Dual Action of FRC8653, a Novel Dihydropyridine Derivative, on the Ba$^{2+}$ Current Recorded From the Rabbit Basilar Artery

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Actions of FRC8653 on the macroscopic and unitary Ba$^{2+}$ currents were studied using the rabbit basilar artery. Application of (±)-FRC8653 (<1 μM) increased the amplitude of the inward current when depolarization pulses more negative than −10 mV were applied but inhibited it when depolarization was more positive than 0 mV (in each case from a holding potential of −80 mV). At a holding potential of −40 mV, (±)-FRC8653 (>0.1 nM) consistently inhibited the inward current. (−)-FRC8653 (>1 nM) inhibited the amplitude of the inward current evoked by a depolarizing pulse more positive than −10 mV (the holding potential being −80 mV). At the holding potential of −80 mV, but not at −40 mV, (±)-FRC8653 (1 μM) enhanced the current amplitude evoked by a depolarizing pulse more negative than −10 mV but inhibited the current evoked by a pulse more positive than 0 mV. (±)-FRC8653 shifted the voltage-dependent inhibition curves to the left, and the slope of the curve became steeper (test pulse of +10 mV). Two types of single Ca$^{2+}$ channel currents (12 and 23 pS) were recorded from the basilar artery by the cell-attached patch-clamp method. Opening of the 12-pS channel occurred with a depolarizing pulse (−20 mV) from a holding potential of −80 mV, but not from one of −60 mV. (+)-FRC8653 activated, and (−)-FRC8653 inhibited, the 23-pS channel. These results indicate that (±)-FRC8653 had dual actions (excitatory and inhibitory) on the Ca$^{2+}$ channels in the rabbit basilar artery. Excitatory actions of (±)-FRC8653 result mainly from the actions of (+)-FRC8653 on the 23-pS channel, but this was reversed to an inhibitory action at a holding potential of −40 mV. Therefore, FRC8653 dominantly acts as a Ca$^{2+}$ channel blocker in the rabbit basilar artery, since this tissue has a resting membrane potential of −50 to −60 mV. (Circulation Research 1990;67:993–1006)

Recently, two types of voltage-dependent Ca$^{2+}$ channels have been revealed in smooth muscle cells.1–6 In smooth muscle cells, dihydropyridine (DHP) derivatives such as nifedipine and nitrendipine are known to inhibit the high-threshold Ca$^{2+}$ channel or L-type channel without effect on the low-threshold Ca$^{2+}$ channel, the T-type.3–5,7 Moreover, it has been reported that DHP derivatives inhibited other ion channels such as the voltage-dependent K$^{+}$ channels and Na$^{+}$ channels in cardiac and smooth muscle cells, although the required concentration was 200–1,000 times higher.8,9 These results indicate that DHP derivatives have selective inhibitory actions on the high-threshold–type (L-type) Ca$^{2+}$ channel in smooth muscle cells. However, the low-threshold Ca$^{2+}$ channel (T-type) recorded from the rat aorta could be inhibited by nifedipine, albeit with higher concentrations than those needed to block the high-threshold Ca$^{2+}$ channels.10

In contrast, some DHP derivatives, namely, Bay K 8644 and 202-791, are reported to enhance the high-threshold Ca$^{2+}$ channels but not the low-threshold one.11,12 Because such augmentation of the Ca$^{2+}$ current by DHP derivatives was reported using nifedipine as well as nitrendipine, this action may be a common property of DHP derivatives and may play a role in their apparent inhibitory actions.13 Therefore, it seems that the potency of the inhibitory actions of DHP derivatives observed in the various vascular tissues may depend on the types of Ca$^{2+}$ channels distributed in the smooth muscle cells of particular vascular tissue and the relative potency of the inhibitory actions of each DHP derivative compared with its excitatory actions.
In guinea pig basilar artery, Fujiwara and Kuriyama\textsuperscript{14} reported that nicardipine, a DHP derivative, inhibited the action potential and the high-$K^+$-induced contraction but not the 5-hydroxytryptamine-induced contraction, and they postulated that nicardipine selectively inhibited Ca$^{2+}$ permeation through the voltage-dependent Ca$^{2+}$ channel in the membrane. However, no patch-clamp investigation has been performed using basilar artery.

$(\pm)$-FRC8653 [2-methoxethyl (E)-3-phenyl-2-propen-1-yl-$\pm$-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate] is a newly synthesized DHP derivative and produces vasodilatation, especially in the basilar and coronary arteries.\textsuperscript{15}

The present experiments were performed to investigate in detail the actions of $(\pm)$-FRC8653 and its enantiomers on smooth muscle cells of the rabbit basilar artery.

