high-speed confocal scanning microscope system (CSU-21; Yokogawa Electric, Tokyo, Japan) attached to an inverted microscope (Olympus IX70, Tokyo, Japan). The modified Nipkow laser confocal scanning microscope system has two mechanically connected disks, an upper microlens-array disk and a lower pinhole-array disk (Genka *et al.* 1999), which are rotated by a motor at 1000–5000 r.p.m. The excitation light emitted from a laser unit (488 nm; argon laser, 4–13 mW) and passed through a fiber optic probe is attenuated by a 10% neutral density filter before reaching the upper disk, which has 20 000 microlenses to focus excitation beams exactly on the corresponding 20 000 pinholes in the lower disk. The microlenses are known to increase light transmission through the pinholes by about 40% from the source (about 2% in the absence of the microlenses). The light passing through the pinholes was focused by an objective (UPlanFl ×40, NA = 0.75; Olympus) to a point on the cells. Fluorescent light beams emitted from a cluster of cells were collected by the same objective and those scanned by the pinholes were reflected by a dichroic mirror, passed through a barrier filter (> 515 nm) and focused on a cooled digital EMCCD camera (C9100-12; Hamamatsu Photonics, Shizuoka, Japan). Two-dimensional images composed of 512 × 512 pixels (204.8 $\mu$m × 204.8 $\mu$m) were obtained at 2 frames $s^{-1}$ with an exposure time of 495 ms. The image analysis and data acquisition (regions of interest as well as background areas of similar size) were carried out on an IBM computer using the Aquacosmos image program (Hamamatsu Photonics). All experiments were conducted at room temperature. Application of the high-$K^+$ solution was done by local ejection from a small-tipped pipette using a pressure ejection device (PicoPump; World Precision Instruments, Sarasota, FL, USA). The high-$K^+$ solution contained (mM): K-$CH_3O_3S$ (potassium methane sulphonate), 150; $CaCl_2$, 1.5 or 2.0; $MgCl_2$, 1; and Hepes, 10, adjusted to pH 7.4 with KOH, so that the $[K^+][Cl^-]$ product and osmolarity stayed as close as possible to those of the standard bath solution (Hodgkin & Horowicsz, 1959). Changes in fluorescence from the cytoplasm (excluding the nucleus) are shown relative to the baseline fluorescence ($\Delta F/F$), where $F$ denotes baseline fluorescence and $\Delta F$ is the change in fluorescence in response to stimulation. Background intensity was subtracted from the raw data before calculating $\Delta F/F$. Results are reported as the means ± S.D. where necessary.

### Electrophysiological recording

Whole-cell $Ca^{2+}$ currents were recorded as described previously (Yamaguchi *et al.* 2003; Takamatsu *et al.* 2003). The bath solution contained (mM): NaCl, 137; KCl, 5.4; $MgCl_2$, 1; glucose, 10; Hepes, 10; and $CaCl_2$, 2, adjusted to pH 7.4 with NaOH, at room temperature. The resistance of the patch pipettes ranged between 2 and 3 MΩ when filled with an internal solution composed of (mM): Cs-$CH_3O_3S$ (caesium methane sulphonate), 120; TEACl, 20; EGTA, 14; MgATP, 5; disodium creatinine phosphate, 5; GTP, 0.2; and Hepes, 10, adjusted to pH 7.3 with CsOH. Whole-cell currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA) via an A/D converter (Digidata 1200; Axon Instruments). Voltage-clamp protocols, including leak subtraction and data acquisitions, were performed using pCLAMP software version 7.0 (Axon Instruments). To obtain current–voltage relationships, depolarizing pulses of 100 ms duration were applied every 2 s from a holding potential of −60 mV to various test potentials, ranging from −60 to +40 mV, in 10 mV increments. The capacitative currents were determined and compensated before each voltage pulse, and series resistance was compensated electronically, as necessary. Currents were low-pass filtered (8-pole Bessel) at a corner frequency of 5 kHz and digitized at 40 $\mu$s intervals. To remove residual linear capacitative and leakage currents from the test currents, four scaled, hyperpolarizing control voltage steps (each one-quarter of the magnitude of the corresponding test step) were administered before applying the test pulses ($P/-4$). It was difficult to determine the reversal potential of the $Ca^{2+}$ current precisely, since the $Ca^{2+}$-activated $K^+$ current developed quickly during the course of an experiment.

