Gadolinium blocks low- and high-threshold calcium currents in pituitary cells

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BIAGI, BRUCE A., AND JOHN J. ENYEART. *Gadolinium blocks low- and high-threshold calcium currents in pituitary cells.* Am. J. Physiol. 259 (Cell Physiol. 28): C515–C520, 1990.—The inhibition of L- and T-type Ca^{2+} currents by Gd^{3+} was studied in the rat pituitary GH_4C_1 cell line. In whole cell patch recordings, Gd^{3+} at concentrations of 50 nM to 5 μM blocked Ca^{2+} current through L-type channels. Block was promoted by prolonged channel activation. With 4.5-s test pulses to + 10 mV, Gd^{3+} at concentrations as low as 200 nM produced near-complete block of L current. At higher Gd^{3+} concentrations (5 μM), complete block occurred with short test pulses and appeared to be independent of channel activation. Gd^{3+} also blocked current through low-threshold T channels in GH_4C_1 cells. Two other trivalent elements, La^{3+} and Y^{3+}, blocked L-type Ca^{2+} channels in GH_4C_1 cells with potency similar to Gd^{3+}. These results indicate that these trivalent cations are effective nonselective inhibitors of both low- and high-threshold Ca^{2+} channels in endocrine cells. In this regard, they are among the most potent inorganic Ca^{2+} antagonists yet discovered.

lanthanum; yttrium; GH_4C_1 cells; inorganic calcium antagonists

EXCITABLE CELLS possess several types of voltage-gated Ca^{2+} channels that can be distinguished by their voltage dependence, kinetics, and pharmacology (1, 25). High-threshold slowly inactivating L-type Ca^{2+} channels are predominant in many types of excitable cells. These channels are blocked by the major organic Ca^{2+} antagonists. Low-threshold rapidly inactivating T channels coexist with L channels in heart, smooth muscle, neurons, and endocrine cells. T channels are insensitive to the major organic antagonists but are blocked by Ni^{2+} and several recently discovered organic antagonists (1, 9).

A third, but more controversial, N-type Ca^{2+} channel possessing characteristics intermediate to T and L channels has been identified in neurons (1, 5, 9, 10, 21). These high-threshold channels inactivate more slowly than T channels but much faster than L channels. N channels are prominent in nerve terminals where they function in the regulation of neurotransmitter and peptide hormone secretion (18, 21, 22). These Ca^{2+} channels are relatively insensitive to organic antagonists but are preferentially blocked by ω-conotoxin (21). However, the degree of selectivity is disputed (1).

A recent report suggests that in neurons Gd^{3+} selectively blocks N-type calcium currents. In a neuroblastoma-glioma hybrid (NG108-15) tumor cell line, a Ca^{2+} current with N-type characteristics is completely blocked by Gd^{3+} at concentrations of 10–20 μM (5). In these same cells, two additional components of Ca^{2+} current, presumably T- and L-type channels, are unaffected by Gd^{3+} concentrations <5 μM. If Gd^{3+} is a selective antagonist of N-type channels in neurons, it may be valuable in defining their role in neuronal function.

Endocrine cells including those of the anterior pituitary possess two types of Ca^{2+} channels with characteristics similar to T- and L-type channels in other excitable cells (1, 9, 17, 20). In particular, Ca^{2+} channels in prolactin-secreting pituitary cells and cell lines have been extensively studied (3, 4, 7, 8, 16, 17). There is currently no evidence suggesting the presence of N-type Ca^{2+} channels in these cells. To assess the specificity of Gd^{3+} as a selective blocker of N-type Ca^{2+} channels and to further characterize the pharmacology of pituitary Ca^{2+} channels, we have studied the effects of Gd^{3+} and two other trivalent elements, La^{3+} and Y^{3+}, on whole cell currents in the GH_4C_1 cell line.

MATERIALS AND METHODS

Materials. Tissue culture media, horse serum, and fetal calf serum were obtained from GIBCO (Grand Island, NY). Culture dishes were purchased from Corning (Corning, NY). Tetrodotoxin, GTP, adenosine 3′,5′-cyclic monophosphate (cAMP), MgATP, and the chloride salt of lanthanum were obtained from Sigma Chemical (St. Louis, MO). The chloride salts of gadolinium (99.999% purity) and yttrium (99.9% purity) were obtained from Aldrich Chemical (Milwaukee, WI).