**Materials and Methods**

Male albino rabbits (1.7–2.0 kg) were anesthetized with sodium pentobarbital (40 mg/kg i.v.; Pitman-Moore Inc., Washington Cross, N.J.) and exsanguinated. The basilar artery was dissected along with brain and brain stem and isolated in physiological salt solution (PSS). Tunica adventitia and surrounding connective tissue were carefully removed, as far as possible, with fine scissors and forceps under a binocular microscope. Endothelium was also removed by gentle rubbing with a small cotton ball. The procedure for cell dispersion was similar to that described by Momose and Gomi\textsuperscript{16} and Terada et al.\textsuperscript{17} In brief, small segments of the tissue were rinsed with Ca$^{2+}$-free PSS, then incubated in warmed Ca$^{2+}$-free PSS containing 0.3% collagenase (Wako Pure Chemicals, Osaka, Japan), 0.1% bovine serum albumin (essentially fatty acid free; Sigma Chemical Co., St. Louis), and 0.1% trypsin inhibitor (type II-S, Sigma) at 36°C for 70 minutes. After collagenase treatment, tissues were transferred to fresh Ca$^{2+}$-free PSS, and single cells were dispersed by gentle agitation with a glass pipette. Dispersed cells were collected by mild centrifugation (600 rpm, 1 minute) after removal of pieces of undigested tissue with fine nylon mesh (100×100 $\mu$m). Dispersed cells were resuspended in fresh 0.5 mM Ca$^{2+}$–0.5 mM Mg$^{2+}$ solution and stored in ice-cold water. Experiments were performed at room temperature (20–25°C).

**Recording of the Membrane Currents**

Recordings of the macroscopic and single-channel currents were made in ways similar to those described by Hamill et al.\textsuperscript{18} One drop of the cell suspension was added to a small chamber (0.2-ml volume) placed on the stage of a differential interference inverted microscope (TMD-Diaphot, Nikon Co., Tokyo). Patch electrodes (o.d., 2.0–3.5 $\mu$m; 3–5 $M\Omega$) were prepared by means of an electrode puller and heat polisher (models PP-83 and MF-83, Narishige Scientific Instruments Labo-

\textsuperscript{ratory, Tokyo) and were manipulated by three-dimensional oil-driven or electric manipulators (model MO-102, Narishige; Manipulator-E, Leitz-Wetzler GmbH, Wetzlar, FRG). A high-resistance seal (>10 G$\Omega$) was obtained by application of negative pressure to the patch electrode (10–30 cm H$_2$O). For single-channel current recording, the pipette was filled with 100 mM Ba$^{2+}$ solution with high-$K^+$ solution being superfused in the bath. For macroscopic current recording, the pipette was filled with high-Cs$^+$ solution (for recording the Ca$^{2+}$ or Ba$^{2+}$ current) or high-K$^+$ solution (for recording the K$^+$ current) with 10 mM Ca$^{2+}$ or Ba$^{2+}$ solution (for the Ca$^{2+}$ or Ba$^{2+}$ current) or PSS (for the K$^+$ current) superfused in the bath.

Membrane currents were monitored on a high-gain digital oscilloscope and a conventional thermowriting pen recorder (models VC-10 and RJG-4124, Nihon Kohden, Tokyo) through a patch-clamp amplifier (model EPC-5 or EPC-7, List Medical Electronics, Darmstadt, FRG) and stored on magnetic tape by a FM tape recorder (model A-65, Sony-Magnesco Inc., Tokyo) or videocassette recorder via a PCM data recording system (model NV-21, National Co., Tokyo; model PCM-501E, Sony Co., Tokyo).

Capacitative and leak currents were subtracted using the $P/n$ ($n=2–4$) method described by Almers and Palade\textsuperscript{19} for measurement of macroscopic currents on a digital oscilloscope (model 4094B, Nicolet Instrument Corp., Madison, Wis.), and a hard copy was obtained with an X-Y plotter (model 7440A, Hewlett-Packard Co., San Diego). For single-channel current recording, capacitative and leak currents were averaged from 30 to 50 tracings with no appearance of the single-channel current (“blank” tracings); then the capacitative and leak currents in the traces with the appearance of the single-channel current (“nonblank” tracings) were subtracted from the averaged capacitative and leak currents on a digital storage oscilloscope (model 4094B, Nicolet).

**Solutions and Drugs**

Ionic millimolar composition of PSS was NaCl 134, KCl 6, CaCl$_2$ 2.5, and glucose 12. The Ca$^{2+}$-free solution was made by replacement of CaCl$_2$ with an equimolar amount of NaCl. The 0.5 mM Ca$^{2+}$–0.5 mM Mg$^{2+}$ solution was made by adding 0.5 mM CaCl$_2$ and 0.5 mM MgCl$_2$ to the Ca$^{2+}$-free solution. The 10 mM Ba$^{2+}$ or Ca$^{2+}$ solution contained BaCl$_2$ 10 mM or CaCl$_2$ 10 mM with tetraethylammonium chloride 135 mM and glucose 10 mM. Ionic millimolar composition of the 100 mM Ba$^{2+}$ solution was BaCl$_2$ 100 and glucose 12. The high-K$^+$ solution (bath solution for single-channel recording) had the following ionic millimolar composition: KCl 145, MgCl$_2$ 5, and EGTA 4.

