellular Ca^{2+} are often associated with simultaneous decreases in extracellular Mg^{2+} (2–6), which might further accentuate activation in these channels.

The current we have described resembles in some aspects that observed in *Xenopus* oocytes. Both currents are activated by lowering Ca^{2+}, both are slowly activating and show little inactivation, and both are mediated by channels that demonstrate little selectivity between Na^+ and K^+ but have little Ca^{2+} permeability and exclude anions (11). Also, like stretch-activated channels, both oocyte and hippocampal currents are highly sensitive to Gd^{3+} (11, 14, 31). The hippocampal channel is also sensitive to flufenamic acid and high concentrations of amiloride, as are stretch-activated cation channels. However, the nonselective cation channel observed in hippocampal neurons demonstrates no intrinsic voltage dependency, and the block by Ca^{2+} and Gd^{3+} is not voltage-dependent in contrast with most other nonselective cation channels (12, 13). Some hemi-gap channels are also voltage-dependent but they are permeable to anions, which differentiates them from the hippocampal channel (32).

The sensitivity of the hippocampal nonselective cation channel to various cations and polyvalent cations is remarkably similar to that reported for the calcium-sensing receptor (30). At least one form of this receptor has been cloned from the rat brain, and it is expressed in cultured hippocampal neurons (33). This receptor is linked to G protein activation and it is most homologous to metabotropic glutamate receptors. However, it is highly unlikely that this receptor itself forms an ion channel (30). Furthermore, Ca^{2+} and polyvalent cations activate this receptor while they inhibit the gating of the nonselective cation channel expressed in hippocampal neurons. The lack of dependence of this nonselective cation channel upon the presence of intracellular GTP also suggests that there is no direct link between this receptor and the nonselective cation channel described in this paper. It seems more likely that both activation of calcium-sensing receptor and gating of the nonselective cation channel are controlled by a similar mechanism, that is, a shielding of negative surface charges.

The high selectivity of Ca^{2+} channels is compromised by lowering Ca^{2+} to concentrations below the micromolar range (16, 34). However, the nonselective cation channels described here could be activated by decreases in Ca^{2+} at a far higher range of concentration (e.g., from 2.0 to 0.5 mM). Furthermore, they demonstrated no voltage dependency, and they were insensitive to at least some Ca^{2+} channel antagonists (e.g., verapamil and nitrendipine). Similarly, the selectivity of K^+ channels (35, 36) may be lost when Ca^{2+} is lowered. These modified K^+ channels were insensitive to Mg^{2+} and were blocked by extracellular K^+. This strongly contrasts with the blockade of hippocampal nonselective cation channels by Mg^{2+} and their high relative permeability to K^+.

Our results demonstrate that the practice of simultaneously lowering Ca^{2+} when applying NMDA may be inappropriate. The relatively slow onset of the nonselective current, together with its lack of inactivation, will tend to contaminate and obscure NMDA-induced inactivation. However, this contamination clearly does not account for all of the effects of lowered Ca^{2+} on inactivation of NMDA currents (Fig. 2*C*, b). The nonselective cation channel also shares a number of other properties with NMDA channels (e.g., both are blocked by divalent cations, spermine, and flufenamic acid). Its presence may have complicated the interpretation of some of these studies (25–27).

Tetanic stimulation of hippocampal afferents depolarizes CA1 neurons sufficiently to relieve the voltage-dependent blockade of NMDA receptors by Mg^{2+}. The resulting sustained influx of Ca^{2+} through these receptors then contributes to the induction of long-term potentiation (1). However, the resulting decrease in extracellular Ca^{2+} could also potentially activate nonselective cation channels, leading to a further depolarization and amplification of the excitatory signal. Such a mechanism could also serve to synchronize and amplify paroxysmal bursting of neurons, which is characteristic of seizure activity. In this respect, the nonselective cation channels might be anticipated to play a role in the epileptogenic-like bursting of neurons that is observed when hippocampal slices are superfused with solutions containing either low concentrations of Ca^{2+} (37) or Mg^{2+} (38).

