

A New Site for the Activation of Cardiac Calcium Channels Defined by the Nondihydropyridine FPL 64176¹

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ABSTRACT

We examined the effects of a new ligand, FPLnM-64176, on L-type Ca⁺⁺ channels in cardiac tissue. FPL 64176 (10–1 μM) enhanced Ca⁺⁺ influx into neonatal rat ventricular myocytes, a response which was blocked by nifedipine. FPL 64176 had no effect on [³H]PN200–110 binding in rat ventricular membranes, but dramatically increased L-type Ca⁺⁺ channel current amplitude. FPL 64176 (1 μM) slowed both the activation and the inactivation kinetics of the L-channel in neonatal rat ventricular

cells. We also noted a hyperpolarizing shift in the threshold and peak potential of the Ca⁺⁺ channel current-voltage relationship in response to the compound. Additionally, the binding site for FPL 64176 appeared to be located on the extracellular face of the channel. We conclude that FPL 64176 is a potent new activator of L-type Ca⁺⁺ channels with a novel mechanism and site of action.

Calcium ions play an important role in regulating the function of excitable tissues. A major means by which Ca⁺⁺ gains entry into a variety of cells is *via* intramembraneous protein known as voltage-dependent Ca⁺⁺ channels. A subtype of these channels known as the L-type Ca⁺⁺ channel (Nowycky *et al.*, 1985; Hofmann *et al.*, 1987) is especially important in controlling excitation-contraction coupling in cardiac tissue. The L-type Ca⁺⁺ channel is also the target for a number of drugs known collectively as the calcium channel antagonists (Janis *et al.*, 1987). These compounds, which include the 1,4-dihydropyridines, the phenylalkylamines and the benzothiazepines, have proved clinically useful in treating a number of cardiovascular disorders. In addition, these drugs have also been instrumental in probing the basic structure and function of L-type Ca⁺⁺ channels.

Although it is common to think of the Ca⁺⁺ channel antagonists as being comprised of only a few distinct structures, it is becoming clear that a host of compounds can antagonize L-type Ca⁺⁺ channel activity (Rampe and Triggle, 1990). In contrast, few ligands are known which act predominantly as L-type Ca⁺⁺ channel activators. Naturally occurring molecules, such as heparin (Knaus *et al.*, 1990) and certain animal toxins (Hamilton and Perez, 1987), have been shown to stimulate L-type channels in various tissues. Of the synthetic Ca⁺⁺ channel

ligands, 1,4-dihydropyridines, such as Bay K 8644 (Hess *et al.*, 1984) and CGP 28392 (Kokubun and Reuter, 1984; Patmore *et al.*, 1990), have been shown to possess predominantly agonist-like effects, and even these compounds may be best described as partial agonists (Triggle and Rampe, 1989).

Recently, a novel nondihydropyridine structure, FPL 64176 (fig. 1), has been synthesized (McKechnie *et al.*, 1989). This benzoyl pyrrole increased ⁴⁵Ca⁺⁺ influx into GH₃ cells and caused positive inotropic activity in both guinea pig atria and aortic smooth muscle. These responses were similar to those seen with Bay K 8644 and consistent with activation of L-type Ca⁺⁺ channels. Utilizing biochemical and electrophysiological approaches, the present study was undertaken to determine what effect FPL 64176 has on L-type Ca⁺⁺ channels in cardiac tissue.

Methods

Neonatal rat heart ventricular myocytes were prepared by a modification of Mark and Strasser (1966). Briefly, ventricular cells were obtained for primary culture by trypsin digestion of 1- to 3-day old neonatal ventricular tissue cut into 1- to 2-mm cubes. For ⁴⁵Ca⁺⁺ uptake experiments, cells were cultured in 35-mm Falcon culture dishes in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco Laboratories, Grand Island, NY) in a water-saturated air atmosphere for 3 to 4 days. Cells used for electrophysiological experiments were cultured in a similar fashion for 12 to 24 hr on glass coverslips. When cells are cultured for this period of time, they remain rounded. The passive electrical properties of the mem-

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ABBREVIATIONS: GH₃, rat anterior pituitary; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid.