The high-K$^+$ solution (pipette solution for macroscopic current recording) was made by adding 5 mM Na$_2$ATP to the high-K$^+$ solution used in the
bath. The high-Cs\textsuperscript+ solution was made by replacement of KCl in the high-K\textsuperscript+ solution (the pipette solution) with an equimolar amount of CsCl. The pH of the solutions was adjusted to 7.25–7.30 by 10 mM HEPES titrated with Tris. The solution in the chamber was perfused at a constant rate (1 ml/min) throughout the experiments. Exchange of the solution was accomplished within 1 minute.

Drugs used in the present experiments were (±)-FRC8653 and its enantiomers (Fuji-Rebio Inc., Tokyo). The chemical structure of (±)-FRC8653 (MW 492.53) is shown in Figure 1. FRC8653 (100 nM) was dissolved in 50% polyethylene glycol (PEG)–50% ethanol solution and diluted with ethanol to keep the final concentration of ethanol constant (0.1%). Although the maximum concentrations of PEG (0.05%) and ethanol (0.1%) used in the present experiments did not produce any effect on the membrane currents, the same concentrations of PEG (0.05%) and ethanol (0.1%) were added in the control solutions.

**Results**

Under control conditions, the Ba\textsuperscript2+ inward current amplitude remained the same for over 30 minutes; therefore, all experiments were performed within 30 minutes. Figure 2 shows the time course of inhibition of the Ba\textsuperscript2+ inward current evoked by a depolarizing pulse of +10 mV from the holding potential of −80 mV. When depolarizing pulses were applied every 20 seconds, (±)-FRC8653 (100 nM) gradually inhibited the amplitude of the Ba\textsuperscript2+ inward current, and maximum inhibition was reached after 4 minutes (Figure 2A, open circles). The Ba\textsuperscript2+ inward current amplitude was not completely restored after removal of (±)-FRC8653 even after 30 minutes.

**Effects of (±)-FRC8653 on Macroscopic Ca\textsuperscript2+ (Ba\textsuperscript2+) Currents in the Rabbit Basilar Artery**

In 10 mM Ca\textsuperscript2+ solution, application of a depolarizing pulse (more positive than −40 mV) evoked an inward current, and the maximum amplitude was evoked by depolarizing pulses of +10 to +20 mV (Figure 3Aa, open circles). Application of (±)-FRC8653 (100 nM) inhibited the inward current amplitude evoked by depolarizing pulses more positive than 0 mV, but slightly enhanced the current amplitude evoked by depolarizing pulses more negative than −10 mV (Figure 3Aa, closed circles). Under the influence of the drug, the maximum inward current amplitude was obtained by depolarizing pulses of 0 to +10 mV. Figure

![Figure 1](image1.png)  
*Figure 1. Chemical structure of (±)-FRC8653 [2-methoxyethyl (E)-3-phenyl-2-propen-1-yl(±)-1,4-dihydro-2,6-dimethyl-4-(3-nitro-phenyl)pyridine-3,5-dicarboxylate].*

![Figure 2](image2.png)  
*Figure 2. Changes in amplitude of the Ba\textsuperscript2+ inward current in the presence or absence of (±)-FRC8653 (100 nM). Depolarizing pulses (100 msec in duration) to +10 mV from the holding potential of −80 mV were applied every 20 seconds. Panel A: Effect of (±)-FRC8653 when the stimulation was unbroken (○) or interrupted for the first 3.5 minutes after application of drug (∆) and stimulation in the absence of drug (●). The amplitude of the Ba\textsuperscript2+ inward current recorded 3 minutes before application of (±)-FRC8653 was normalized as 1.0. Values of control are shown as mean±SD (n=3). Panel B: Tracings of the Ba\textsuperscript2+ inward current recorded before (a) and during (b and c) application and after removal (d) of 100 nM (±)-FRC8653. Tracings (a–d) were obtained at the time indicated by each letter in panel A.*
3Ab shows typical inward current tracings recorded in the absence or presence of (±)-FRC8653 (100 nM). (±)-FRC8653 consistently inhibited the inward current amplitude measured at peak or at the end of the depolarizing pulse (100 msec in duration and more positive than 0 mV). However, (±)-FRC8653 more profoundly inhibited the current amplitude measured at the end of depolarizing pulse than it did that measured at the peak amplitude. As shown in the top tracing in Figure 3Ab, the augmentation induced by (±)-FRC8653 is seen only in the early phase of the inward current evoked by a depolarizing pulse of −20 mV.

