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# Selectivity of felodipine for depolarized ventricular myocytes: a study at the single-cell level

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**Abstract**

We studied the effects of felodipine (a second-generation dihydropyridine $Ca^{2+}$ channel blocker) on excitation-contraction coupling (E-C coupling) in single isolated guinea-pig ventricular myocytes, using the whole-cell perforated patch-clamp technique or the Ca indicator, indo-1. Felodipine inhibited both L-type $Ca^{2+}$ channel currents ($I_{Ca}$) and cell contractions in a concentration-dependent manner (10 pM to 100 nM) when we used a holding potential of $-80$ mV or $-40$ mV. The potency of felodipine was sharply dependent on a holding potential. Namely, use of a more depolarized holding potential markedly increased the potency of felodipine for inhibition of $I_{Ca}$ and cell contraction. Next we current-clamped cells and obtained the resting membrane potential of $-82 \pm 8$ mV. When cells were current-injected at 0.1 Hz, exposure to 10 nM felodipine slightly but significantly diminished the amplitude of cell contractions ($7.2 \pm 1.6$ to $6.7 \pm 1.7$ μm, $P < 0.05$) within 10 min. When cells were field stimulated, exposure of cells to 10 nM felodipine also slightly diminished the amplitude of cell shortening ($5.1 \pm 2.0$ to $4.6 \pm 1.9$ μm, $P < 0.05$) and $[Ca^{2+}]_i$ transients. We observed clear voltage-dependent blockade of E-C coupling by felodipine in ventricular myocytes. Thus, therapeutic concentrations (1–10 nM) of felodipine could inhibit E-C coupling in depolarized ventricular myocytes, which might simulate an ischemic or failing heart.

*Keywords:* Felodipine; Ventricular cell, guinea pig; $Ca^{2+}$ channel, L-type; Voltage-dependent block; E-C (excitation-contraction) coupling

## 1. Introduction

L-type $Ca^{2+}$ channels represent a major route by which $Ca^{2+}$ enters cardiac myocytes or vascular smooth muscle cells, and play an important role in excitation-contraction coupling (E-C coupling). To clarify the effects of vascular selective $Ca^{2+}$ channel blockers on cardiac myocytes, it seems important to know the relationship between $I_{Ca}$ and cell contraction during exposure to $Ca^{2+}$ channel blockers. Dihydropyridine $Ca^{2+}$ channel blockers including nifedipine, nitrendipine, nisoldipine and amplodipine generally show tissue selectivity (Fleckenstein, 1983; Godfraind et al., 1988; Sun and Triggle, 1995). Particularly newly developed dihydropyridine $Ca^{2+}$ channel blockers at therapeutic concentrations have been shown to dilate peripheral arteries without depressing cardiac function (Burges et al., 1987; Cheng et al., 1994). These reports suggested that second-generation dihydropyridine $Ca^{2+}$ channel blockers may have a potential beneficial effect in the treatment of congestive heart failure. Other vasodilators including prazosin and nifedipine did not improve the prognosis or symptoms of patients with congestive heart failure in spite of their short-term beneficial effects on hemodynamics (Cohn et al., 1986; Elkayam et al., 1990). The $\alpha_1$ subunits of the L-type Ca channels found in cardiac and smooth muscles are homologous proteins and are splicing products of the type 2 calcium channel gene, which is distinct from the type 1 gene encoding the skeletal muscle $\alpha_1$ subunit. Despite this analogy, these newly developed Ca channel blockers are 100 times more selective for excised artery ring than for papillary muscle (Ljung et al., 1987). Thus, the tissue selectivity appears to be mainly due to the less polarized membrane potential of vascular smooth muscle cells than of cardiac myocytes (Green et al., 1985; Lumley

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and Robertson, 1987; Sun and Triggle, 1995) although different molecular structures may partly contribute to tissue selectivity (Welling et al., 1993). Dihydropyridine $Ca^{2+}$ channel blockers show voltage-dependent blockade of L-type $Ca^{2+}$ channels in vascular smooth muscle cells (Nelson and Worley, 1989). The therapeutic dose of dihydropyridine $Ca^{2+}$ channel blockers was thought not to affect cardiac performance, but actually worsened congestive heart failure despite the afterload reduction (Elkayam et al., 1990; Jezek et al., 1990). These results also suggest that the tissue selectivity of dihydropyridine $Ca^{2+}$ channel blockers depends mainly on the membrane potential of cells (Sun and Triggle, 1995). If so, depolarized cardiac myocytes (ischemic or under cardiac glycoside treatment) can be as susceptible to 'vascular selective dihydropyridine $Ca^{2+}$ channel blockers' as vascular smooth muscle cells. Earlier studies showed voltage-dependent blockade of $I_{Ca}$ by various $Ca^{2+}$ channel blockers (Bean, 1984; Sanguinetti and Kass, 1984; Wibo, 1989; Herzig et al., 1992). However, voltage-dependent inhibition of contraction has not been directly shown although earlier works showed that cells depolarized by exposure to high $K^+$ were more sensitive to dihydropyridine $Ca^{2+}$ channel blockers (Herzig et al., 1992; Sun and Triggle, 1995). In those studies, membrane potentials were not measured. As far as we know, no studies have directly shown the effects of membrane potentials on $I_{Ca}$ or cell contraction during exposure to felodipine. We, therefore, studied the effects of felodipine on $I_{Ca}$ and cell contraction while the membrane potential was varied.