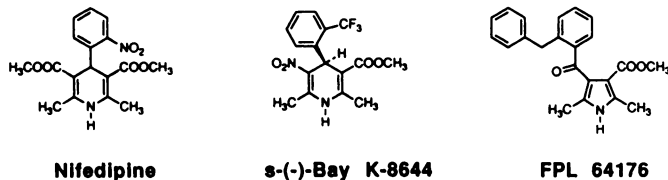


Fig. 1. Structural formulae of FPL 64176, nifedipine and *s*(-)-Bay K 8644.

branes are consistent with those of a simple sphere, thus helping to ensure electrical control of the cells (Kunze *et al.*, 1985).

For ⁴⁵Ca²⁺ influx experiments, ventricular cells were removed from the incubator and the culture media was aspirated off and replaced by 1 ml of an EGTA solution containing (millimolar): NaCl, 110; KCl, 5.4; EGTA, 0.2; HEPES, 20 and glucose, 25 (pH 7.4). After 5 min, this solution was aspirated off and replaced by 1 ml of a low K⁺ solution containing (millimolar): NaCl, 110; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 1.0; HEPES, 20 and glucose, 25 (pH 7.4) or a high K⁺ solution containing 42 mM KCl in equimolar substitution for NaCl. Both these solutions also contained 2 μCi of ⁴⁵Ca²⁺/ml. Uptake was allowed to proceed for 10 sec, at which time the ⁴⁵Ca²⁺-containing solutions were aspirated off and the cells washed with 3 × 1.5-ml aliquots of ice-cold lanthanum solution containing (millimolar): NaCl, 110; KCl, 5.4; LaCl₃, 1.0; HEPES, 20 and glucose, 25 (pH 7.4). All ⁴⁵Ca²⁺ influx experiments were carried out at room temperature and appropriate concentrations of vehicle (0.1% ethanol final concentration) or drugs were present in all uptake solutions. After ⁴⁵Ca²⁺ uptake, cells were digested with 0.5% NaOH and radioactivity was quantitated using liquid scintillation spectroscopy. Proteins were determined by the method of Lowry *et al.* (1951).

[³H]PN200-110 binding was carried out as follows. Male Sprague-Dawley rats were decapitated and their ventricles removed, washed free of blood, minced with scissors and placed in 15 to 20 v/g wet weight of ice-cold 50 mM Tris buffer (pH 7.4 at 25°C). The tissue was disrupted with two 30-sec bursts in a Brinkman polytron (setting 7), and then with 10 passes in a glass-Teflon homogenizer. The homogenate was centrifuged at 1000 × *g* for 15 min. The supernatant was collected and recentrifuged at 42,000 × *g* for 30 min. The resultant pellet was resuspended in 50 mM Tris and used for radioligand binding experiments. [³H]PN200-110 binding (50 pM final concentration) was carried out in 1 ml of 50 mM Tris buffer for 75 min at room temperature. Nonspecific binding was determined in duplicate sets of tubes by inclusion of 10⁻⁶ M nifedipine. Incubation was terminated by rapid filtration through Whatman GF/B filter strips using a Brandel (Gaithersburg, MD) cell harvester. Filters were washed with 3 × 5 ml of ice-cold 50 mM Tris buffer. Radioactivity was quantitated using liquid scintillation spectroscopy.

Ion currents were recorded at room temperature *via* the gigaseal patch clamp technique as described by Hamill *et al.* (1981) utilizing an axopatch-1B amplifier (Axon Instruments, Burlingame, CA). Electrodes were fashioned from TW150 glass capillary tubes (World Precision Instruments, New Haven, CT) and had resistances of 2 to 5 megaohms when filled with internal solution. Pipettes used for intracellular recordings were filled with the following solution (millimolar): CsOH, 130; aspartic acid, 80; EGTA, 15; 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid, 5; MgCl₂, 11.5; Na₂ATP, 3; Na₂GTP, 0.1 and HEPES, 10, pH 7.4 with CsOH. Seals were made in Tyrode's solution. After seal formation, the extracellular solution was replaced with a Ca²⁺ channel recording solution containing (millimolar): N-methyl-D-glucamine, 110; aspartate, 140; Ba(OH)₂, 10; HEPES, 10; tetraethylammonium hydroxide, 30 and 4-aminopyridine, 5, pH 7.4 with methylsulfonic acid. In some cases, 10 mM Ca(OH)₂ was substituted for Ba(OH)₂ in the external solution. Cell currents were conditioned by a four-pole low-pass filter with a cutoff frequency of 1 KHz. Data were corrected by an on-line summing procedure that added a variable, predetermined number (usually four) of scaled and inverted pulses to a single depolarizing pulse. Currents were stored and analyzed