To enable further study of the actions of (±)-FRC8653, 10 mM Ba²⁺ solution was superfused in the bath instead of the 10 mM Ca²⁺ solution, because the Ca²⁺ current has been reported to be inactivated by an increase in concentration of intracellular Ca²⁺, as well as by depolarization of the membrane.20-22 In the 10 mM Ba²⁺ solution, the Ba²⁺ inward current amplitude was larger and the decay was slower than their equivalents in the Ca²⁺ inward current experiments. However, the shape of the current-voltage relation in the absence of (±)-FRC8653 was almost the same as that observed in the presence of 10 mM Ca²⁺, also without (±)-FRC8653 (open circles, Figure 3Ba versus Figure 3Aa). In 10 mM Ba²⁺ solution, (±)-FRC8653 (100 nM) reduced the inward current amplitude evoked by depolarizing pulses more positive than 0 mV but
increased the amplitude evoked by depolarization pulses more negative than −10 mV (holding potential, −80 mV; Figure 3Ba, closed circles). Enhancement of the inward current induced by (+)-FRC8653 (100 nM) was seen only during the early phase of the inward current (Figure 3Bb, top tracing), and decay of the inward current evoked by any given depolarizing pulse was steeper than that observed in the control (Figure 3Bb).

At a holding potential of −40 mV, the Ba²⁺ inward current amplitude evoked by depolarizing pulses more positive than −20 mV was reduced with no detectable change in the shape of the Ba²⁺ inward current (compare open symbols of Figures 3C and 3B). (+)-FRC8653 (10 nM) reduced the amplitude but not the decay of the Ba²⁺ inward current evoked by any given depolarizing pulse (Figures 3Ca and Cb).

Figure 4 shows the relation between the concentration of (±)-FRC8653 and the relative amplitude of the Ba²⁺ inward current evoked by depolarizing pulses of +10 mV or −20 mV from holding potentials of either −80 mV or −40 mV. Figure 4A shows that at the holding potential of −80 mV, (±)-FRC8653 (>0.1 nM) inhibited the Ba²⁺ inward current evoked by a depolarizing pulse of +10 mV, but that even application of 20 μM (±)-FRC8653 did not completely inhibit the Ba²⁺ inward current. The 50% inhibitory concentration (IC₅₀) of (±)-FRC8653 on the Ba²⁺ inward current evoked by the depolarizing pulse of +10 mV was 1 μM at the holding potential of −80 mV. On the other hand, at the holding potential of −40 mV, (±)-FRC8653 potently inhibited the Ba²⁺ inward current; application of 1 μM (±)-FRC8653 completely inhibited the Ba²⁺ inward current, and the IC₅₀ value was 1 nM. In contrast, an enhancement of the Ba²⁺ inward current amplitude occurred in a concentration-dependent manner (1 nM to 1 μM), when the effects of (±)-FRC8653 were explored using a depolarizing pulse of −20 mV from the holding potential of −80 mV (Figure 4B). A higher concentration of (±)-FRC8653 (20 μM) reduced the Ba²⁺ inward current amplitude to less than the control value.

**Effects of (+)- and (−)-FRC8653 on the Ba²⁺ Inward Current in the Rabbit Basilar Artery**

Application of 100 nM (−)-FRC8653 reduced the amplitude of the Ba²⁺ inward current evoked by a depolarizing pulse more positive than +10 mV. However, augmentation of the Ba²⁺ inward current was not clearly observed when a depolarizing pulse more negative than −20 mV was applied (Figure 5A). On the other hand, 100 nM (+)-FRC8653 did enhance the Ba²⁺ inward current amplitude evoked by any depolarizing pulses more negative than −10 mV but inhibited the current evoked by pulses more positive than 0 mV (Figure 5B). The peak of the current-voltage curve was shifted in a more negative direction (20 mV) by (+)-FRC8653; however, the threshold potential of the inward current remained the same (−40 mV) whether or not (+)-FRC8653 was present. Both (+)- and (−)-FRC8653 accelerated the decay of the inward current. When the membrane potential was held at −40 mV, (+)-FRC8653 produced no enhancement of the Ba²⁺ inward current amplitude (Figure 5C).

Figure 6 shows the relation between the relative amplitude of the Ba²⁺ inward current evoked by two different depolarizing pulses (+10 mV and −20 mV) and the concentration of (+)- or (−)-FRC8653. The inhibitory action of (−)-FRC8653 on the Ba²⁺ inward current was more potent than that of the (+) enantiomer [IC₅₀ of (−)-FRC8653, 60 nM; IC₅₀ of (+)-FRC8653, 1.5 μM; Figure 6A]. When a depolarizing pulse of −20 mV from the holding potential of −80 mV was applied, (−)-FRC8653 (<10 μM) inhibited, but the (+) enantiomer (<100 nM) augmented, the Ba²⁺ inward current amplitude in a concentration-dependent manner. A higher concentration of (+)-FRC8653 (10 μM) caused no increase above control in the Ba²⁺ current amplitude (Figure 6B).
These results indicate that the augmentation of the Ba\(^{2+}\) inward current amplitude observed on application of \((\pm)-FRC8653\) is closely related to the presence of the (+) enantiomer and is seen only when depolarizing pulses of less than \(-10 \text{ mV}\) are applied from a low holding potential level of \(-80 \text{ mV}\).