## Results

### Voltage-dependent activation and inactivation of $Ca^{2+}$ transient

In the present study, we first asked whether depolarization causes $Ca^{2+}$ entry into cultured parathyroid cells. If these cells expressed voltage-dependent $Ca^{2+}$ channels, membrane depolarization would cause an inward $Ca^{2+}$ current and a resultant increase in $[Ca^{2+}]_i$. To examine this, cells were loaded with fluo-3 AM and the membrane was depolarized to near 0 mV (assuming cytoplasmic $K^+$ concentration to be around 140 mM) by external exposure to a 150 mM $K^+$ solution. Surprisingly, a transient increase in fluo-3 fluorescence was detected in most cells tested following 10 s exposure to the 150 mM $K^+$ solution containing 2 mM $Ca^{2+}$ ($n$ > 170 cells from > 20 patients). This indicated that membrane depolarization caused a transient increase in $[Ca^{2+}]_i$ in the cultured parathyroid cells (Fig. 1*A*, left). The slow onset of the $Ca^{2+}$ transient following pressure application was due to gentle application of the solution from a small-tipped pipette, so as to not stimulate stretch-activated

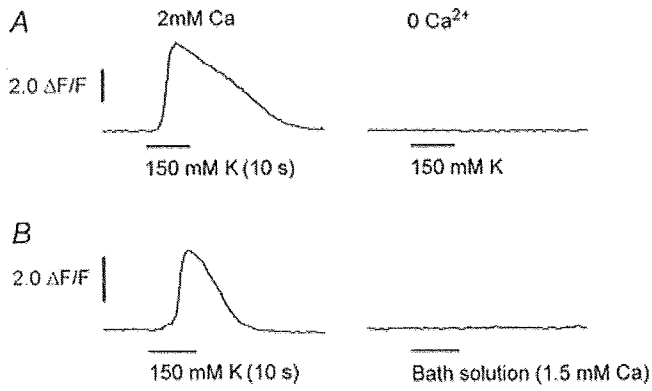


**Figure 1. Voltage-dependent activation of fluo-3 $Ca^{2+}$ transients in human parathyroid cells**
*A*, left, application of a 150 mM $K^+$ solution (2 mM $Ca^{2+}$) evoked a transient increase in fluo-3 fluorescence; right, application of the nominally $Ca^{2+}$-free 150 mM $K^+$ solution failed to increase fluo-3 fluorescence. *B*, left, application of 150 mM $K^+$ solution (the bath contained 1.5 mM $Ca^{2+}$); right, application of the bath solution.

channels and distort confocal imaging. At present, we cannot completely exclude the possibility that $IP_3$-induced $Ca^{2+}$ release was in part involved in the high-$K^+$-induced $Ca^{2+}$ transient, because intracellular $Ca^{2+}$ stores were not depleted in our experimental conditions. It should be noted, however, that the application of 30 mM caffeine failed to induce a $Ca^{2+}$ transient, indicating that ryanodine-sensitive $Ca^{2+}$ stores were not expressed in the cultured parathyroid cells, hence $Ca^{2+}$-induced $Ca^{2+}$ release was not involved in the high-$K^+$-induced $Ca^{2+}$ transient. The response was remarkable after 2 and 3 days of primary culture. In a similar manner, nominally $Ca^{2+}$-free saline containing 150 mM $K^+$ was gently applied to cells immersed in either 1 mM $Ca^{2+}$ or nominally $Ca^{2+}$-free saline. However, depolarization failed to evoke a $Ca^{2+}$ transient in the absence of $[Ca^{2+}]_o$ (Fig. 1*A*, right; $n = 22$). Since application of the bath solution *per se* did not induce a $Ca^{2+}$ transient (Fig. 1*B*, right; $n = 10$), it is unlikely that the $Ca^{2+}$ transient induced by the high-$K^+$ solution was due to opening of stretch-activated $Ca^{2+}$-permeable channels.