using a laboratory computer (IBM-AT) and pCLAMP software (Axon Instruments).

(+)-[³H]PN200-110 (85.9 Ci/mmol) and ⁴⁵CaCl₂ (20.4 Ci/g) were obtained from New England Nuclear (Boston, MA). Nifedipine and *s*(-)-Bay K 8644 were obtained from Miles Laboratory (New Haven, CT). FPL 64176 was the generous gift of Fisons Pharmaceuticals (Leicestershire, UK). All other materials were obtained from commercial sources.

Results

Neonatal rat ventricular myocytes respond to elevated extracellular K⁺ concentrations with an increase in Ca²⁺ uptake. We have previously demonstrated that this K⁺-stimulated Ca²⁺ influx is blocked by the 1,4-dihydropyridine Ca²⁺ channel antagonists as well as other classes of L-type Ca²⁺ channel antagonists (Rampe *et al.*, 1989a). Figure 2 illustrates the effects of FPL 64176 on Ca²⁺ uptake in neonatal rat ventricular cells. FPL 64176 (10 nM to 1 μM) significantly (*P* < .02 Student's *t* test) increased Ca²⁺ influx into these cells under conditions of both elevated extracellular [K⁺] (42 mM) and resting (5.4 mM) extracellular [K⁺]. Figure 2 also illustrates that the stimulatory effects of 1 μM FPL 64176 were abolished when 1 μM nifedipine was present during the uptake experiments.

The 1,4-dihydropyridine group of Ca²⁺ channel ligands displays specific high-affinity binding to a number of excitable tissues, including heart (for review see Janis *et al.*, 1987). Figure 3 shows the effects of a variety of ligands on the binding of [³H]PN200-110 to adult rat ventricular membranes. Both the Ca²⁺ channel antagonist nifedipine and the Ca²⁺ channel activator *s*(-)-Bay K 8644 potently displaced [³H]PN200-110 binding consistent with previous results (Janis *et al.*, 1987; Rampe *et al.*, 1989b). The IC₅₀ and Hill slope values for nifedipine were 1.13 ± 0.08 nM and -1.01 ± 0.02, respectively, whereas these values were 4.17 ± 0.35 nM and -1.04 ± 0.06 for *s*(-)-Bay K 8644. In contrast, FPL 64176 had no significant effect on [³H]PN200-110 binding even at concentrations as high as 1 μM.

We next turned to whole cell patch clamp electrophysiology to directly examine the effects of FPL 64176 on L-type Ca²⁺ currents in neonatal rat ventricular cells. Under the present culture conditions, we found peak L-channel amplitudes of approximately 50 to 150 pA per cell using 5 to 10 mM Ba²⁺ or Ca²⁺ as the charge carrier (Lacerda and Brown, 1989; Rampe