**Effects of \((\pm)-FRC8653\) on Voltage-Dependent Inactivation in Smooth Muscle Cells of the Rabbit Basilar Artery**

When various amplitudes of long conditioning pulses (5 seconds) were applied just before application of the test pulse (depolarization to \(+10 \text{ mV}\), 20-msec interval, 100 msec in duration), the Ba\(^{2+}\) inward current amplitude evoked by the test pulse was decreased depending on the amplitude of the conditioning pulse (Figure 7A). However, even with application of a conditioning pulse of \(+20 \text{ mV}\), about 15% of the Ba\(^{2+}\) inward current was not inactivated (noninactivating component\(^{13,23}\)). When a Boltzmann's distribution was adopted for the present voltage-dependent inactivation curve, the curve did not fit with a single slope factor, as shown in Figure 7A (continuous line with open circles). \((\pm)-FRC8653\) (100 nM) inhibited the noninactivating component completely and shifted the voltage-dependent inactivation curve to the left. When similar experiments were performed using a test pulse of \(-20 \text{ mV}\) (Figure 7B), \((\pm)-FRC8653\) (100 nM) augmented the Ba\(^{2+}\) inward current evoked by...
conditioning pulses more negative than −60 mV but inhibited the current evoked by conditioning pulses more positive than −50 mV.

Effects of (±)-FRC8653 on the K⁺ Outward Current in Smooth Muscle Cell Membranes of the Rabbit Basilar Artery

To investigate the effects of (±)-FRC8653 on the K⁺ outward current, various concentrations of (±)-FRC8653 were applied to the cells in a PSS bath. As shown in Figure 8A, (±)-FRC8653 (3 and 10 μM) inhibited the amplitude of the outward current evoked by any given depolarizing pulse (−30 to +40 mV; holding potential, −60 mV). The inhibition of the outward current was completely reversible after removal of the drug. Applications of (±)-FRC8653 (>1 μM) inhibited the outward current in a concentration-dependent manner (Figure 8B). The IC₅₀ value of (±)-FRC8653 for the K⁺ outward current was about 3 μM; this value was three times higher than that for the Ba²⁺ inward current measured at the holding potential of −80 mV, but 3,000 times higher than that measured at the holding potential of −40 mV (see Figure 4).

Recording of the Single Ba²⁺ Channel Current From Smooth Muscle Cells of the Rabbit Basilar Artery

Figure 9A shows the current-voltage relations of the two different amplitudes of the single Ba²⁺ channel current. The values of the large and small single Ba²⁺ channel conductance were 23 and 12 pS, respectively. When the holding potential was elevated to −60 mV, the 12-pS channel was not evoked. Figure 9B shows the amplitudes of the single-channel currents evoked by various intensities of depolarizing pulses (−30 to +10 mV) from the holding potential of −80 mV in 100 mM Ba²⁺ solution. When the membrane was depolarized to −30 to −10 mV, two different amplitudes of single-channel currents were recorded in the same membrane patch. On the other hand, when depolarizing pulses more positive than 0 mV were applied in the same membrane patch, the small amplitude inward current ceased. In Figure 9C, summated single-channel currents of the 12- and 23-pS channels are shown. The amplitude of the summated current of the 12-pS channel declined during the depolarization (150 msec in duration), while that of the 23-pS channel was sustained at the same level.

Effects of (+)- or (−)-FRC8653 on the Single Ba²⁺ Channel Current in Smooth Muscle Cells of the Rabbit Basilar Artery

Figure 10 shows the effects of (+)-FRC8653 on the 12-pS channel current evoked by repetitively applied depolarizing pulses to −20 mV from the holding potential of −80 mV (20-second interval, 150 msec in duration). In the absence of (+)-FRC8653, 43 of 72 depolarization pulses evoked channel opening (nonblank tracings), but the other 29 pulses did not (blank tracings). Essentially, the same result was obtained in the presence of 100 nM (+)-FRC8653 (42 nonblank and 31 blank tracings from 73 depolarizing pulses). In Figure 10, the lower tracings show summated currents of the 12-pS channel in the absence or presence of 100 nM (+)-FRC8653. There is no significant difference in the amplitude or the shape before or after application of (+)-FRC8653. Table 1 shows the proportion of nonblank tracings, mean open and closed times, open probability, and averaged number of events (channel opening) per tracing recorded from two different membrane patches. (+)-FRC8653 (100 nM) did not modify the above parameters. This means that (+)-FRC8653 does not modify the activity of the 12-pS channel currents in smooth muscle cells of the rabbit basilar artery.