Cell culture. GH_4C_1 cells were grown as a monolayer in Ham's F-10 medium supplemented with 15% horse serum and 2.5% fetal calf serum at 37°C in a humidified atmosphere as previously described (24). In addition to sera, amino acids, and other nutrients, this medium contained the following (in mM): 0.36 $CaCl_2$, 3.82 KCl, 0.61 KH_2PO_4, 0.62 Mg_2SO_4, 14.3 $NaHCO_3$, and 126 NaCl, with pH buffered to 7.4.

Patch-clamp experiments. For patch-clamp experi-

ments, GH_4C_1 cells were transferred to the recording chamber (2-ml volume) filled with a protein-free solution containing (in mM) 135 NaCl, 5 CsCl, 10 $CaCl_2$, 2 $MgCl_2$, and 5 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), buffered to pH 7.4. The chloride salts of gadolinium, lanthanum, or yttrium were added to this solution from 10 mM stock solutions. Tetrodotoxin (2 μM) was added to all solutions to eliminate Na^+-channel currents.

Patch electrodes were filled with a solution designed to eliminate all K^+-channel currents and to minimize Ca^{2+}-channel rundown. Electrode-filling solution contained (in mM) 120 CsCl, 2 $MgCl_2$, 11 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 1.0 $CaCl_2$, 10 HEPES, 1 MgATP, 0.1 cAMP, and 0.04 GTP, with the pH adjusted to 7.2 with CsOH. Electrodes with resistances between 2 and 5 MΩ were used for voltage clamping. Electrodes were fabricated from RC-6 glass (Garner Glass, Claremont, CA) by pulling on a Brown-Flaming P-80 micropipette puller (Sutter Instruments, San Francisco, CA). Whole cell Ca^{2+} currents were recorded following the procedure of Hamill et al. (12), using a List EPC-7 patch-clamp amplifier (Medical Systems, Great Neck, NY). Pulse generation, data acquisition, subtraction of linear leak and capacitance currents, and data analysis were done using PCLAMP software (Axon Instruments, Burlingame, CA).

Cells were placed in the recording chamber and approached with fire-polished electrodes containing the internal solution. After a gigaseal was formed, the patch was ruptured by gentle suction to give a whole cell clamp. Test solutions were applied by gravity perfusion of the recording chamber at a rate of 2–5 ml/min.

RESULTS

In whole cell patch-clamp experiments, Gd^{3+} produced a concentration-dependent block of Ca^{2+} currents through L-type channels. L-type channels were selectively activated by applying test pulses from holding potentials of −40 or −35 mV, where T channels are completely inactivated (3). Figure 1 shows current-voltage relationships obtained from a holding potential of −40 mV immediately before and 7 min after superfusing a GH_4C_1 cell with 5 μM Gd^{3+}. At this time, the amplitude of the maximum peak current had been reduced by 90% (Fig. 1, *inset*), with no marked shift in the current-voltage relationship.