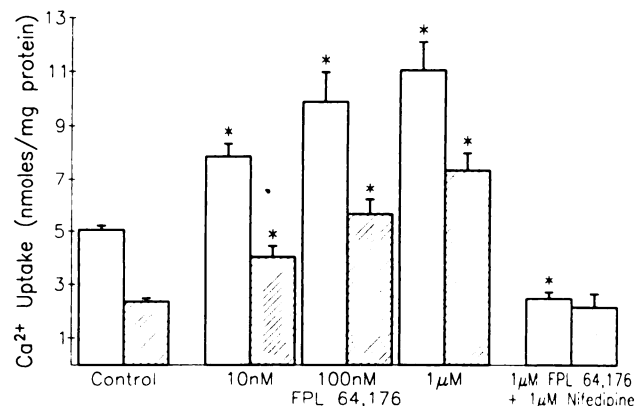


Fig. 2. Effects of FPL 64176 on Ca²⁺ uptake in neonatal rat ventricular myocytes. Uptake was performed as described under "Methods." (□), indicate uptake in 42 mM extracellular K⁺; (▨), indicate uptake in 5.4 mM extracellular K⁺; (*), indicate significantly different from control value (*P* < .02 *t* test). Error bars indicate S.E.M. (*n* = 3).

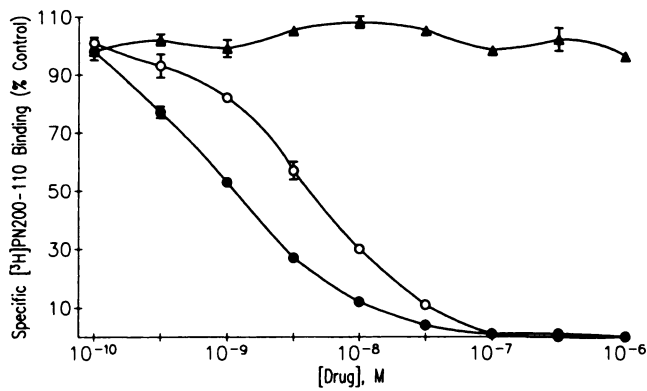


Fig. 3. Effects of nifedipine (●), s(-)-Bay K 8644 (○) and FPL 64176 (▲) on [³H]PN200-110 binding in adult rat ventricular membranes. Binding was carried out using 50 pM [³H]PN200-110 as described under "Methods." The IC_{50} and Hill coefficient values for nifedipine were 1.13 ± 0.08 nM and -1.01 ± 0.02 , respectively. These values for s(-)-Bay K 8644 were 4.17 ± 0.35 nM and -1.04 ± 0.06 , respectively. Bars indicated S.E.M. ($n = 3$). Where no bars are present error is contained within the symbol.

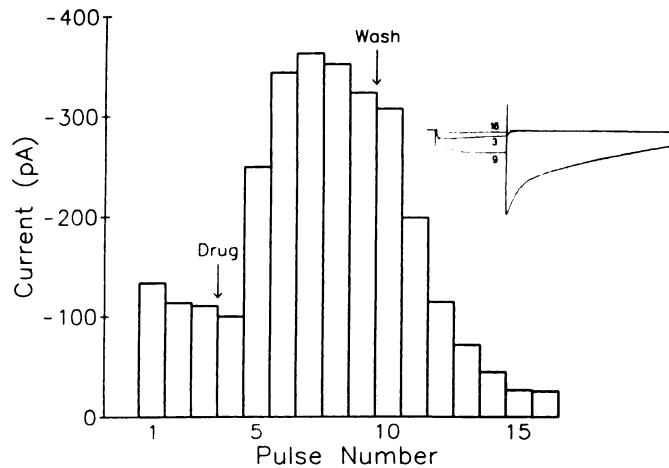


Fig. 4. Effects of FPL 64176 (1 μ M) on L-type Ca^{2+} channel currents in neonatal rat ventricular myocytes. Current was induced by 170-msec clamp pulses to 0 mV from a holding potential of -60 mV every 20 sec. FPL 64176 was added to the bath where indicated. Currents were recorded in 10 mM extracellular Ba^{2+} and were sampled 100 msec into each pulse. Inset: Current traces from pulse numbers 3, 9 and 16. The lower current amplitudes observed after washout are likely due to some degree of run-down of the Ca^{2+} channels.