## 2. Materials and methods

### 2.1. Preparation of isolated single ventricular myocytes

$Ca^{2+}$-tolerant single ventricular myocytes were isolated from the ventricles of 250–400 g adult female guinea pigs using an enzymatic method as described elsewhere (Kohmoto et al., 1994). Adult guinea pigs were deeply anesthetized with pentobarbital sodium (35 mg/kg), and the hearts were removed via a thoracotomy. The heart was immediately attached to an aortic cannula, which provided continuous retrograde coronary artery perfusion at 37°C with constant flow (5–10 ml/min). The heart was perfused with solution A for 5 min, immediately followed by 12 min of recirculating perfusion with solution B. The heart was then perfused with solution C for 5 min. The heart was then detached from the cannula, and the ventricles were cut into small pieces and gently shaken for 10 min at 37°C in solution C to disperse the cells. The cells were resuspended in a HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)) buffered physiological solution (solution D). Isolated myocytes were attached to the bottom of a cell bath with CR-laminin (Collaborative Research, Bedford, MA, USA) and viewed with an inverted microscope (Diaphot, Nikon, Tokyo, Japan).

Solution A contained (in mM) 126.0 NaCl, 22.0 dextrose, 4.4 KCl, 5.0 $MgCl_2$, 20.0 taurine, 5.0 creatine, 1.0 $NaH_2PO_4$, 24.0 HEPES and 12.5 NaOH. Solution B contained solution A + 0.1 mM $CaCl_2$, 1.0 mg/ml collagenase (class II, Worthington Biomedical, Freehold, NJ, USA) and 0.1 mg/ml protease (type XIV, Sigma, St. Louis, MO, USA). Solution C contained solution A + 0.1 mM $CaCl_2$. Solution D contained (mM) 138.0 NaCl, 11.0 dextrose, 4.4 KCl, 1.0 $MgCl_2$, 2.7 $CaCl_2$, 12.0 HEPES and 6.4 NaOH. The pH was 7.35 in all solutions. All the experiments were performed at room temperature (21–23°C). We added 150 μM nystatin to the pipette solution just before use. We made fresh nystatin pipette solution every hour to ensure its activity. We obtained giga seals by using the conventional method, then waited for 10 min to obtain intracellular access with perforated patch.

### 2.2. Simultaneous measurement of intracellular free calcium and cell motion

$[Ca^{2+}]_i$ was measured with the $Ca^{2+}$-sensitive fluorescent dye indo-1 (Grynkiewicz et al., 1985). The 'loading stock' of 100 μM indo-1 AM was prepared as described by DuBell et al. (1988). Coverslips of isolated guinea-pig ventricular cells were incubated at room temperature in normal Tyrode solution containing 5 μM indo-1 AM for 20 min and then washed in indo-1-free solution for 15 min. After dye loading, a coverslip was placed in a flow-through cell chamber and continuously superfused with HEPES-buffered normal Tyrode solution.

The instrumentation used for fluorescence measurement has been described (Peeters et al., 1987). Briefly, the system uses a high-pressure mercury-arc lamp as the excitation source. The fluorescent light was collected by the objective lens and divided with a dichroic mirror system (Model FM-1000, Rincon Scientific, Palo Alto, CA, USA) to permit simultaneous measurement of both 400 and 500 nm wavelengths by use of two separate photomultiplier tubes. The ratio emitted fluorescence (400/500 nm) was obtained on-line through an analog divider circuit. Cell shortening of isolated adult ventricular myocytes was measured by tracking the end of a single cell with a video motion analyzer (Crescent Electronics, Salt Lake City, UT, USA) (Steadman et al., 1988). Cells were paced with field stimulation as described previously (Kohmoto et al., 1990).

### 2.3. Whole-cell voltage clamp

Membrane currents and potentials were recorded using the whole-cell variant of the patch-clamp technique (Axopatch 200A, Axon Instruments, Burlingame, CA, USA). Suction micropipettes, with tip diameters 3–5 μm (0.5–1.5 MΩ resistance when filled), were pulled (model P-87, Sutter Instrument Co., Novato, CA, USA) from

borosilicate capillary tubing (Corning 7052, 1.65 mm o.d., 1.2 mm i.d., A-M Systems, Everett, WA, USA) and lightly fire-polished. For current-clamp experiments, microelectrodes were filled with dialyzing solution containing (mM) 110.0 K-gluconate, 20 KCl, 10 NaCl, 0.5 $MgCl_2$, 5.0 $K_2ATP$, 5.5 dextrose and 10 HEPES; they also contained 20 μM EGTA and no added $Ca^{2+}$. For voltage-clamp experiments, microelectrodes were filled with dialyzing solution containing (mM) 130.0 CsCl, 0 or 10 NaCl, 0.5 $MgCl_2$, 5.0 $K_2ATP$, 5.5 dextrose and 10 HEPES; they also contained 20 μM EGTA (ethylene glycol-bis(β-aminoethlyl ether) and no added $Ca^{2+}$. The pH was adjusted to 7.1 with KOH.