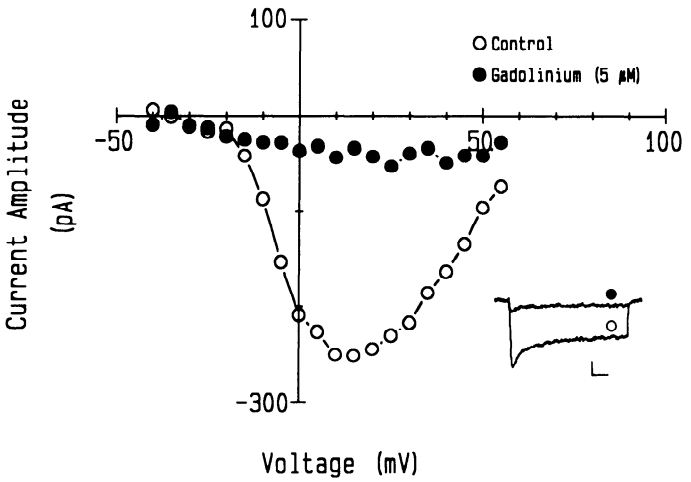


FIG. 1. Blockade of L-type Ca^{2+} currents by Gd^{3+}. Current-voltage relationships were obtained before and 7 min after superfusing a GH_4C_1 cell with saline containing 5 μM Gd^{3+}. Test pulses (300 ms) of increasing magnitude were applied at 0.1 Hz in 5-mV increments from a holding potential of −40 mV. Peak currents were plotted as a function of test potential. Test pulses to +10 mV were applied from −40 mV at 0.1 Hz during Gd^{3+} application to determine when a steady-state block was achieved. *Inset:* maximum peak current records in the absence and presence of Gd^{3+} as indicated. Scale bars represent 50 pA and 40 ms.

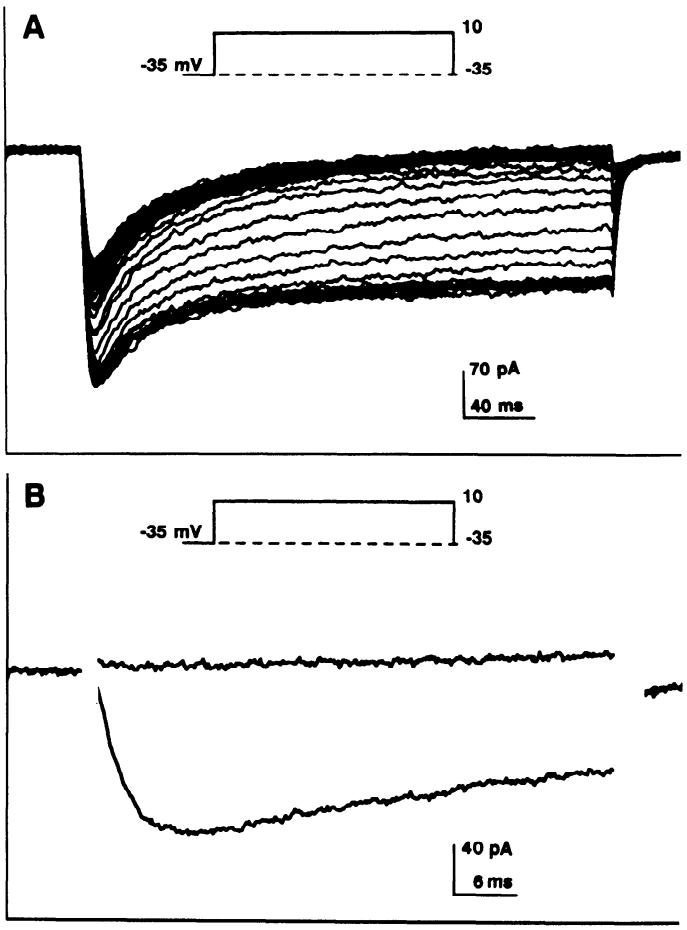


FIG. 2. Characteristics of Gd^{3+} block vary with concentration and test pulse duration. *A*: inhibition by 0.5 μM Gd^{3+}; test pulses of 300-ms duration were continuously applied from −35 mV at 0.1 Hz. After recording 5 control currents, cell was superfused with saline containing 0.5 μM Gd^{3+}. Progressive inhibition of Ca^{2+} current occurred during the next 80–90 s, after which a steady-state block was attained. *B*: inhibition by 5.0 μM Gd^{3+}; test pulses of 50-ms duration were applied at 0.1 Hz from a holding potential of −35 mV before (*bottom trace*) and 2 min after (*top trace*) superfusing cell with saline containing 5 μM Gd^{3+}. Each trace is the average of 3 currents, with 2 ms blanked at the beginning and end of each test pulse.