Since the $Ca^{2+}$ transient evoked by the high-$K^+$ solution decayed gradually during continuous exposure to the high-$K^+$ solution for 30 s (Fig. 2*B*), it would appear that the $Ca^{2+}$ entry system entered an inactivated state during long-lasting depolarization. Indeed, a second application of the high-$K^+$ solution no longer induced a $Ca^{2+}$ transient following washout of the initial high-$K^+$ solution ($n = 10$). As seen in Fig. 2*C*, the response recovered following a 10 min rest interval, again supporting the idea that the cell had not deteriorated but experienced a voltage-dependent inactivation process.

## The high-$K^+$-induced $Ca^{2+}$ transient is sensitive to L-type $Ca^{2+}$ channel modulators

We then examined whether the $Ca^{2+}$ entry system is sensitive to L-type $Ca^{2+}$ channel modulators. As shown in Fig. 3*A*, the depolarization-induced $Ca^{2+}$ transient was increased significantly in the presence of 10 $\mu$M FPL-64176 (by $2.4 \pm 0.6$ times; $n = 9$), a potent agonist for L-type $Ca^{2+}$ channels, which is known to bind to a domain distinct from that of the dihydropyridines and exhibits exclusive $Ca^{2+}$-channel agonistic action, even at very high concentrations (Rampe & Lacerda, 1991; Yamaguchi *et al.* 2003). In addition, the slow upstroke of the control $Ca^{2+}$ transient in Fig. 3*A* was due to low (1.2 mM) $[Ca^{2+}]_o$ to illustrate the effect of the drug. By contrast, exposure to the high-$K^+$ solution failed to induce a noticeable $Ca^{2+}$ transient in the presence of dihydropyridine antagonists. With 1 $\mu$M nitrendipine, about 60% of cells tested (13 out of 20) showed complete abrogation of the $Ca^{2+}$ transient (Fig. 3*B*). With 10 $\mu$M nitrendipine, about 95% of cells

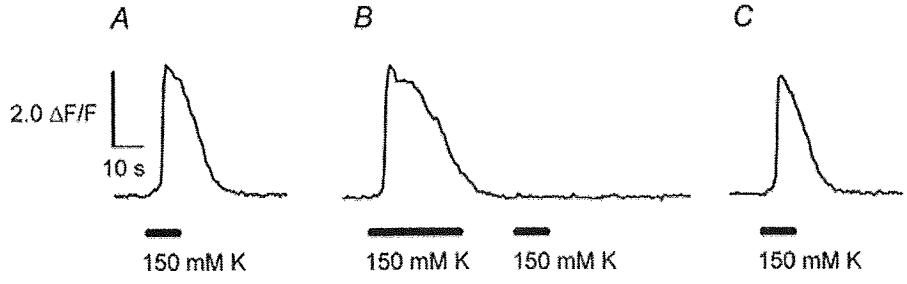


**Figure 2. Voltage-dependent inactivation of $Ca^{2+}$ transient in a human parathyroid cell**
*A*, the first fluo-4 $Ca^{2+}$ transient evoked by the 150 mM $K^+$ solution for 10 s (the bath contained 1.5 mM $Ca^{2+}$). *B*, inactivation of the high-$K^+$-induced $Ca^{2+}$ transient during 30 s exposure to the 150 mM $K^+$ solution. Following washout of the high-$K^+$ solution for about 15 s, a third application of the high-$K^+$ solution no longer induced the $Ca^{2+}$ transient. *C*, recovery of the high-$K^+$-induced $Ca^{2+}$ transient following a 10 min rest.

tested (21 out of 22) showed complete abrogation of the $Ca^{2+}$ transient. Similar results were obtained with nicardipine. With 1 $\mu$M nicardipine, about 90% of cells tested (18 out of 20) showed complete abrogation of the high-$K^+$-induced $Ca^{2+}$ transient. Complete or partial abrogation of the high-$K^+$-induced $Ca^{2+}$ transient was also observed in the presence of 0.5 mM $Cd^{2+}$ ($n = 20$), a well-known inorganic blocker of L-type $Ca^{2+}$ channels (Swandulla & Armstrong, 1989; Chow, 1991).

Taken together, these results suggest that human parathyroid cells do express L-type-like $Ca^{2+}$ channels. Examples of the effect of L-type $Ca^{2+}$ channel modulators on fluo-3 fluorescence in response to the high-$K^+$ solution are shown in Table 1.