### 2.4. Simultaneous measurement of the $Ca^{2+}$ current and cell contraction

Depolarizing steps from a holding potential of −40 mV triggered L-type $Ca^{2+}$ channel currents ($I_{Ca}$). We used a holding potential of −80 mV in some experiments. An initial inward $I_{Ca}$ was activated every 10 or 60 s by depolarizing the cell to +10 mV for 0.4 s and produced a phasic contraction of ventricular myocytes. Two-step depolarization from −80 mV to −40 mV for 0.1 s and subsequently to +10 mV for 0.4 s also activated $I_{Ca}$ and a phasic contraction. For voltage-current and -motion experiments, a conditioning protocol was not used because single depolarization may enhance dihydropyridine $Ca^{2+}$ channel blockers binding to $Ca^{2+}$ channels. Instead to maintain sarcoplasmic reticulum $Ca^{2+}$ content constant, we applied a depolarizing pulse at 0.1 Hz. At this rate of stimulation, we did not observe a positive or negative staircase phenomenon. We estimated the peak $I_{Ca}$ by using the difference between the peak inward current and the current at 350 ms ($I_{peak} - I_{350}$). It is possible that $I_{Ca}$ may not be fully inactivated at 350 ms. Other ions (particularly $K^+$) may contribute to the time-dependent currents measured under voltage clamp because of the complex nature of the intracellular and extracellular solution necessary to permit cell contraction with depolarization. We used a high concentration of $Cs^+$ as intracellular perfusate to avoid con-

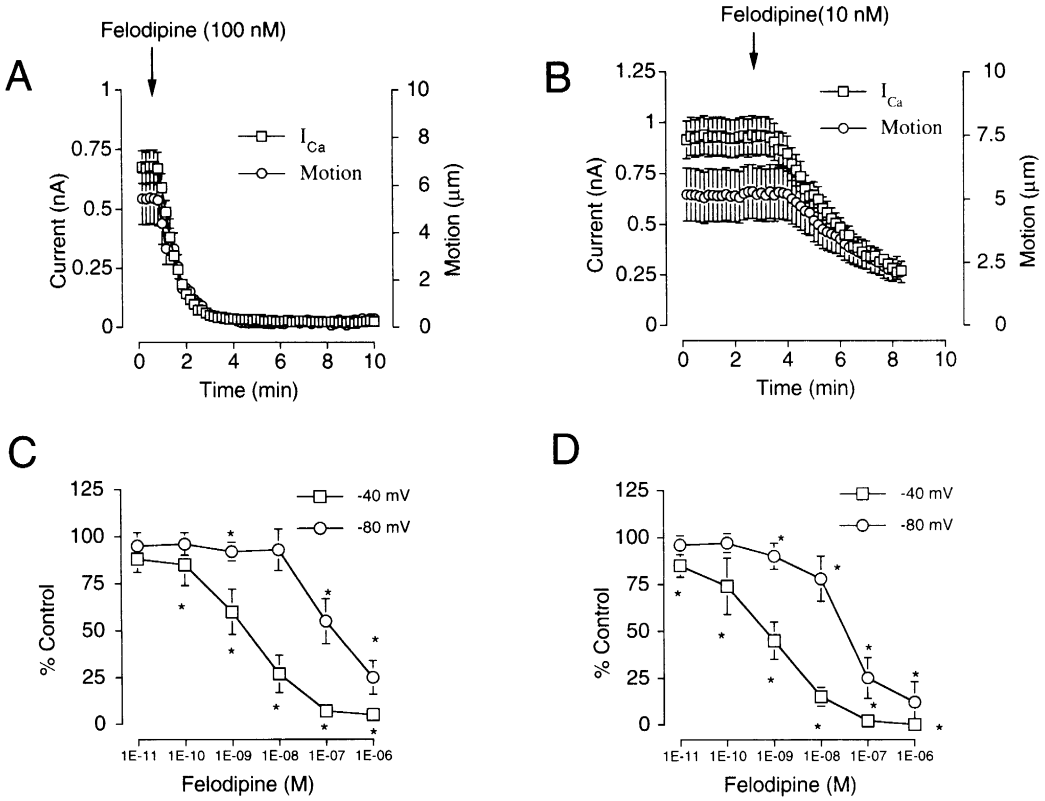


Fig. 1. (A and B) Time-course of the amplitude of cell shortening and $I_{Ca}$ when cells were exposed to 100 nM (A) or 10 nM (B) felodipine at a holding potential of −40 mV. Cells were depolarized at 0.1 Hz using 0.4 s pulses to +10 mV from the holding potential. Felodipine was added to the chamber at the time indicated by the arrow. There was a decrease in both the amplitude of cell (open circle) contraction and $I_{Ca}$ (open square). Each point is the mean (±S.E.M.) of 3–5 observations. Lower panels: Concentration-response relationships for felodipine-induced inhibition of peak calcium channel currents (C) and the amplitude of cell contraction (D) at −40 mV (□) or at −80 mV (○) in voltage-clamped cells. Cells were depolarized every 10 s using 0.4 s pulse to +10 mV from a holding potential of −40 mV (single depolarization step) or −80 mV (two-step depolarization from −80 mV to −40 mV then to +10 mV). The average amplitude of cell contractions or peak current was measured at each concentration of drug in each cell 5 min after exposure to test solutions. Results are normalized with respect to the control values in the absence of any drug. Each point is the mean (±S.E.M.) of 3–5 observations. Each point was compared with the control value. * $P < 0.05$.

tamination by outward $K^+$ current. Contractions were simultaneously measured as changes in cell edge position with a video-motion detector (Steadman et al., 1988).