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1. Bliss, T. V. P. & Collingridge, G. L. (1993) *Nature (London)* **361,** 31–39.
2. Heinemann, U., Lux, H. D. & Gutnick, M. J. (1977) *Exp. Brain Res.* **27,** 237–243.
3. Heinemann, U., Stabel, J. & Rausche, G. (1990) *Prog. Brain Res.* **83,** 197–214.
4. Heinemann, U. & Hamon, B. (1986) *Exp. Brain Res.* **65,** 1–10.
5. Kohr, G. & Heinemann, U. (1989) *Epilepsy Res.* **4,** 187–200.
6. Krnjevic, K., Morris, M. E. & Reiffenstein, R. J. (1982) *Can. J. Physiol. Pharmacol.* **60,** 1643–1657.
7. Hille, B. (1992) *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA).
8. Zhou, W. & Jones, S. W. (1995) *J. Gen. Physiol.* **105,** 441–462.
9. Hess, P. & Tsien, R. W. (1984) *Nature (London)* **309,** 453–456.
10. Hablitz, J. J., Heinemann, U. & Lux, H. D. (1986) *Biophys. J.* **50,** 753–757.

11. Arellano, R. O., Woodward, R. M. & Miledi, R. (1995) *J. Physiol. (London)* **484,** 593–604.
12. Root, M. J. & MacKinnon, R. (1993) *Neuron* **11,** 459–466.
13. Frings, S., Seifert, R., Godde, M. & Kaupp, U. B. (1995) *Neuron* **15,** 169–179.
14. Yang, X. & Sachs, F. (1989) *Science* **243,** 1068–1071.
15. Malchow, R. P., Qian, H. & Ripps, H. (1993) *J. Neurosci. Res.* **35,** 237–245.
16. Almers, W. & McCleskey, E. W. (1984) *J. Physiol. (London)* **353,** 585–608.
17. Clark, G. D., Clifford, D. B. & Zorumski, C. F. (1990) *Neuroscience* **39,** 787–797.
18. Vyklicky, L., Jr. (1993) *J. Physiol. (London)* **470,** 575–600.
19. Legendre, P., Rosenmund, C. & Westbrook, G. L. (1993) *J. Neurosci.* **13,** 674–684.
20. Rosenmund, C. & Westbrook, G. L. (1993) *J. Physiol. (London)* **470,** 705–729.
21. Ehlers, M. D., Zhang, S., Bernhardt, J. P. & Huganir, R. L. (1996) *Cell* **84,** 745–755.
22. MacDonald, J. F., Mody, I. & Salter, M. W. (1989) *J. Physiol. (London)* **414,** 17–34.
23. Wang, L.-Y. & MacDonald, J. F. (1995) *J. Physiol.* **486.1,** 83–95.
24. Hoth, M. (1995) *Pflügers Arch.* **430,** 315–322.
25. Lerma, J. & Del Río, R. M. (1992) *Mol. Pharmacol.* **41,** 217–222.
26. Lerma, J. (1992) *Neuron* **8,** 343–352.
27. Benveniste, M. & Mayer, M. L. (1993) *J. Physiol. (London)* **464,** 131–163.
28. Lane, J. W., McBride, D. W., Jr., & Hamill, O. P. (1992) *Br. J. Pharmacol.* **106,** 283–286.
29. Eismann, E., Bonigk, W. & Kaupp, U. B. (1993) *Cell. Physiol. Biochem.* **3,** 332–351.
30. Brown, E. M., Gamba, G., Riccardi, D., Lombardl, M., Butters, R., Klfor, O., Sun, A., Hedlger, M. A., Lytton, J. & Hebert, S. C. (1993) *Nature (London)* **366,** 575–580.
31. Sackin, H. (1995) *Annu. Rev. Physiol.* **57,** 333–353.
32. De Vries, S. W. & Schwartz, E. A. (1992) *J. Physiol.* **445,** 201–230.
33. Ruat, M., Molliver, M. E., Snowman, A. M. & Snyder, S. H. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3161–3165.
34. Ellinor, P. T., Yang, J., Sather, W. A., Zhang, J. F. & Tsien, R. W. (1995) *Neuron* **15,** 1121–1132.
35. Armstrong, C. M. & Lopez-Barneo, J. (1987) *Science* **236,** 712–714.
36. Armstrong, C. M. & Miller, C. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7579–7582.
37. Jefferys, J. G. R. & Haas, H. L. (1982) *Nature (London)* **300,** 448–450.
38. Mody, I., Lambert, J. D. C. & Heinemann, U. (1987) *J. Neurophysiol.* **57,** 869–888.