et al., 1989a). Figure 4 shows a diary of the effects of 1 μ M FPL 64176 on L-type Ca^{2+} channel currents carried by 10 mM Ba^{2+} (I_{Ba}). For these experiments, I_{Ba} was elicited by a 170-msec test pulse to 0 mV from a holding potential of -60 mV. As can be seen, 1 μ M FPL 64176 dramatically increased I_{Ba} in these cells, an effect which was reversible upon washing the cell with drug-free solution (chamber volume 750 μ l, flow rate 2 ml/min). In four cells tested, peak I_{Ba} was increased an average of $162 \pm 41\%$ (range = 65–266%) 1 min after the addition of 1 μ M FPL 64176. In addition to increasing I_{Ba} during the test pulse, FPL 64176 had further effects. First, we observed greatly prolonged tail currents in all drug-treated cells (see inset, fig. 4). Second, the current kinetics during the test pulse were changed. Most obvious was a dramatic slowing of the activation of I_{Ba} . When current activation was fit with a single exponential, the time constant for activation, $\tau_{act.}$, was significantly ($P < .05$, paired t test) prolonged from 2.7 ± 0.3 msec in control to 12.0 ± 1.6

msec 1 min after addition of 1 μ M FPL 64176 ($n = 4$). Furthermore, after FPL 64176 treatment, there was no apparent inactivation of I_{Ba} during the pulse. These effects on current kinetics were also reversible upon washing the cell with drug-free solution (inset, fig. 4).

Figure 5 shows the current-voltage (I-V) relationship of the L-type Ca^{2+} channel in the presence and absence of 1 μ M FPL 64176. The amplitude of I_{Ba} was increased at all test potentials, although this enhancement was more pronounced at negative test potentials. Furthermore, FPL 64176 shifted both the threshold and the peak of the I-V relationship in the hyperpolarizing direction. The shift in the peak of the I-V relationship ranged from approximately 5 to 15 mV in six cells tested.

As figure 4 demonstrates, the L-type Ca^{2+} channel current decays very slowly when Ba^{2+} is used as the charge carrier. For this reason, we next conducted experiments using 10 mM external Ca^{2+} as the charge carrier in order to further examine the effects of FPL 64176 on L-type Ca^{2+} channel current kinetics in the myocytes. Figure 6A illustrates the effects of 1 μ M FPL 64176 on L-channel Ca^{2+} current (I_{Ca}) elicited by a 750-msec test pulse to 0 mV from a holding potential of -50 mV. As was the case with I_{Ba} , FPL 64176 greatly increased the magnitude of I_{Ca} . In five cells tested, the average increase in peak I_{Ca} was $325.6 \pm 84.0\%$. Furthermore, activation kinetics were slowed in the presence of FPL 64176. The time constant for activation ($\tau_{act.}$) in control cells was 2.1 ± 0.3 msec, whereas after addition of 1 μ M FPL 64176, this value significantly ($P < .05$, paired t test) increased to 13.1 ± 2.9 msec ($n = 5$). To test the effects of FPL 64176 on current inactivation, we found it necessary to give even more prolonged test pulses. Figure 6B shows the effects of FPL 64176 on I_{Ca} elicited by a 1440 msec test pulse to 0 mV from a holding potential of -50 mV. Under these conditions, we were able to successfully resolve two components of inactivation in agreement with previous reports (Kass and Sanguinetti, 1984; Markwardt and Nilius, 1988). In control cells, the time constant for the fast component of inactivation ($\tau_{1\text{inact.}}$) was 25.1 ± 4.5 msec, whereas the value for the slow component ($\tau_{2\text{inact.}}$) was 185.4 ± 7.3 msec ($n = 6$). After application of 1 μ M FPL 64176, both $\tau_{1\text{inact.}}$ and $\tau_{2\text{inact.}}$