### 2.5. Solutions

Cells were superfused with normal Tyrode solution containing (in mM) 137.0 NaCl; 3.7 KCl; 0.5 $MgCl_2$; 1.8 $CaCl_2$; 5.6 glucose; and 4.0 HEPES (free acid) titrated to pH 7.35 with 2.1 mM NaOH. Felodipine (gift from Hoechst Japan, Tokyo) was dissolved in ethanol to give a stock solution of 10 mM. Final concentration of ethanol was adjusted in every test solution.

### 2.6. Data acquisition and statistics

We digitized the membrane current, voltage, or $[Ca^{2+}]_i$ and cell shortening with a 12-bit A/D converter (Digidata 1200) and stored the digitized data with Clampex or Axotape software (Axon Instruments, Burlingame, CA, USA) on a Pentium personal computer (Fujitsu, Tokyo, Japan). We analyzed the data with Clampfit or Fetchan software (Axon Instruments). Values are expressed as means ± S.E.M. Comparison of two groups was performed by the paired *t*-test. $P < 0.05$ was considered statistically significant.

## 3. Results

To obtain a dose–response curve for felodipine-induced inhibition of $I_{Ca}$ and cell contraction with different holding potentials, we voltage-clamped the cells with a holding potential of −40 mV or −80 mV. First we depolarized cells from −40 mV to +10 mV for 0.4 s at 0.1 Hz to produce $I_{Ca}$ and a phasic contraction. Exposure of these cells to 100 nM felodipine rapidly inhibited both $I_{Ca}$ and cell contraction within 1 min. Representative tracings are shown in Fig. 1A. With a lower concentration (10 nM), it took longer to obtain a reduction in $I_{Ca}$ and in the amplitude of cell contraction (Fig. 1B). We repeated the same protocols with various concentrations of felodipine (10 pM–100 nM). Thus felodipine (10 pM–100 nM) produced a concentration-dependent inhibition of $I_{Ca}$ and of cell contraction in single isolated guinea-pig ventricular myocytes held under voltage clamp at a holding potential of −40 mV. Open squares in Fig. 1C and 1D indicate the dose response of felodipine-induced inhibition when cells were held at −40 mV. Next we repeated the above protocols using a holding potential of −80 mV. Cells were depolarized from a holding potential of −80 mV to −40 mV for 0.1 s and then to +10 mV for 0.4 s at 0.1 Hz. Exposure of cells to various concentrations of felodipine inhibited both $I_{Ca}$ and cell contraction. However, a much