At lower Gd^{3+} concentrations, the extent of current block was dependent on the frequency and duration of test pulses. Figure 2*A* shows the effect of 0.5 μM Gd^{3+} on Ca^{2+} currents recorded at 0.1 Hz from a holding potential of −35 mV. In control saline, five consecutive test pulses elicited nearly identical currents that include a characteristic component of inactivation always observed with Ca^{2+} as the charge carrier. Upon exposing the cell to Gd^{3+}, the current amplitude declined continuously with successive test pulses until a steady-state

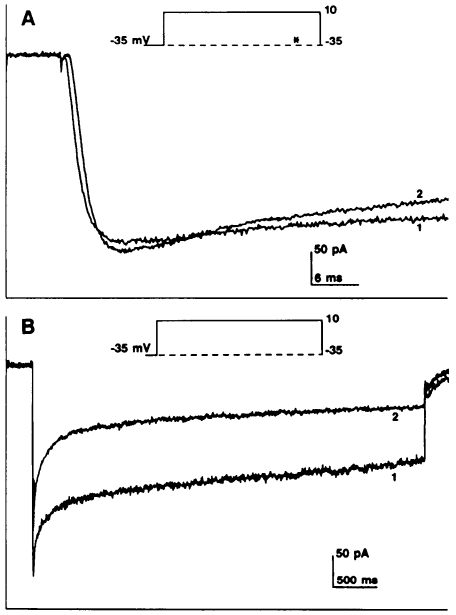


FIG. 3. Block of L-type Ca^{2+} current is facilitated by channel activation at low Gd^{3+} concentration. Ca^{2+} currents were first recorded in control saline in response to 50-ms (*A1*) and then 4.5-s (*B1*) test pulses. Three minutes after superfusing cell with 0.05 μM Gd^{3+}, currents were again recorded in response to short (*A2*) and then long (*B2*) test pulses. Test pulses were applied from a holding potential of −35 mV either at 0.1 (*A*) or 0.05 Hz (*B*). Each trace is the average of 3 individual currents.

block was achieved within ~2 min. The inhibition by Gd^{3+} was distinctive in that it increased during the course of individual pulses. When the steady-state block was achieved, the peak current was diminished by 55%, whereas current measured at the end of the individual test pulses had disappeared completely.

When Gd^{3+} was used at higher concentrations, complete block of L-type current was observed even with very short test pulses and appeared to be independent of channel activation. Figure 2*B* shows currents activated by 50-ms test pulses before and 2 min after superfusing the cell with 5 μM Gd^{3+}. In the presence of 5 μM Gd^{3+}, current was blocked completely and was maximum with the initiation of the first test pulse.

The variation in Gd^{3+} potency with test-pulse duration was clear in experiments in which block of L current was compared in the same cell with short and extremely long test pulses. In Fig. 3, currents activated by short (*A1*) and then long (*B1*) test pulses were recorded in control saline. After a 3-min exposure of 50 nM Gd^{3+}, the procedure was repeated (Fig. 3, *A2* and *B2*). Gd^{3+} blocked Ca^{2+} current during the short test pulse by a maximum of 12%. In contrast, during the long test pulse, block reached 77%.

Blockade of L-type Ca^{2+} channels by Gd^{3+} was not easily reversed by extensive washing in Gd^{3+}-free saline. A significant reversal was observed in 6 of 10 cells. In these cells, the peak control currents were 213 ± 23.1 pA (N = 6). After completely blocking Ca^{2+} currents with Gd^{3+} at concentrations up to 10 μM, extensive washing of the chamber with a 10- to 20-fold volume of saline resulted in a recovery of only 39 ± 11 pA or 18% of control currents.

Recovery was markedly enhanced by the addition of 0.1 mM EGTA to the bathing medium. Figure 4 illustrates the time-dependent block of Ca^{2+} (*A*) or Ba^{2+} (*B*)