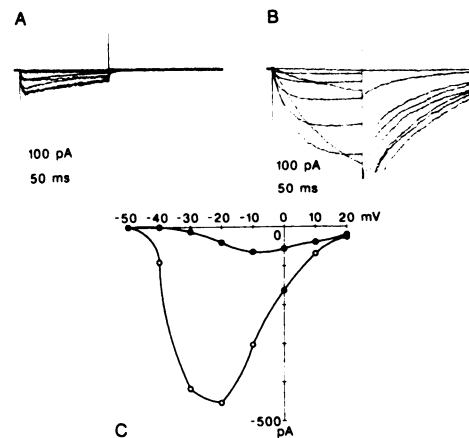


Fig. 5. Effects of FPL 64176 on the L-type Ca^{2+} channel current-voltage relationship in neonatal rat ventricular myocytes. Current was induced by 170-msec clamp pulses from a holding potential of -60 mV every 10 sec using 10 mM Ba^{2+} as the charge carrier. A) Control current traces. B) Current traces in the same cell 1 min after the addition of 1 μ M FPL 64176. C) Calcium channel I-V relationships for the experiments illustrated in A (●) and B (○). Currents were sampled 100 msec into each pulse to generate the I-V relationship.

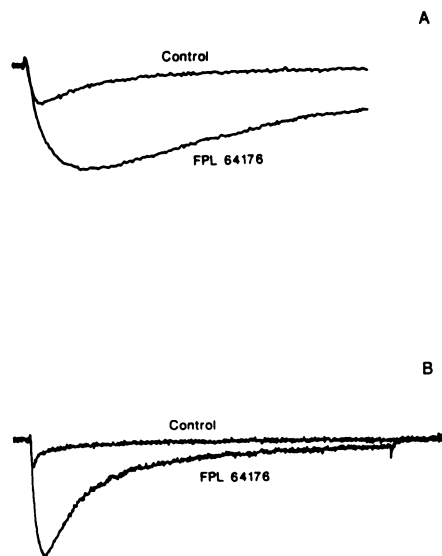


Fig. 6. Effects of FPL 64176 on L-type Ca⁺⁺ channel currents (I_{Ca}) in neonatal rat ventricular myocytes. A) This figure shows a typical Ca⁺⁺ current before and after the addition of FPL 64176 (1 μ M). Current was induced by a 750-msec clamp pulse to 0 mV from a holding potential of -50 mV. To highlight the effects of FPL 64176 on the activation of I_{Ca} , only the first 213 msec of the pulse are shown. Peak amplitude of the control current was -88 pA, whereas in the presence of FPL 64176 it was -235 pA. The time constant of activation (τ_{act}) in control was 2.5 msec, whereas after addition of FPL 64176 this value was 12.3 msec. B) Effects of FPL 64176 (1 μ M) on the inactivation of I_{Ca} . Current was induced by a 1440-msec clamp pulse to 0 mV from a holding potential of -50 mV. Peak currents under control and FPL 64176 treated conditions were -43 pA and -179 pA, respectively. The fast ($\tau_{1\ inact}$) and slow ($\tau_{2\ inact}$) components of inactivation in control were 13.3 msec and 183.4 msec, respectively. These values were 102.8 msec and 526.9 msec after addition of FPL 64176.

TABLE 1

Effects of FPL 64176 (1 μ M) on L-Type Ca⁺⁺ channel current (I_{Ca}) kinetics

Current	$\tau_{act} \pm$ S.E.M.	$\tau_{1\ inact} \pm$ S.E.M.	$\tau_{2\ inact} \pm$ S.E.M.
	msec		
I_{Ca} control	2.1 \pm 0.3	25.1 \pm 4.5	185.4 \pm 7.3
I_{Ca} + FPL 64176	13.1 \pm 2.9*	78.8 \pm 7.8*	455.2 \pm 95.0*

* Significantly different from corresponding control value $P < .05$ paired t test.

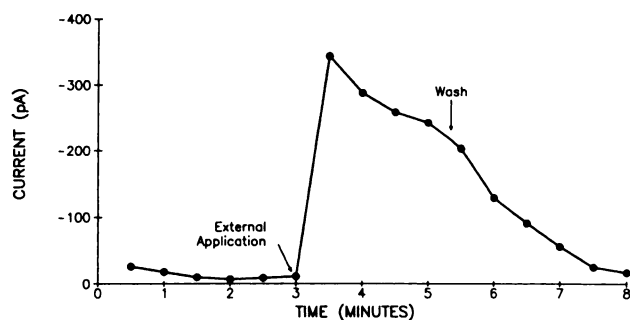


Fig. 7. Effects of internally vs. externally applied FPL 64176 on whole cell I_{Ba} in neonatal rat ventricular myocyte. Cell was held at -60 mV and pulsed to 0 mV for 175 msec. Current was sampled at the end of each pulse. 1 μ M FPL 64176 was present in the pipette solution throughout the experiment. External application of 1 μ M FPL 64176 and its subsequent washout are indicated.

were significantly ($P < .05$, paired t test) prolonged to 78.8 ± 7.8 msec and 455.2 ± 95.0 msec, respectively. The effects of FPL 64176 on I_{Ca} kinetics are summarized in table 1.