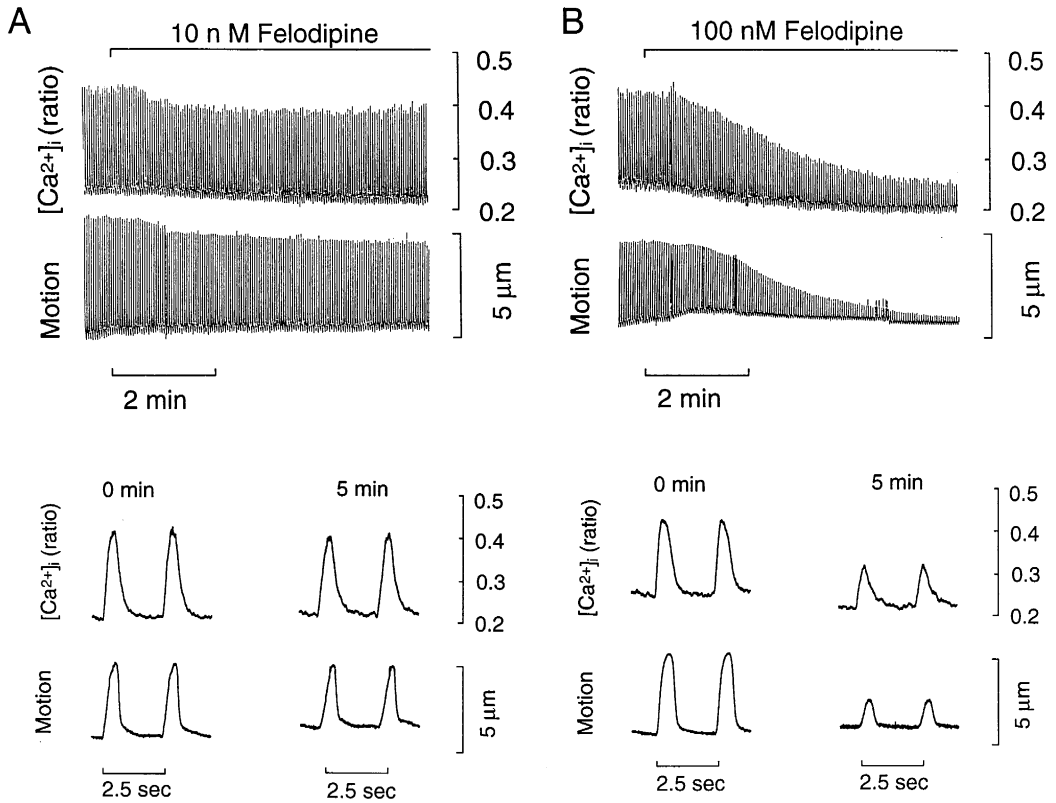


Fig. 2. Representative tracings of simultaneous records of cell contractions and $[Ca^{2+}]_i$ transients during exposure to felodipine. Cells were indo-1-loaded and field-stimulated at 0.4 Hz. (A) 10 nM felodipine: There were slight reductions in the amplitude of cell contraction and $[Ca^{2+}]_i$ transients during exposure to 10 nM felodipine. (B) 100 nM felodipine: There were rapid reductions both in the amplitude of cell contraction and $[Ca^{2+}]_i$ transients.

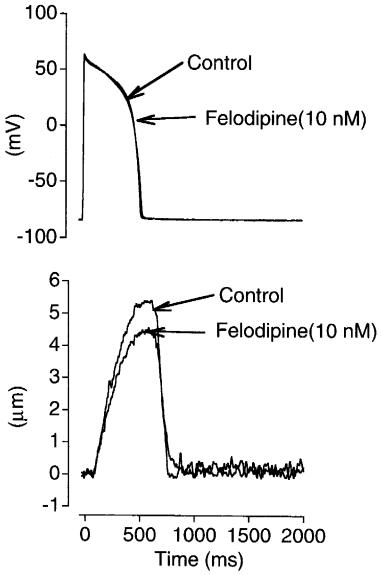


Fig. 3. Representative tracings of membrane potential and cell contraction before and 5 min after exposure to 10 nM felodipine are shown. Cells were current-injected at 0.1 Hz under current-clamp mode conditions. Exposure to 10 nM felodipine slightly decreased the amplitude of cell contraction without change in the action potential.

higher concentration was necessary to obtain a similar extent of inhibition. Open circles in Fig. 1C and 1D indicate the dose response of felodipine-induced inhibition when cells were held at −80 mV. We observed marked differences in the potency of felodipine for inhibition of $I_{Ca}$ and cell contraction when the holding potential was varied. When we used a holding potential of −40 mV instead of −80 mV, the dose–response curves were dramatically shifted to the left (Fig. 1C and 1D).

Next, we studied the effects of felodipine in beating ventricular myocytes with field stimulation. Cells were loaded with indo-1 and field-stimulated at 0.4 Hz. We simultaneously measured cell shortening and $[Ca^{2+}]_i$ transients. Exposure of cells to 10 nM felodipine slightly but significantly diminished the amplitude of cell contraction ($5.1 \pm 2.0$ to $4.6 \pm 1.9$ μm, $n = 5$, $P < 0.05$) or $[Ca^{2+}]_i$ transients within 5 min. Representative tracings are shown in Fig. 2A. Exposure to a higher dose of felodipine (100 nM) steadily inhibited both cell contraction ($5.8 \pm 2.1$ to $0.5 \pm 0.4$ μm, $n = 5$, $P < 0.001$) and $[Ca^{2+}]_i$ transients (Fig. 2B). These results are consistent with the dose–response curves shown in Fig. 1. To confirm the resting membrane potential of these indo-1-loaded cells, we patch-clamped the cells and obtained the resting membrane potential of $-82 \pm 8$ mV ($n = 5$) using the current-clamp method. To study the effects of felodipine on the action potential and cell contraction, cells were current-injected at 0.1 Hz. After the action potential and cell contraction were recorded under control conditions, cells were exposed to solutions containing 10 nM felodipine. Representative tracings are shown in Fig. 3A. The amplitude of cell contraction was slightly but significantly decreased within 6 min ($7.2 \pm 1.6$ to $6.7 \pm 1.7$ μm, $n = 4$, $P < 0.05$). The half-width of action potentials was not significantly changed ($474 \pm 24$ to $462 \pm 28$ ms, $n = 4$, N.S.). Data are summarized in Fig. 3B and 3C. When cells were voltage-clamped at a holding potential of −40 mV, 10 nM felodipine markedly inhibited cell contraction as shown above. In contrast, 10 nM felodipine produced subtle effects on the amplitude of cell contraction when cells were field-stimulated or current-clamped. This discrepancy appears to be due to differences in resting membrane potentials. Dihydropyridine $Ca^{2+}$ channel blockers bind rapidly with 'inactivated' L-type $Ca^{2+}$ channels (Bean, 1984; Green et al., 1985) and block $I_{Ca}$ more effectively in depolarized cells, although this has not been proven with felodipine.