Figure 11A shows the effects of (+)-FRC8653 (100 nM) on the 23-pS channel current evoked by a depolarizing pulse of −20 mV. In this membrane patch, both 12- and 23-pS channels were simultaneously opened, and in 56 of 72 depolarizing pulses, the 23-pS channel opened. In the presence of 100 nM (+)-FRC8653, the number of depolarizing pulses needed to record 50 nonblank tracings was reduced to 58. As shown in the bottom tracings, the amplitude of the summated currents was increased by 100 nM
(+)-FRC8653; however, no decay of the current was seen during the membrane depolarization (150 msec in duration). (+)-FRC8653 did not modify the amplitude of the 23-pS channel current, but it did increase the proportion of nonblank tracings, the mean open time, the averaged open probability, and the number of channel openings per tracing. On the other hand, 100 nM (+)-FRC8653 reduced the fast component of the closed time but not the slow component (Table 2). Figure 11B shows the effects of (−)-FRC8653 on the 23-pS channel current evoked by a depolarizing pulse to +10 mV from the holding potential of −80 mV (mean ± SD, n=4). The lines were drawn theoretically using the following equation: $I = (I_{\text{max}} - C)/[1 + \exp((V - V_{0.5})/k)] + C$, where $I$, $I_{\text{max}}$, $V$, $V_{0.5}$, $k$, and $C$ are the relative amplitudes of the Ba$^{2+}$ inward currents observed at various amplitudes of the conditioning pulse ($V$) and that where the amplitude of the Ba$^{2+}$ inward current was reduced to half ($V_{0.5}$), slope factor ($k$) and fraction of the noninactivating component of the Ba$^{2+}$ inward current ($C$). The curves in the absence or presence of (−)-FRC8653 were drawn using the following values: (control) $I_{\text{max}}=1.0$, $V_{0.5}=-22$ mV, $k=9$ mV, and $C=0.15$; (±)-FRC8653 $I_{\text{max}}=0.61$, $V_{0.5}=-43$ mV, $k=9$ mV, and $C=0.02$. Tracings of the Ba$^{2+}$ inward current recorded with various amplitudes of the conditioning pulse (−100, −40, −20, and +20 mV) are shown on the right. Panel B: Amplitude of the Ba$^{2+}$ inward current evoked by the test pulse depolarization to −20 mV (100 msec in duration) was plotted against the amplitude of the conditioning pulse (5 seconds in duration). ○, Absence of (±)-FRC8653; ●, presence of 100 nM (±)-FRC8653 (mean ± SD, n=4). The amplitude of the Ba$^{2+}$ inward current was measured at peak. The Ba$^{2+}$ inward current amplitude evoked by the conditioning pulse of −100 mV in the absence of (±)-FRC8653 was normalized as a relative amplitude of 1.0. Curves were drawn by eye. Tracings recorded with conditioning pulses of −100, −50, −30, and −10 mV, are shown on the right.
absence or presence of (−)-FRC8653 recorded on application of depolarizing pulses to +10 mV from the holding potential of −80 mV. The inhibitory action of (−)-FRC8653 observed at +10 mV was mainly due 1) to an increase in the number of blank tracings [proportion of nonblank tracings: control, 79.1%; but in the presence of (−)-FRC8653, 47.5%] and 2) to a reduction in the number of events per tracing [control, 8.5 ± 1.0, n = 3; (−)-FRC8653, 4.2 ± 0.8, n = 3]. However, the mean open and closed times were not modified (control, τo = 2.1 ± 0.3 msec, τc,fast = 4.3 ± 0.4 msec, τc,slow = 14.1 ± 0.8 msec, n = 3; (−)-FRC8653, τo = 2.0 ± 0.3 msec, τc,fast = 4.0 ± 0.7 msec, τc,slow = 15.1 ± 0.9 msec, n = 3). As a consequence, mean open probability was reduced to 0.03 ± 0.01 from 0.09 ± 0.02 in the presence of 100 nM (−)-FRC8653 (n = 3). (−)-FRC8653 prolonged the time between the final closing of the 23-pS channel and the end of the depolarizing pulse (control, 20.5 ± 5.0 msec, n = 3; (−)-FRC8653, 57.5 ± 4.6 msec, n = 3).

**Discussion**

In dispersed smooth muscle cells of the rabbit basilar artery, two types of voltage-dependent Ca2+ channels were present with single-channel conductances of 12 or 23 pS (in the 100 mM BaCl2 solution). The summated current of the 12-pS channel was inactivated within 100 msec, but that of the 23-pS channel was long lasting. Both 12- and 23-pS channel currents were activated by depolarizing pulses above −30 mV from the holding potential of −80 mV; however, the 12-pS channel was not evoked by depolarizing pulses from the holding potential of −60 mV. These characteristics are consistent with the properties reported as belonging to the T- and L-types of voltage-dependent Ca2+ channels in various excitable cells.3,7,24 In the whole-cell voltage-clamp experiments, however, no clear evidence for the existence of T- or L-type voltage-dependent Ca2+ channels was obtained. For example, no additional peak on the low membrane potential level was observed in the current-voltage relation, and a large rapidly inactivating component was not observed on the decay phase of the inward current at the holding potential of −80 mV. The resting membrane potential of the rabbit basilar artery has been reported to be −50 to −60 mV25,26; therefore, almost all T-type Ca2+ channels might be inactivated at the resting membrane potential level. It is not yet clear what the role of the T-type Ca2+ channel is, but whatever it is, modification of the T-type Ca2+ channel may not be involved in the vasodilating actions of (±)-FRC8653.