**Table 1. Effect of $Ca^{2+}$ channel modulators on the high-$K^+$-induced $Ca^{2+}$ transient**

| | Control | With drug | No. of cells |
|---|---|---|---|
| FPL-64176 (10 $\mu$M) | 2.7 ± 1.2 | 4.4 ± 1.6 | 10 |
| Nitrendipine (1 $\mu$M) | 3.0 ± 1.2 | 0.3 ± 0.3 | 19 |
| Nicardipine (1 $\mu$M) | 2.8 ± 1.5 | 0.4 ± 0.6 | 18 |
| $Cd^{2+}$ (0.5 mM) | 2.6 ± 1.4 | 0.6 ± 0.7 | 10 |

In each row, data (maximal change in $\Delta F/F$ from baseline fluo-3 fluorescence following 10 s exposure to the 150 mM $K^+$ solution) were obtained from the population of a single primary culture dish before and after exposure to the drug, and are presented as the means ± S.D. The $[Ca^{2+}]_o$ in the bath solution was 2 mM, except in the case of FPL-64176, for which $[Ca^{2+}]_o$ was 1.2 mM to highlight the effect of the drug. For both FPL-64176 and $Cd^{2+}$, data with a remarkable increase in baseline fluorescence were excluded fromthe table.

## Electrophysiological and pharmacological properties of whole-cell $Ca^{2+}$ and $Ba^{2+}$ currents

To directly track the entry of extracellular $Ca^{2+}$, whole-cell patch-clamp recording was performed on the cultured parathyroid cells. When a 100 ms depolarizing pulse was applied from a holding potential of −60 mV to a test potential of 0 mV, an inward $Ca^{2+}$ current was detected, indicating that positively charged $Ca^{2+}$ ions indeed entered during depolarization (Fig. 4*A*). A $[Ca^{2+}]_o$ of 2 mM was routinely used to represent nearly the highest level of serum $Ca^{2+}$ concentration in physiological conditions and because 2 mM $[Ca^{2+}]_o$ was not able to induce $Ca^{2+}$ transients unless the plasma membrane was depolarized (the threshold $[Ca^{2+}]_o$ for inducing $Ca^{2+}$ transients was 2.5 mM when the bath contained 1.5–2.0 mM $Ca^{2+}$). As seen in the same figure, the inward current was significantly enhanced in the presence of 10 $\mu$M FPL-64176. By contrast, the inward current was strongly reduced in the presence of nitrendipine (Fig. 4*B*) or 0.2 mM $Cd^{2+}$. An inward current was also detected when $Ba^{2+}$ was used as the charge carrier. The current–voltage relationship showed a bell-shaped voltage dependence with a peak current around 0 mV (Fig. 4*C*), which exhibited a 10–15 mV negative shift in the presence of 10 $\mu$M FPL-64176 ($n = 4$). These electrophysiological properties are similar to those of L-type $Ca^{2+}$ channels. Collectively, these results are consistent with the results obtained from $Ca^{2+}$-related fluorescence measurements and strongly support the notion that the L-type-like $Ca^{2+}$ channel is responsible for the high-$K^+$-induced $Ca^{2+}$ entry into cultured parathyroid cells.