Thus, we performed experiments to show the voltage-dependent blockade of cardiac L-type $Ca^{2+}$ channels by felodipine (Fig. 4). It is well known that dihydropyridine $Ca^{2+}$ channel blockers bind more rapidly to its receptors when cells are depolarized (Sanguinetti and Kass, 1984; Pérez-Vizcaíno et al., 1993). During the initial 3 min after exposure to felodipine, the membrane potential was held at

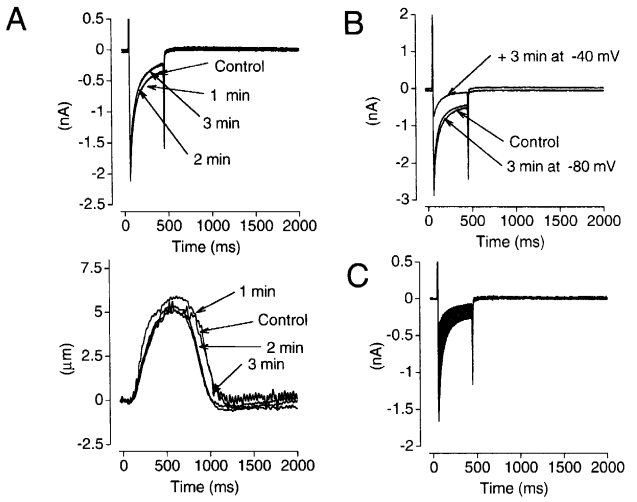


Fig. 4. The effects of holding potential on the action of 10 nM felodipine in voltage-clamped ventricular myocytes. (A) Representative tracings of $I_{Ca}$ and cell contraction are shown when cells were exposed to 10 nM felodipine at a holding potential of −80 mV for 3 min. An initial depolarization from −80 mV to −40 mV for 0.1 s and subsequent depolarization from −40 mV to +10 mV for 0.4 s every minute produced $I_{Ca}$ (upper traces) and phasic contraction (lower traces). As expected from a previous series of experiments, exposure to 10 nM felodipine did not produce a significant decrease in the amplitude of cell contraction and $I_{Ca}$. (B) Effects of holding potential on felodipine-induced inhibition of $I_{Ca}$ and cell contraction. Cells were voltage-clamped at a holding potential of −80 mV initially. We first measured $I_{Ca}$ and cell contraction under control conditions. Then we exposed cells to 10 nM felodipine. During the 3-min exposure we did not observe any changes in $I_{Ca}$ or cell contraction. Then the holding potential was changed from −80 mV to −40 mV. After another 3 min, we observed marked reductions in $I_{Ca}$ and the amplitude of cell contraction. (C) This graph shows tracings of $I_{Ca}$ during 3 min after changing a holding potential from −80 mV to −40 mV in the presence of 10 nM felodipine. All traces were superimposed. In contrast to an initial 3 min, $I_{Ca}$ was steadily inhibited by felodipine.

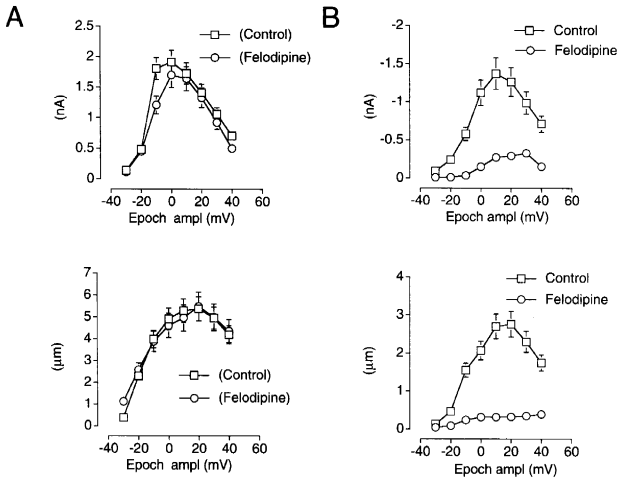


Fig. 5. The effects of felodipine on calcium channel currents and cell contraction in guinea-pig ventricular myocytes when cells were held at a holding potential of −80 mV (A) or −40 mV (B). Cells were depolarized every 10 s from a holding potential of −80 mV to −40 mV for 0.1 s then depolarized to test potentials (−30, −20, −10, 0, +10, +20, +30, +40 mV) for 0.4 s (A) or from a holding potential of −40 mV to test potentials (−30, −20, −10, 0, +10, +20, +30, +40 mV) for 0.4 s (B). Peak calcium channel currents and cell contraction were recorded. Subsequently cells were exposed to 10 nM felodipine for 3 min. We again measured the voltage-current and voltage-shortening relationships. Upper panels show the effects of felodipine on the voltage-current relationship while lower panels show the effects of felodipine on the voltage-shortening relationship. We observed subtle effects on these relationships at a holding potential of −80 mV (A). At every epoch amplitude except −10 mV step, changes in the amplitude of cell contraction or $I_{Ca}$ were not statistically significant (A). In contrast, when we used the holding potential (B), we observed a marked downward shift in these relationships. Changes were statistically significant ($P < 0.05$) except at the −30 mV step. Each point is the mean (±S.E.M.) of 4–5 observations.