The characteristics of (±)-FRC8653, a novel DHP derivative, as determined in the present experiments may be summarized as follows. 1) (±)-FRC8653 had a dual action, producing both excitatory and inhibitory effects, on the Ba2+ inward current. The (+) enantiomer mainly had excitatory, and the (−) enantiomer inhibitory, actions. The excitatory action of the (+) enantiomer was easily reversed to an inhibitory action by elevation of the membrane potential.
(to −40 mV). 2) (+)-FRC8653 did not act on the 12-pS channel, which was classified as a T-type Ca²⁺ channel,⁷ and thus both the excitatory and inhibitory actions induced by (+)-FRC8653 result from actions on the 23-pS channel in the rabbit basilar artery.

Although the T- and L-type Ca²⁺ channels are present in the smooth muscle cell membrane of the rabbit basilar artery, both the excitatory and inhibitory actions of (+)-FRC8653 were caused by actions of the drug on the 23-pS channel (L-type Ca²⁺ channel). It is well known that many DHP derivatives, such as Bay K 8644, PN200-110, nifedipine, and nilnidipine, and newly synthesized derivatives, such as CV-4093 or benidipine, have both agonistic and antagonistic actions on the L-type Ca²⁺ channels in various muscle cells. Furthermore, one of the enantiomers has a predominantly agonistic action,¹³,¹⁷,²⁷⁻²⁹ In this respect, (+)-FRC8653 appears to be a typical DHP derivative; that is, (+)-FRC8653 has a predominantly agonistic action, but that of the (−) enantiomer is marginal.

One of the interesting properties of (+)-FRC8653 is that this drug can inhibit the voltage-dependent Ca²⁺ channel over a very wide range of concentrations (<0.1 nM to >20 μM) (Figure 4, at a holding potential of −80 mV). When the Ba²⁺ current amplitude was measured at peak with a depolarizing pulse of +10 mV from the holding potential of −80 mV, (+)-FRC8653 did not completely inhibit the inward current, presumably because of activation of the T-type Ca²⁺ channel and partly because of participation of the agonistic action of the (+) enantiomer. However, the contribution of the former may be small, because (−)-FRC8653 (10 μM) produced a nearly complete block of the Ba²⁺ current. On the other hand, a step observed between 10 and 100 nM in the concentration-inhibition curve (as shown in Figure 4A at a holding potential of −80 mV) may reflect a contamination of the agonistic action of the drug, because such a step was not observed in the concentration-inhibition curve observed at the holding potential of −40 mV (Figure 4A). Similarly, the turning down of the augmentation curve of the Ba²⁺ current (depolarizing pulse, −20 mV; holding potential, −80 mV) observed after application of (+)-FRC8653 and of the (+) enantiomer (>10 μM) also may be due to contamination of antagonistic actions. Therefore, the overall actions of (+)-FRC8653 can be expressed as the sum of the agonistic and antagonistic actions of the (+) and (−) enantiomers.

The voltage-dependent inactivation curves showed that 15% of the inward current was not inactivated above +10 mV of conditioning depolarization and that (+)-FRC8653 (100 nM) inhibited this component, as reported in other smooth muscle cells.¹³,²³ (+)-FRC8653 shifted the voltage-dependent inactivation curve to the left (17 mV), suggesting that this drug binds at the inactivated state with high affinity.³⁰,³¹ However, in the present experiments (see Figure 7), the voltage-dependent inactivation curve in the absence of (+)-FRC8653 did not fit with Boltzmann’s equation with a single slope factor; that is, the upper part of the curve fitted with a slope factor of 16 but the lower part with a factor of 9. These results may be due to contamination of the activation of the T-type Ca²⁺ channel at lower conditioning potentials (<−40 mV, upper part of the curve). Various Ca²⁺ antagonists, such as diltiazem, verapamil, flunarizine, and many DHP derivatives, are known to shift the voltage-dependent inactivation curve
Figure 10. Effects of 100 nM (+)-FRC8653 on the 12-pS channel current. Seventy-two repetitive depolarizing pulses (150 msec in duration, 20-second interval) to −20 mV in the absence of (+)-FRC8653 were applied; then 100 nM (+)-FRC8653 was superfused. Ten minutes after application of drug, 73 pulses were applied. Tracings shown were obtained from pulses 33 to 43 in control and from 20 to 30 in the presence of (+)-FRC8653. Bottom tracings are two summated currents superimposed in the absence or presence of (+)-FRC8653. Fraction numbers in the bottom tracings indicate (number of nonblank tracings)/(total number of tracings).