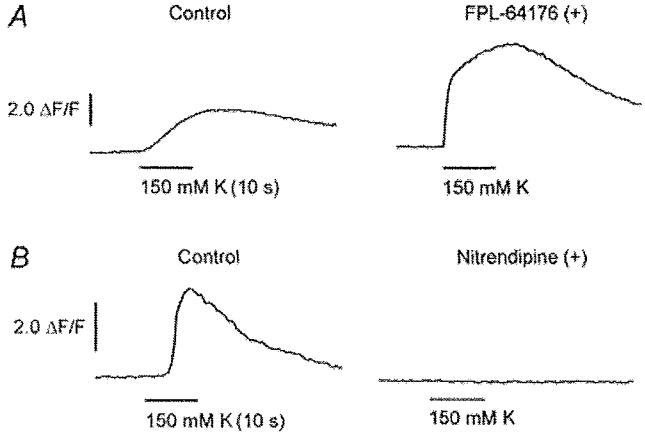


**Figure 3. The high-$K^+$-induced $Ca^{2+}$ transient is sensitive to L-type $Ca^{2+}$ channel modulators**
*A*, the bath contained 1.2 mM $Ca^{2+}$ to highlight the effect of the drug. Left panel shows that application of the 150 mM $K^+$ solution caused a small increase in fluo-3 fluorescence. Right panel shows that in the presence of FPL-64176, fluo-3 fluorescence was dramatically increased upon exposure to the high-$K^+$ solution. *B*, left, in the absence of nitrendipine, application of the 150 mM $K^+$ solution evoked a transient increase in fluo-3 fluorescence (the bath contained 2 mM $Ca^{2+}$); right, in the presence of 1 $\mu$M nitrendipine, exposure to the high-$K^+$ solution failed to increase fluo-3 fluorescence.

## Inhibition of the high-$[Ca^{2+}]_o$-induced $Ca^{2+}$ transient by $Ca^{2+}$ channel inhibitors

We finally investigated the effects of L-type $Ca^{2+}$ channel inhibitors on the high-$[Ca^{2+}]_o$-induced increase in $[Ca^{2+}]_i$, which is a characteristic feature of parathyroid cells. A $[Ca^{2+}]_o$ of greater than 2.5 mM was able to induce this type of response in our experimental conditions. This threshold for inducing $Ca^{2+}$ transients was substantially lower than 7.0–7.5 mM (Garrett *et al.* 1995), the half-maximal activation of human CaR in *in vitro* conditions. In this study, therefore, we used 3 mM $[Ca^{2+}]_o$ to evoke $Ca^{2+}$ transients without effectively activating the CaR. In addition, application of 10 mM $[Ca^{2+}]_o$ induced remarkably larger $Ca^{2+}$ transients than 3 mM $[Ca^{2+}]_o$. As shown in Fig. 5*A* (left), the external application of a 3 mM $[Ca^{2+}]_o$ solution to cells bathed in a 2 mM $[Ca^{2+}]_o$ solution induced a transient increase in fluo-3 fluorescence (here

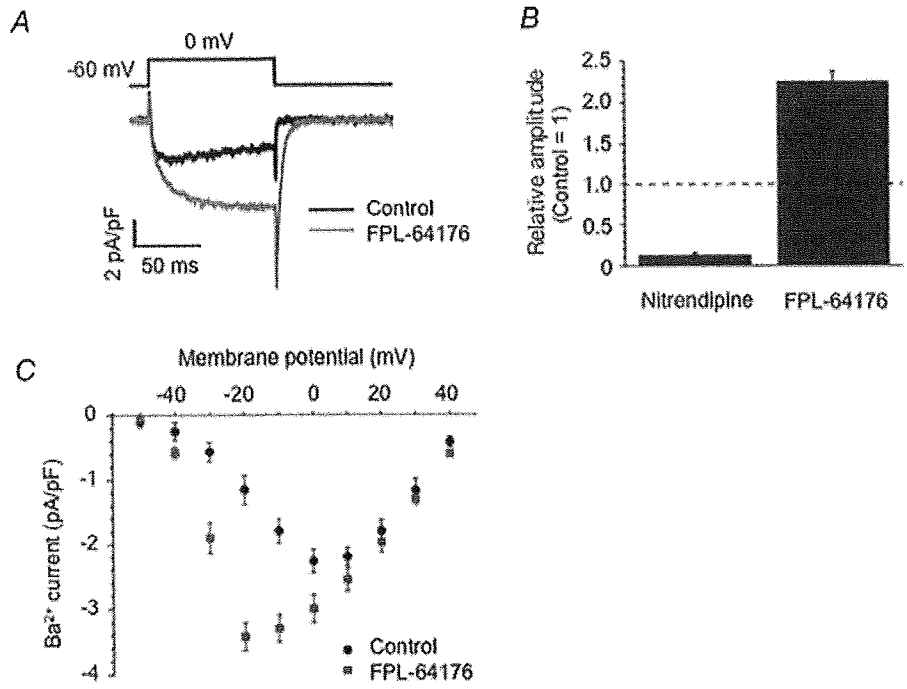


**Figure 4. Electrophysiological and pharmacological properties of the whole-cell inward current**
The bath contained 2 mM $Ca^{2+}$ (in *A* and *B*) or $Ba^{2+}$ (in *C*). *A*, inward $Ca^{2+}$ currents were evoked by depolarizing the membrane from a holding potential of −60 mV to a test potential of 0 mV. *B*, inhibition and enhancement of the inward $Ca^{2+}$ current by 1 $\mu$M nitrendipine and 10 $\mu$M FPL-64176, respectively. *C*, current–voltage relationship of the inward $Ba^{2+}$ current in the presence (squares) and absence (circles) of FPL-64176. Data represent the means ± S.E.M. ($n = 6$ for control and $n = 4$ for FPL-64176).