−80 mV and then it was changed to −40 mV. Cells were held quiescent except when recording $I_{Ca}$ and cell motion. We observed no changes in $I_{Ca}$ during the first 3 min after exposure to felodipine (Fig. 4B). Three minutes after a holding potential was set −40 mV (6 min after felodipine administration), $I_{Ca}$ was markedly reduced, as shown in Fig. 4B and 4C. Fig. 5A shows current-voltage and motion-voltage relationships before and after exposure to 10 nM felodipine when a holding potential was held at −80 mV. Fig. 5B shows the effects of 10 nM felodipine on current-voltage and motion-voltage relationships when a holding potential was held at −40 mV (compare Fig. 5A and 5B). This result indicates that felodipine inhibited $I_{Ca}$ and cell contraction in depolarized cells much more effectively than in polarized cells.

There is another possible mechanism that may explain why felodipine did not inhibit effectively field-stimulated or current-injected cells. Cell contraction is possibly triggered by both $I_{Ca}$ and $Na^+$ currents ($I_{Na}$) induced $Ca^{2+}$ influx through Na-Ca exchange under physiological conditions (as suggested by LeBlanc and Hume, 1990). Thus we recorded the membrane current and cell shortening when cells were depolarized from −80 mV to +10 mV and from −40 mV to +10 mV for 0.4 s. In control cells, both depolarization protocols produced a similar amplitude of cell contraction (Fig. 6A). Depolarization from −80 to +10 mV provoked both $I_{Na}$ and $I_{Ca}$ while depolarization from −40 mV to +10 mV provoked only $I_{Ca}$. Series resistance was approximately 70% corrected. Voltage control error was negligible when $I_{Ca}$ was evoked by depolarization from −40 mV to +10 mV because it was estimated as approximately 0.9 mV = 3 MΩ (series resistance) × 0.3 × 1 nA ($I_{Ca}$) However, voltage control error was probably significant when depolarization from −80 mV to +10 mV was used. The voltage control error was estimated as approximately 18 mV = 3 MΩ (series resistance) × 0.3 × 20 nA. These cells were then held at a holding potential of −40 mV and exposed to 10 nM felodipine. Five minutes after exposure, the same depolarization protocols were applied to the same cells. Depolarization from −80 mV to +10 mV triggered a similar amplitude of cell contraction to that of control cells but with a shorter contraction duration. However, depolarization from −40 mV to +10 mV triggered a smaller $I_{Ca}$ and a smaller amplitude of contraction (~50% reduction). Under these conditions, L-type $Ca^{2+}$ channels were partially blocked by felodipine. Even if depolarization from −80 mV to +10 mV causes significant voltage control errors, this protocol should not be an optimal condition to evoke $I_{Ca}$ compared with −40 mV to +10 mV. Thus larger contractions evoked by depolarization from −80

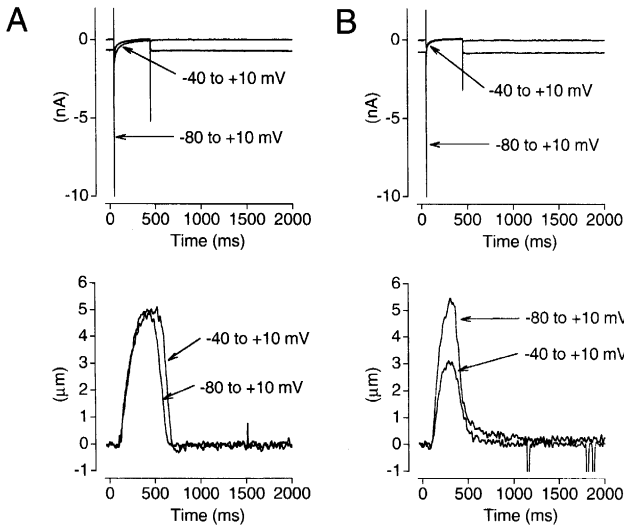


Fig. 6. (A) Control: Representative tracings of simultaneous recordings of membrane current and cell contraction when cells were depolarized from −80 mV to +10 mV for 0.4 s and from −40 mV to +10 mV for 0.4 s. Both depolarization protocols produced a similar amplitude of contraction. (B) Five minutes after exposure to 10 nM felodipine at a holding potential of −40 mV: Depolarization from −80 mV to +10 mV for 0.4 s produced a similar amplitude of contraction as control. But depolarization from −40 mV to +10 mV for 0.4 s evoked a smaller amplitude of contraction than that from −80 mV to +10 mV. $I_{Ca}$ was consistently diminished after exposure to 10 nM felodipine.

mV to +10 mV could not be due to enhanced $Ca^{2+}$ influx through L-type $Ca^{2+}$ channels. When $I_{Ca}$ is partially inhibited, $Ca^{2+}$ influx except through L-type $Ca^{2+}$ channels might become more important in E-C coupling. In beating heart, association of felodipine with dihydropyridine receptors may be slower than in voltage-clamped cells at a holding potential of −40 mV. $Ca^{2+}$ influx except through L-type $Ca^{2+}$ channels could also trigger contractions in field-stimulated or current-injected but not in voltage-clamped cells at a holding potential of −40 mV.