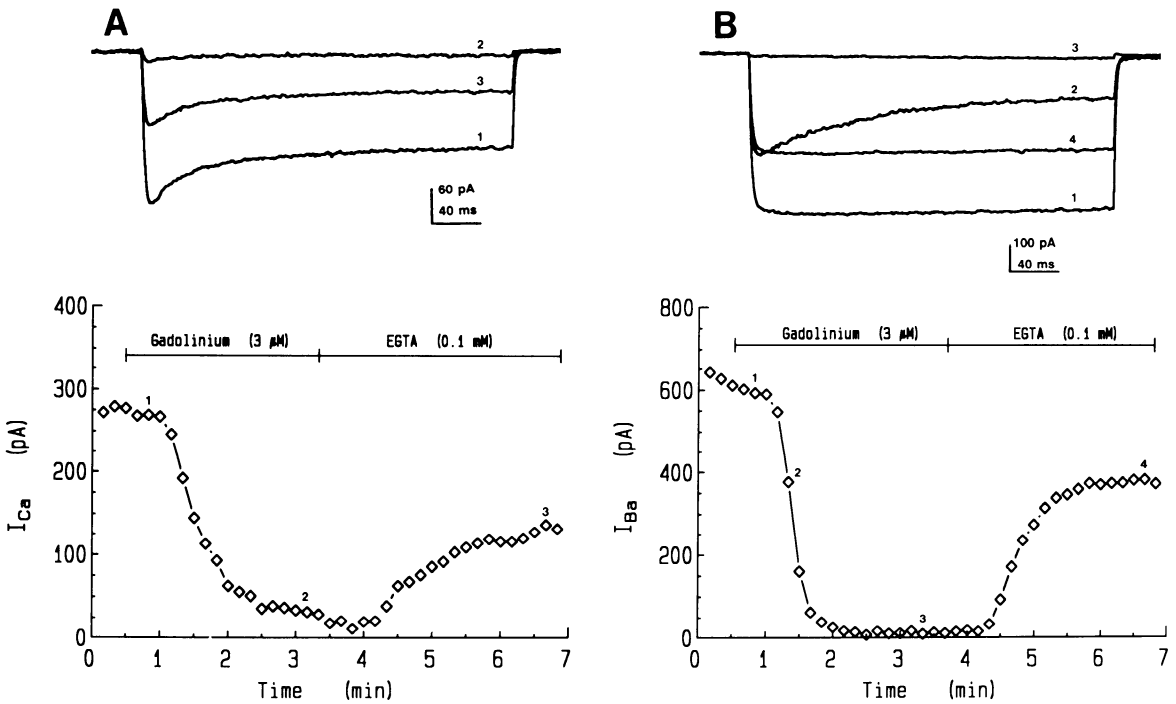
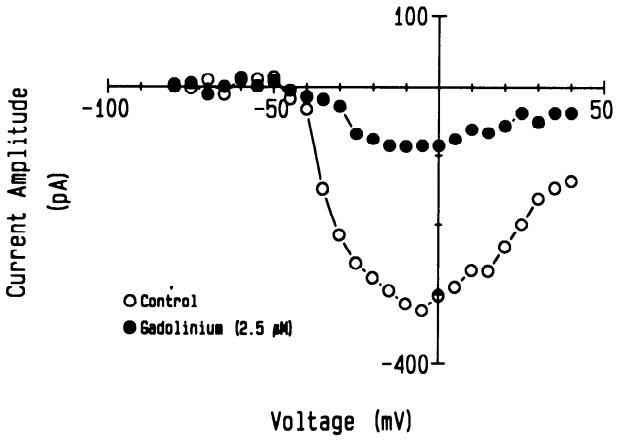


FIG. 4. Reversal of Gd^{3+} block by EGTA. Ca^{2+} (I_{Ca}; *A*) or Ba^{2+} (I_{Ba}; *B*) currents were activated by test pulses to +10 mV applied at 0.1 Hz from a holding potential of −40 mV. After recording control currents, cells were superfused with saline containing Gd^{3+} (3 μM). When steady-state block was achieved, cells were washed with EGTA-containing saline while continuing test pulses. *A*, *top*: Ca^{2+} current records at times corresponding to numerals on *bottom*. Peak Ca^{2+} current amplitudes are plotted vs. time. *B*: experimental protocol as in *A* but with Ba^{2+} as charge carrier.



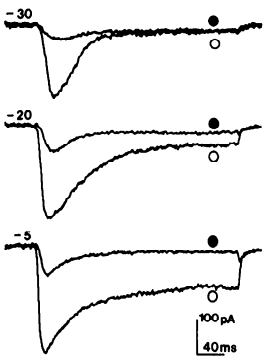


FIG. 5. Gd^{3+} blocks low- and high-threshold Ca^{2+} currents. Current-voltage relationships were obtained from a holding potential of −80 mV before and 6 min after superfusing a GH_4C_1 cell with saline containing 2.5 μM Gd^{3+}. During Gd^{3+} application, test pulses to +10 mV were applied at 0.1 Hz to ensure that a steady-state block was achieved. *Left*: peak currents are plotted as a function of test potential. *Right*: selected currents recorded at test potentials of −30, −20, and −5 mV before and after Gd^{3+} perfusion.