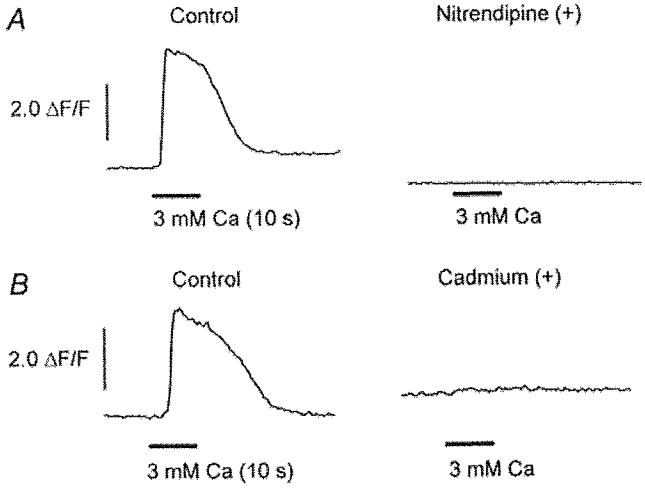


**Figure 5. Reduction of the high-$[Ca^{2+}]_o$-induced $Ca^{2+}$ transient by L-type $Ca^{2+}$ channel inhibitors**
The bath contained 2 mM $Ca^{2+}$. *A*, application of 3 mM $[Ca^{2+}]_o$ solution for 10 s evoked a $Ca^{2+}$ transient (left). Following > 25 min treatment with 1 $\mu$M nitrendipine, application of 3 mM $[Ca^{2+}]_o$ solution no longer induced a $Ca^{2+}$ transient (right). *B*, in the presence of 0.2 mM $Cd^{2+}$, application of 3 mM $[Ca^{2+}]_o$ solution failed to induce a $Ca^{2+}$ transient (right).

again the slow onset of the $Ca^{2+}$ transient was due to the gentle application of the high-$[Ca^{2+}]_o$ solution from a small-tipped pipette), but not following > 25 min treatment with 1 $\mu$M nitrendipine (Fig. 5*A*, right). In this population, all cells tested showed complete abrogation of the 3 mM $[Ca^{2+}]_o$-induced $Ca^{2+}$ transient ($n = 20$). The high-$[Ca^{2+}]_o$-induced $Ca^{2+}$ transient was also strongly inhibited by 0.2 mM $Cd^{2+}$ (Fig. 5*B*, right), where the average amplitude of fluo-3 fluorescence was decreased significantly from 3.9 (±1.1) to 0.44 (±0.6; $n = 20$).

## Voltage-dependent inactivation of the high-$[Ca^{2+}]_o$-induced $Ca^{2+}$ transient

Figure 6*B* shows that exposure to a 3.0 mM $[Ca^{2+}]_o$ failed to induce a noticeable $Ca^{2+}$ transient following 30 s exposure to the 150 mM $K^+$ solution ($n = 13$), which was the protocol used to inactivate the depolarization-induced $Ca^{2+}$ entry (see Fig. 2). Essentially the same results were obtained when a 2.5 mM $[Ca^{2+}]_o$ solution was applied externally to cells bathed in 1.5 mM $[Ca^{2+}]_o$ solution. Furthermore, an inhibitory effect was observed when 1 mM $Ca^{2+}$ was added to the high-$K^+$ solution to reduce the amplitude of the $Ca^{2+}$ transients. Thus, it would appear that this type of inhibition was due neither to store depletion nor to inhibition of $IP_3$-induced $Ca^{2+}$

release by the preceding long-lasting $Ca^{2+}$ transient. These results raise the possibility that dihydropyridine-sensitive $Ca^{2+}$ channels are involved in the high-$[Ca^{2+}]_o$-induced increase in $[Ca^{2+}]_i$ in human parathyroid cells.