## 4. Discussion

It has been established that some vasodilators improve the prognosis of patients with heart failure. The V-HeFT I and II studies provided information about treatment of congestive heart failure with conventional therapy, and have been used to evaluate the long-term effects of vasodilators (Cohn et al., 1986, 1991). In the V-HeFT I study, the combination of hydralazine and isosorbide dinitrate showed a beneficial effect on prognosis in heart failure. The V-HeFT II study demonstrated that enalapril had a more favorable effect on 2-year survival than a combination of hydralazine plus isosorbide dinitrate. The V-HeFT studies showed that although not all vasodilators are alike, their differing effects might be beneficial when used in combination. First-generation calcium channel blockers including nifedipine do not have beneficial effects in the treatment of congestive heart failure, probably due to negative inotropic action (Elkayam et al., 1990). Second-generation dihydropyridine $Ca^{2+}$ channel blockers including felodipine and amlodipine do not show significant negative inotropic effects over the therapeutic range (Burges et al., 1987; Ljung et al., 1987). Amlodipine improves the prognosis of congestive heart failure with nonischemic cardiomyopathy (Packer et al., 1996). Felodipine without angiotensin converting enzyme inhibitors does not show beneficial effects on congestive heart failure (Tan et al., 1987; Littler and Sheridan, 1995). However, the efficacy of its combination with angiotensin converting enzyme inhibitors has not been clarified. Determination of the potential additive effect of felodipine when used in combination with an angiotensin converting enzyme inhibitor, is the major goal of the V-HeFT III study. Thus, we studied whether felodipine at therapeutic concentrations may produce negative inotropic effects in adult mammalian ventricular myocytes.

When cells were voltage-clamped at a holding potential of −40 mV, 10 nM felodipine (maximal therapeutic concentration) produced a marked reduction both in $I_{Ca}$ and in the amplitude of cell contraction. However, when a holding potential was held at −80 mV, we observed subtle changes in $I_{Ca}$ and in the amplitude of cell contraction after exposure of cells to 10 nM felodipine. These results suggest that in normally polarized cells therapeutic concentrations of felodipine do not depress cardiac performance. When cells loaded with indo-1 were field-simulated, we observed the effects of 10 nM felodipine on $[Ca^{2+}]_i$ transients and cell contraction. Felodipine (10 nM) only slightly diminished the amplitude of $[Ca^{2+}]_i$ transients and cell contraction. But when cells were exposed to felodipine (100 nM), there were marked reductions in the amplitude of $[Ca^{2+}]_i$ transients and cell contraction. Felodipine (10 nM) only slightly changed the amplitude of cell shortening when cells were current-clamped and current-injected. These results indicate that blockade of $I_{Ca}$ and cell contraction by felodipine is voltage dependent and tissue specificity depends on tissue-specific membrane potential. If cardiac myocytes are depolarized (treatment with cardiac glycoside or ischemia), therapeutic concentrations of felodipine possibly inhibit cardiac performance. When administered to patients with effort angina, felodipine at usual doses may not act on normally polarized ventricular myocytes. But during an angina episode, part of the myocardium becomes ischemic and depolarized. Felodipine may effectively bind to $Ca^{2+}$ channels on depolarized myocytes and decrease oxygen demand. However, when given to patients with congestive heart failure, felodipine might depress cardiac function and worsen congestive heart failure if the failing myocardium should be somehow depolarized. We do not know exactly the membrane potential of failing human heart or ischemic heart. Nabauer et al. (1993) reported that single ventricular myocytes isolated from end-stage failing human heart had a similar resting potential as that of non-failing human hearts. From our study, in depolarized cardiac myocytes, therapeutic concentrations of felodipine can still depress cardiac function. The sensitivity of ventricular cells to felodipine may vary among species. The PRAISE study (Packer et al., 1996) showed that amlodipine improved the prognosis of patients with dilated cardiomyopathy but not with ischemic cardiomyopathy. This beneficial effect in contrast to first-generation dihydropyridine $Ca^{2+}$ channel blockers may be obtained by strict tissue selectivity or the absence of reflex activation of the adrenergic system (very slow onset of action). Amlodipine exerts voltage-dependent blockade of L-type $Ca^{2+}$ channels in vascular smooth muscle cells and cardiac myocytes as do first-generation dihydropyridine $Ca^{2+}$ channel blockers (Kass et al., 1991; Hughes and Wijetunge, 1993). Felodipine also produced voltage-dependent blockade of L-type $Ca^{2+}$ channels in cardiac myocytes, as shown above and by Sun and Triggle (1995). The tissue selectivity of felodipine appears to depend on the membrane potential. If this selectivity should be lost in failing hearts, therapeutic doses of felodipine would exert negative inotropic effects. If intracellular $Na^+$ concentrations were to be elevated in failing hearts, $Na^+$ current-induced contraction would play a more important role in E-C coupling because $Na^+$ current-induced contraction is assumed to depend heavily on the intracellular $Na^+$ concen-

tration. Due to the complex nature of E-C coupling in the failing myocardium, we cannot make a simple speculation regarding the effects of felodipine on failing heart on the basis of our data. Preliminary results of the V-HeFT III study showed that felodipine in combination with angiotensin converting enzyme inhibitors appears not to improve the prognosis of the patients with congestive heart failure. This clinical outcome is consistent with our in vitro data. However, it is well known that coronary occlusion produces myocardial ischemia and membrane depolarization. Therefore therapeutic concentrations of felodipine may inhibit ventricular contraction and protect the ischemic part of the myocardium without compromising the systolic function of the normoxic part of the heart.

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