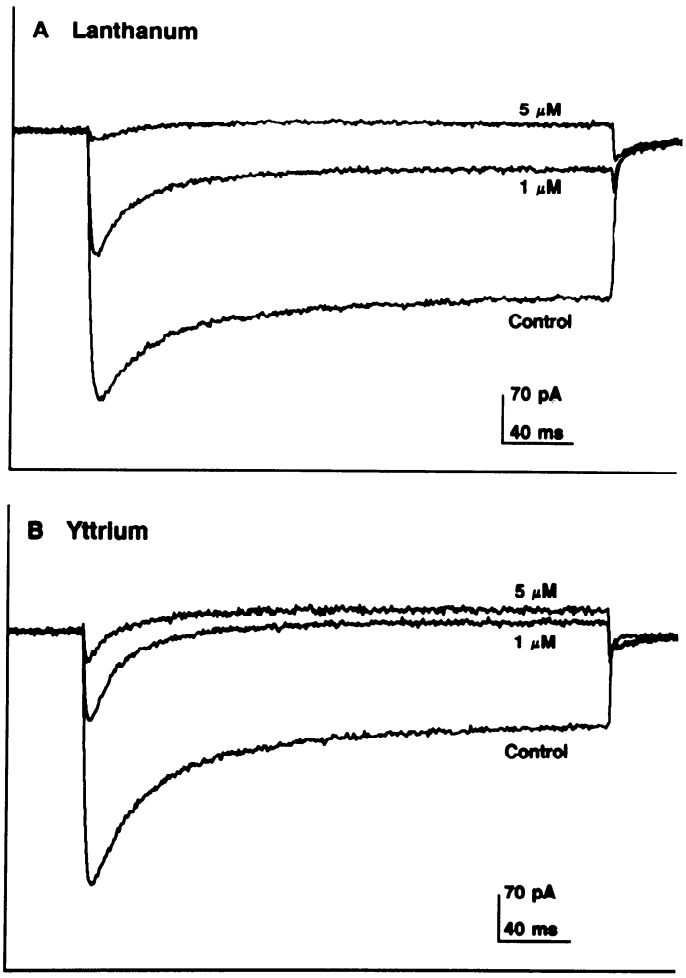


FIG. 6. Concentration-dependent block of L-type Ca^{2+} current by La^{3+} and Y^{3+}. Test pulses to +10 mV were applied from −35 mV at 0.1 Hz. Each trace is the average of 5 currents obtained initially (control) and after steady-state block by 1 and 5 μM La^{3+} or Y^{3+}.

currents by Gd^{3+} and subsequent recovery upon superfusing cells with saline containing 0.1 mM EGTA. In these two cells, 3 μM Gd^{3+} produced complete inhibition of L-type current with either Ba^{2+} or Ca^{2+} as the charge carrier. A 3-min wash with EGTA-containing saline restored 45 and 63% of the original Ca^{2+} and Ba^{2+} currents.

When test pulses are applied from a holding potential of −80 mV, both low-threshold T and high-threshold L channels are available for activation. In these experiments Gd^{3+} was found to block T- as well as L-type channels. In the results shown in Fig. 5, current-voltage relationships were obtained from a holding potential of −80 mV before and 6 min after exposing the cell to 2.5 μM Gd^{3+}. In control saline, test pulses to −30 mV activated primarily T-channel current while stronger depolarizations activated, in addition, the noninactivating L current (Fig. 5, *right*). Both current components were markedly reduced by Gd^{3+} with little shift in the current-voltage curve. The T current activated at a test pulse to −30 mV was blocked completely.

Other trivalent elements were found to block Ca^{2+} currents in GH_4C_1 cells. The records in Fig. 6 illustrate the effects of 1 and 5 μM La^{3+} and Y^{3+} on L-type Ca^{2+} current activated by test pulses applied at 0.1 Hz from a holding potential of −35 mV. Both elements show a concentration-dependent block that is nearly complete at a concentration of 5 μM.