## Discussion

In the present study, we have succeeded in directly detecting dihydropyridine-sensitive extracellular $Ca^{2+}$ entry in cultured human parathyroid cells. The results presented in Fig. 1 suggest that the 150 mM $K^+$-induced $Ca^{2+}$ transient, as observed in the presence of millimolar concentrations of $[Ca^{2+}]_o$, reflects $Ca^{2+}$ entry through voltage-dependent $Ca^{2+}$ channels and that the high $K^+$ *per se* is not the cause of $Ca^{2+}$ release from the endoplasmic reticulum via direct activation of the CaR. In these types of experiments, $[Ca^{2+}]_o$ of 1.5–2.0 mM was used because such $[Ca^{2+}]_o$ levels are considered physiological and the restimg membrane potential is $< -50$ mV (Bruce & Anderson, 1979; López-Barneo & Armstrong, 1983), which should not inactivate L-type $Ca^{2+}$ channels. Voltage-dependent inactivation of the high-$K^+$-induced $Ca^{2+}$ transient (Fig. 2) supports this idea. Further confirmation comes from the results in Fig. 4, where a whole-cell inward current was elicited by membrane depolarization with $Ca^{2+}$ or $Ba^{2+}$ as the charge carrier. The fluorescence measurement data presented in Fig. 3 and Table 1 suggest that voltage-dependent extracellular $Ca^{2+}$ entry is sensitive to L-type $Ca^{2+}$ channel modulators. In the experiments using 1 $\mu$M dihydropyridine antagonists, cells were pretreated with the drugs for > 25 min because the dihydropyridine-binding domains are not easily accessible at relatively hyperpolarized membrane potentials (Bean, 1984; Sanguinetti & Kass, 1984*a*; Bannister *et al.* 2009). In fact, the resting membrane potentials of murine parathyroid cells are reported to fall between -75 and -60 mV in the presence of 1.2–1.5 mM $[Ca^{2+}]_o$ (Bruce & Anderson, 1979; López-Barneo & Armstrong, 1983), the physiological $[Ca^{2+}]_o$ level. The cultured parathyroid cells were subjected to excitation light only during fluorescence measurements to avoid inactivation of the drugs, although both nitrendipine and nicardipine are resistant to light illumination, even at shorter wavelengths (Sanguinetti & Kass, 1984*b*). However, earlier reports refuting the dihydropyridine-sensitive $Ca^{2+}$ entry did not address either of the above points.

The data presented in Fig. 6 provide additional evidence that the dihydropyridine-sensitive $Ca^{2+}$ channels are involved in the regulation of $[Ca^{2+}]_i$ by $[Ca^{2+}]_o$. One may argue that the basal $[Ca^{2+}]_i$ level might be crucial in the regulation of PTH secretion. Although precise evaluation of the time-dependent change in basal $[Ca^{2+}]_i$ in the continuous (> 90 min) presence of $Ca^{2+}$ channel modulators was difficult in our experimental conditions (due to a slight but irreversible elevation of $[Ca^{2+}]_i$ caused presumably by exposure to an argon laser followed by > 60 min of inevitable time to ensure the drugs' effect), we occasionally noticed that the basal fluorescence levels were decreased by $Ca^{2+}$ channel antagonists (e.g. Fig. 5; in one population, 12 out of 20 cells decreased) and increased by FPL-64176 (e.g. Fig. 1*A*; in one population, 12 out of 20 cells increased). The exception was that the basal fluorescence level was clearly elevated in the presence of 0.2 mM $Cd^{2+}$ (e.g. Fig. 5*B*; in one population, 15 out of 15 cells elevated), possibly reflecting a CaR-mediated $IP_3$-induced $Ca^{2+}$ release from internal $Ca^{2+}$ stores.