Because the stimulatory effects of FPL 64176 on Ca⁺⁺ channels were easily reversed upon washing the cell with drug-free solution, we suspected that its site of action may be on or near the extracellular face of the channel. Figure 7 shows the typical effects of intracellularly, as opposed to extracellularly, applied FPL 64176 on I_{Ba} in a cardiac myocyte. In this experiment, 1 μ M FPL 64176 was present in the patch pipette and, hence, had free access to the intracellular face of the channel throughout the duration of the experiment. However, we did not see any significant effect of the drug until it was added to the outside of the cell (external application). At this time, large currents with slow activation and very prolonged tails were immediately observed, which were reversible upon washing the cell with drug-free external solution.

Discussion

Drugs which interact with L-type Ca⁺⁺ channels have proved invaluable as tools for studying the structure and function of these ion channels. A wide range of compounds, including nitrendipine, verapamil and diltiazem, are known to interact at pharmacologically distinct sites on or around the L-channel (Janis *et al.*, 1987). Although under some circumstance these drugs can activate Ca⁺⁺ channels (Brown *et al.*, 1986; Scott and Dolphin, 1987; McDonald *et al.*, 1989), their predominant activity is one of Ca⁺⁺ channel blockade. In fact, structures which act mainly as L-channel activators have been largely confined to the *s*-enantiomers of the 1,4-dihydropyridine group of ligands (Bechem *et al.*, 1988; Langs *et al.*, 1989). Recently, however, a new nondihydropyridine molecule, FPL 64176, has been synthesized and shown to have pharmacological properties consistent with L-channel activation (McKechnie *et al.*, 1989). The present study was undertaken to detail some of the effects of FPL 64176 on cardiac L-type Ca⁺⁺ channels.

We found FPL 64176 to potentially enhance dihydropyridine-sensitive Ca⁺⁺ uptake into neonatal rat ventricular cells. This enhancement was evident at concentrations as low as 10 nM. Ca⁺⁺ uptake was enhanced at both physiological K⁺ levels (5-4 mM) and elevated K⁺ levels (42 mM). This finding is in contrast to that reported by McKechnie *et al.* (1989) for GH₃ cells, where no increase in Ca⁺⁺ uptake was observed under resting K⁺ conditions. However, comparisons between these results may not be appropriate, because the heart cells used here are spontaneously active at physiological K⁺ concentrations and, thus, not truly at rest. Furthermore, the effects of FPL 64176 on Ca⁺⁺ influx were completely blocked by 1 μ M nifedipine, implying a pharmacological interaction at the L-type Ca⁺⁺ channel. Although a functional interaction between FPL 64176 and the dihydropyridines is evident, we could not demonstrate any such interaction at the receptor level. Thus, although nifedipine and *s*-(-)-Bay K 8644 inhibited [³H] PN200-110 binding in ventricular membranes, FPL 64176 had no effect on binding even at concentrations as high as 1 μ M. The results presented here for nifedipine and *s*-(-)-BayK 8644 are consistent with most published reports, which describe these compounds as competitive species (Janis *et al.*, 1987). However, it is apparent that FPL 64176 activates L-type Ca⁺⁺ channels in the heart at a site which is distinct from that of the 1,4-dihydropyridines.