DISCUSSION

A principal finding of this study was that Gd^{3+} potently blocks both T- and L-type Ca^{2+} channels in the GH_4C_1 pituitary cell line. These results contrast sharply with the reported selective block by Gd^{3+} of Ca^{2+} channels with N-type characteristics in a neuronal hybrid cell line, wherein L and T channels were insensitive (5). Gd^{3+} has been reported not to block Ca^{2+} influx through dihydropyridine-sensitive L-type channels in other neuronal cell lines (11). One interpretation of the combined results is that Ca^{2+} channels in neurons and endocrine cells differ markedly with respect to sensitivity to Gd^{3+} and other lanthanide elements. Regardless, our findings in GH_4C_1 cells clearly demonstrate that among Ca^{2+} channels in excitable cells, Gd^{3+} is not a specific antagonist of N-type channels. Accordingly, in cultured bovine chromaffin cells which possess only L-type Ca^{2+} channels, 50 μM Gd^{3+} completely blocks depolarization-dependent Ca^{2+} uptake (2).

In some of our experiments, Gd^{3+} blocked L-type Ca^{2+} currents in GH_4C_1 cells at concentrations 10–100 times lower than those required to inhibit N-type current in the neuronal cell line (5). This apparent disparity in potency is complicated by our observation that blockade of L-type channels is strongly promoted by channel activation. At low Gd^{3+} concentrations, the extent of

block was markedly enhanced by long depolarizing pulses. Within the framework of the "modulated receptor hypothesis," this finding suggests that Gd^{3+} preferentially binds to Ca^{2+} channels that are in a conformation other than that represented by the rested state (13). In this respect, inhibition of L currents by Gd^{3+} in GH_4C_1 cells resembles block by organic Ca^{2+} antagonists including the dihydropyridine nimodipine and the diphenylbutylpiperidine penfluridol (3, 6). With Ba^{2+} as the charge carrier, L-type Ca^{2+} channels in GH_4C_1 cells show little or no time- or voltage-dependent inactivation. Because Gd^{3+} was equally effective at blocking noninactivating Ba^{2+} current, it appears likely that Gd^{3+} like nimodipine (3) preferentially block open, rather than inactivated, channels.

Although the extent of Ca^{2+} current block by Gd^{3+} could increase during the course of a single depolarizing pulse, some recovery of current was observed upon initiation of the next pulse in a train (Fig. 2*A*). It is possible that a fraction of the channels are reactivated during the short period between membrane repolarization and channel closing. According to this scheme, blocking Gd^{3+} might be forced into the cell through open channels upon repolarization. In sensory neurons, Cd^{2+} can be cleared from blocked Ca^{2+} channels by stepping the cell to a negative voltage. Once cleared of Cd^{2+}, the channels conduct transiently upon reopening but are rapidly reblocked (23).

Blockade of L-type channels in GH_4C_1 cells was not specific for the lanthanide Gd^{3+}. Other trivalent cations including La^{3+} and Y^{3+} block these channels with similar potency. La^{3+} also blocks L-type channels in heart cells (15, 19). Inhibition of L-type Ca^{2+} channels in pituitary cells by each of the trivalents was poorly reversed by washing with saline. These channels were rapidly reactivated in the presence of the chelator EGTA. A similar pattern of inhibition and EGTA-mediated recovery has been observed for N-type Ca^{2+} channels blocked by Gd^{3+} in sympathetic neurons (14). In contrast, block of the N-type channels by divalent cations like Cd^{2+} was rapidly reversible by washing in saline.

Compared with L channels, relatively few agents have been discovered that potently block T-type Ca^{2+} channels in excitable cells. Among inorganic blockers, Ni^{2+} is effective at blocking low-threshold channels in heart and neurons at concentrations of 40–100 μM (1, 9). In contrast, 2.5 μM Gd^{3+} blocked current through these channels almost completely in our experiments. It will be interesting to determine whether Gd^{3+} blocks T channels in excitable cells other than pituitary.

In conclusion, we have shown that Gd^{3+} and other lanthanides block low- and high-threshold channels in an endocrine cell line. Inhibition of cellular functions by these agents in any excitable cell must be interpreted as potentially involving multiple types of Ca^{2+} channels.

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