The reason we were able to detect dihydropyridine-sensitive $Ca^{2+}$ channel activity is not clear. It should be noted, however, that channel activity (i.e. the high-$K^+$-induced $Ca^{2+}$ transient) was remarkable after 2 and 3 days of primary culture (since it took at least 1–2 days for the cells to become tightly adherent to the coverslips, we tested the high-$K^+$-induced response starting after 2 days of primary culture; prior to this time point, the response appeared to be small). Moreover, the response was not remarkable when cells were cultured in a low-serum (e.g. 2% fetal bovine serum) or low-$Ca^{2+}$ medium (K. Yokoyama *et al.*, unpublished observations). Since the high-$K^+$-induced $Ca^{2+}$ transient has also been observed in cells plated on collagen-coated coverslips in our laboratory (Ritter *et al.* 2004), it is unlikely that cellular contact with gelatin is necessary for expression of the $Ca^{2+}$ channels. In earlier reports, electrophysiological recordings were

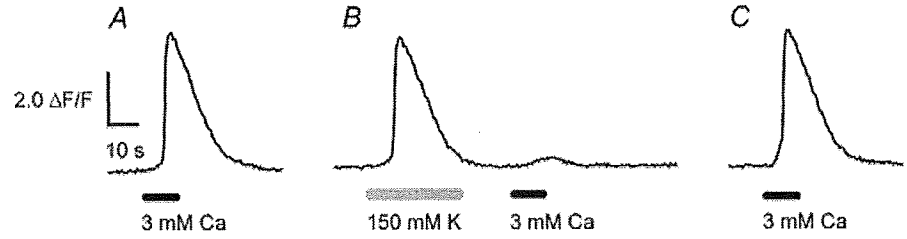


**Figure 6. Voltage-dependent inactivation of the high-$[Ca^{2+}]_o$-induced $Ca^{2+}$ transient**
*A*, the fluo-4 $Ca^{2+}$ transient induced by 10 s exposure to 3 mM $[Ca^{2+}]_o$. *B*, following 30 s exposure to 150 mM $K^+$ solution, the subsequent application of 3 mM $[Ca^{2+}]_o$ solution failed to induce a noticeable $Ca^{2+}$ transient. *C*, recovery of the 3 mM $[Ca^{2+}]_o$-induced $Ca^{2+}$ transient following a 10 min rest.

mostly performed on the day of isolation of bovine parathyroid cells without leak subtraction (e.g. Chang *et al.* 1995, 2001). Thus, culture medium together with the duration of culture after plating the cells appears to be critical for expression of the dihydropyridine-sensitive $Ca^{2+}$ channels in cultured parathyroid cells. In this respect, we cannot totally rule out the possibility that the expression of dihydropyridine receptor could simply be a response to the challenge of the culture environment.

The mechanism by which $Ca^{2+}$ channels become activated in physiological conditions remains to be elucidated. The resting membrane potentials of murine parathyroid cells have been reported to fall between −75 and −60 mV in the presence of 1.2–1.5 mM $[Ca^{2+}]_o$. In principle, therefore, the membrane must be depolarized in order for the $Ca^{2+}$ channels to become effectively activated. It is possible that an increase in extracellular $Ca^{2+}$ activates the CaR, which mildly depolarizes the plasma membrane through inhibition of the $Ca^{2+}$-activated $K^+$ channels (Bruce & Anderson, 1979; Komwatana *et al.* 1994; Vassilev *et al.* 1997), probably with a high affinity for $Ca^{2+}$. Alternatively, some transient receptor potential (TRP) channels might be responsible for the initial mild depolarization of the membrane in the presence of high $[Ca^{2+}]_o$. While extracellular $Ca^{2+}$ entry *per se* may further depolarize the membrane, $Ca^{2+}$-activated $K^+$ channels, probably with a low affinity for $Ca^{2+}$, may compete with this effect (Komwatana *et al.* 1994; Kanazirska *et al.* 1995; Vassilev *et al.* 1997), resulting in relatively mild and slow membrane potential change during exposure to high $[Ca^{2+}]_o$ (Bruce & Anderson, 1979; López-Barneo & Armstrong, 1983). In addition, the possibility that the CaR or TRP channels directly or indirectly activate voltage-dependent $Ca^{2+}$ channels without causing membrane depolarization cannot be ruled out.

Regardless of the mechanism by which the dihydropyridine-sensitive $Ca^{2+}$ channels are activated in physiological conditions, the present study demonstrates, for the first time, that human parathyroid cells express a dihydropyridine- and voltage-sensitive $Ca^{2+}$ entry system. This system may provide a compensatory pathway for the negative feedback regulation of PTH secretion, especially in hyperplastic conditions in which CaR is poorly expressed.

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