Table 1. Effects of 100 nM (+)-FRC8653 on the Single-Channel Activities of the 12-pS Channel

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Proportion of N (N/T)</th>
<th>Mean open time N</th>
<th>Mean closed time (N)</th>
<th>Mean open probability (N)</th>
<th>No. of events/tracing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fast (N)</td>
<td>Slow (N)</td>
<td></td>
</tr>
<tr>
<td>1 (Control)</td>
<td>58.3% (42/72)</td>
<td>3.4 msec</td>
<td>2.8 msec</td>
<td>19.5 msec</td>
<td>0.09</td>
</tr>
<tr>
<td>1 (FRC)</td>
<td>58.9% (43/73)</td>
<td>2.8 msec</td>
<td>2.8 msec</td>
<td>18.9 msec</td>
<td>0.09</td>
</tr>
<tr>
<td>2 (Control)</td>
<td>60.7% (34/56)</td>
<td>2.5 msec</td>
<td>2.9 msec</td>
<td>23.7 msec</td>
<td>0.04</td>
</tr>
<tr>
<td>2 (FRC)</td>
<td>61.1% (33/55)</td>
<td>2.2 msec</td>
<td>2.7 msec</td>
<td>22.5 msec</td>
<td>0.05</td>
</tr>
<tr>
<td>1 (FRC/control)</td>
<td>1.01</td>
<td>0.82</td>
<td>1.00</td>
<td>0.97</td>
<td>1.00</td>
</tr>
<tr>
<td>2 (FRC/control)</td>
<td>1.01</td>
<td>0.88</td>
<td>0.93</td>
<td>0.94</td>
<td>1.25</td>
</tr>
</tbody>
</table>

N, nonblank tracings; T, total tracings; FRC, (+)-FRC8653.
curve to the left in a parallel manner. In the present experiments, (+)-FRC8653 shifted the voltage-dependent inactivation curve and also changed the slope of the upper part of the curve. This may suggest that (+)-FRC8653 has a more potent agonistic action than other Ca\textsuperscript{2+} antagonists.

Enhancement of the Ba\textsuperscript{2+} inward current amplitude was reversed to an inhibition by elevation of the holding potential (-40 mV). When the voltage-dependent inactivation curves (conditioning pulse, 5 seconds in duration) were produced using a short test pulse of -20 mV at which (+)-FRC8653 produced the largest augmentation of the Ba\textsuperscript{2+} inward current; see Figure 3B), these curves in the presence or absence of (+)-FRC8653 crossed at near -50 mV (Figure 7B). With the microelectrode technique, the mean resting membrane potential of the rabbit basilar artery has been reported to be -50 to -60 mV. Although the conditions for these measurements of the resting membrane potential and the Ba\textsuperscript{2+} inward current were quite different, augmentation of the Ba\textsuperscript{2+} current may not play an important role in the action of this drug under physiological conditions. Furthermore, augmentation of the Ca\textsuperscript{2+} inward current by this drug was smaller than that observed in the same concentration of Ba\textsuperscript{2+}; this may reinforce the above postulation.

Inoue et al\textsuperscript{33} studied the inhibitory action of nifedipine on the single Ba\textsuperscript{2+} channel current in rabbit intestinal smooth muscle cells and reported that nifedipine both increases the proportion of blank tracings and reduces the mean open time; however, the former action is more important than the latter in the overall inhibitory actions of nifedipine. They also reported that nifedipine has a voltage-dependent inhibitory action on the single Ba\textsuperscript{2+} channel current, since an increase in the slope of the current decay is thought to be due to the occurrence of a long shut in.
the early phase of the depolarizing pulse. In the present experiments, (-)-FRC8653 had similar actions on the 23-pS channel current evoked by a depolarizing pulse of +10 mV, except for changes in the mean open and closed times, which may be due to a contribution from the agonistic action of (-)-FRC8653. The present experiments showed that application of 100 nM (-)-FRC8653 reduces the mean open probability to 0.3–0.4 times the control, because of an increase in the number of blank tracings and a decrease in the number of events (channel opening) per tracing. On the other hand, the increase in the mean open probability that occurred after application of (+)-FRC8653 may be caused by an increase in the proportion of nonblank tracings, mean open time, and number of events per tracing.

In conclusion, (+)-FRC8653 shares the common properties of DHP derivatives, including the different actions of the two enantiomers. Because (+)-FRC8653 had a moderate agonistic action, the actions of the racemic form of this drug, (+)-(-)-FRC8653, would be modified by the resting membrane potential of various smooth muscle cells. Therefore, this drug may selectively relax certain vascular tissues in which the cells have a relatively less negative resting membrane potential.

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References


KEY WORDS • Ca++ antagonist • dihydropyridine derivative • Ca++ channel • basilar artery • Ca++ agonist
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