FPL 64176 was shown to have a number of effects on L-channel currents in neonatal rat ventricular cells. Current amplitudes during step depolarizations to 0 mV were greatly enhanced after application of 1 μ M FPL 64176, regardless of whether Ba⁺⁺ or Ca⁺⁺ was used as the charge carrier. Inward tail currents also decayed more slowly after a repolarizing step. Additionally, the threshold and the peak of the current-voltage relationship were shifted in the hyperpolarizing direction in the presence of FPL 64176. Taken together, these observations on whole cell currents resemble those made previously in cardiac cells for the classical Ca⁺⁺ channel activator Bay K 8644 (Hess *et al.*, 1984; Sanguinetti *et al.*, 1986; Hamilton *et al.*, 1987). Indeed, given these findings, we predict that FPL 64176 may have effects on single-channel currents similar to Bay K 8644. Perhaps the most striking effect of Bay K 8644 on the single-channel level is a dramatic prolongation of Ca⁺⁺ channel open time (Hess *et al.*, 1984). However, increases in the probability of channel opening and in channel conductance are also seen (Lacerda and Brown, 1989). It is likely that one or more of these effects underlies the activity of FPL 64176 observed here on whole cell currents. Future studies at the single-channel level will be necessary to further explore these possibilities.

Although we find many similarities in activity between FPL 64176 and Bay K 8644, we must also note some important differences. Bay K 8644 has previously been shown to speed the activation of cardiac L-channels. Using 10 mM Ba⁺⁺ as the charge carrier, Markwardt and Nilius (1988) have demonstrated that Bay K 8644 significantly shortens the time to peak of the L-channel in guinea pig ventricular myocytes. This effect was evident over a wide range of test potentials. Similar results have been obtained in our laboratory for I_{Ca} in neonatal rat ventricular cells (Lacerda and Brown, 1989). In contrast, FPL 64176 prolonged the activation τ of both I_{Ca} and I_{Ba} by several-fold. A second important difference between Bay K 8644 and FPL 64176 is seen in their effects on Ca⁺⁺ channel inactivation. It is well established that Bay K 8644 enhances the rate of current decay during a step pulse (Hess *et al.*, 1984; Sanguinetti *et al.*, 1986; Lacerda and Brown, 1989). In guinea pig ventricular cells, Markwardt and Nilius (1988) found that Bay K 8644 sped the fast component of channel inactivation while having no discernable effect on the slow component. Even the stimulatory enantiomer of Bay K 8644 is known to accelerate the time course of Ca⁺⁺ current inactivation (Kass, 1987). In contrast, FPL 64176 slowed both components of Ca⁺⁺ channel inactivation. In this regard, FPL 64176 displays more agonist-like activity than Bay K 8644. Thus, the interaction of FPL 64176 with the L-channel results in a stimulatory activity which is distinct from that of the 1,4-dihydropyridine class of Ca⁺⁺ channel agonists.

Because FPL 64176 had little effect on Ca⁺⁺ channel currents when applied intracellularly, we feel that the site that binds FPL 64176 resides on the extracellular face of the channel or, at least, is accessed most easily *via* the extracellular surface. This is very different from the binding sites of other Ca⁺⁺ channel ligands, such as the dihydropyridines and the phenylalkylamines, which have now been shown to be located on the intracellular face of the Ca⁺⁺ channel (Striessnig *et al.*, 1990; Regulla *et al.*, 1991). We speculate that the site described here may be an important recognition site on the Ca⁺⁺ channel for potential endogenous ligands (Triggle, 1988; Callewaert *et al.*, 1989) or animal toxins (Hamilton and Perez, 1987), many of

which are peptides and are, therefore, unlikely to cross the cell membrane to access the intracellular face of the channel.

In summary, we have described some of the action of a new ligand, FPL 64176, on cardiac cells. This compound displays stimulatory activity on L-type Ca⁺⁺ channels, which in some respects resembles the 1,4-dihydropyridine Bay K 8644. However, FPL 64176 has unique effects on Ca⁺⁺ channel current kinetics and does not interact at the dihydropyridine binding site at pharmacologically relevant concentrations. Furthermore, its site of action appears to be located on the extracellular face of the channel. We feel that FPL 64176 represents an important new class of Ca⁺⁺ channel ligand. Future studies should help further define the mechanisms of action of this novel compound.

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