Actions of FRC-8653 on Smooth Muscle Cells of the Rabbit Mesenteric Artery

Mikio Nakashima, Makoto Kou, Hikaru Hashitani, Guifa Chen, Hideharu Ono, Hirosi Kuriyama and Hikaru Suzuki

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan
Department of Anesthesiology, Saga Medical School, Saga 849, Japan

Address for correspondence: Department of Physiology, Nagoya City University Medical School, Mizuho-ku, Nagoya 467, Japan

ABSTRACT — In ring preparations of the rabbit mesenteric artery, the Ca-contraction, but not the noradrenaline (NA)-contraction, was inhibited by FRC-8653 (10^{-9} - 10^{-5} M) in a concentration-dependent manner, although with a potency 50-100 times weaker than that of nicardipine. The actions of FRC-8653 on Ca-contraction appeared more slowly (over 1 hr) than those of nicardipine. FRC-8653 (up to 10^{-5} M) and nicardipine (up to 10^{-7} M) did not change the resting membrane potential of smooth muscles. The amplitude of the evoked excitatory junction potential (e.j.p.) was inhibited by FRC-8653, but not by nicardipine, with no alteration in the facilitation process of the e.j.p.s. The inhibition by FRC-8653 of the e.j.p. appeared rapidly and was reversible. FRC-8653 inhibited the membrane depolarization of smooth muscles elicited by ATP, but not by NA or high [K^+]_o solution. ATP-induced contractions were also decreased by FRC-8653, with no significant change in the electrotonic potentials. Thus, FRC-8653 has properties similar to those of the dihydropyridine Ca-antagonists, but differs from them in that 1) its inhibitory actions on Ca influx appear slowly and 2) sympathetic transmission is inhibited, possibly by inhibition of the postjunctional events for e.j.p. generation.

A novel dihydropyridine derivative, FRC-8653 (2-methoxyethyl(E)-3-phenyl-2-propen-1-yl(±)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) pyridine-3,5-dicarboxylate), possesses hypotensive actions in experimentally hypertensive rats (1). FRC-8653 inhibits KCl-induced contractions in isolated rat superior mesenteric artery (2). The Ca-influxes into cultured smooth muscle cells are also inhibited by this drug (2). Furthermore, an increase in [Ca^{2+}], elicited by membrane depolarization can be blocked by FRC-8653, with a potency similar to that of nifedipine or nicardipine (2). These results suggest that FRC-8653 inhibits voltage-dependent Ca channels and that the hypotensive action is mainly due to inhibition of the influx of Ca into vascular smooth muscle cells. However, compared to those of other Ca-antagonists, the inhibitory actions of FRC-8653 on Ca-channels appear slowly and last for a long time (2).

Drugs that inhibit the Ca influx through Ca channels (i.e., Ca-antagonists, 3) have been classified into several subgroups based on their chemical structures; and the drugs in one of the subgroups, dihydropyridine derivatives,
can inhibit Ca-channels with no detectable change in the membrane potential (4). The lack of inhibitory action of dihydropyridine Ca-antagonists on the prejunctional Ca-influx related to the release of transmitter substances from nerve terminals has also been reported in many tissues (5).

We investigated the effects of FRC-8653 mainly on the electrical properties of smooth muscle cell membranes in the rabbit mesenteric artery, using the intracellular microelectrode technique. Experiments were carried out first to confirm the Ca-antagonistic properties of FRC-8653 on this artery. This was done by comparing its effects with those of nicardipine, a known dihydropyridine derivative Ca-antagonist (6), on the mechanical responses of smooth muscles. The effects of FRC-8653 on sympathetic transmission in this artery were then estimated from its effects on the excitatory junction potential (e.j.p.) produced by electrical stimulation of perivascular nerves.

MATERIALS AND METHODS

Male albino rabbits, weighing 2.0–2.5 kg, were anesthetized by Na-pentobarbitone (40 mg/kg, i.v. injection). After exsanguination via the femoral artery, the mesenteric vascular beds distributing to the ileal region were dissected out and kept in Krebs solution at room temperature. The mesenteric arteries at the third and fourth branches (diameter of approximately 150–300 \( \mu \text{m} \)) were removed, together with the mesenteric veins, lymphatic vessels, and mesenteric membranes. For experiments involving the recording of electrotonic potentials from arterial smooth muscle, the mesenteric artery was isolated from the surrounding tissues. This procedure, however, for some reason caused about a 10-mV reduction in the resting membrane potential of the smooth muscle cells (a depolarization from around \(-70 \text{ mV}\) to \(-60 \text{ mV}\)).

The experimental chamber for the microelectrode experiments was made of lucite plates and had a capacity of about 2 ml. The bottom was covered with a silicon rubber plate (KE-66, Shin-etsu Kagaku, Tokyo, Japan), and the isolated arteries were mounted on this rubber plate using fine pins, to minimize the movement of the tissue. The tissues were superfused with warmed (35.5°C) Krebs solution at a flow rate of about 3 ml/min. The point stimulation method was used for electrical stimulation of the perivascular nerves. This involved a silver wire (diameter of 0.5 mm) coated with enamel and the distal end cut flat with a knife. The cut end was touched gently onto the proximal part of the mesenteric artery, and square current pulses of 30–50 \( \mu \text{sec} \) duration and 20–50 V intensity were applied between this wire and an indifferent electrode that was placed at the end of the chamber (7). For direct electrical stimulation of the smooth muscle, current was applied using the suction electrode (8); briefly, a segment of the mesenteric artery (diameter of about 200 \( \mu \text{m} \), length of about 1 cm) was drawn into a glass tube whose tip had a diameter similar to that of the artery, and square current pulses of 1–2 sec duration and 1–5 V intensity were applied through the glass tube which was filled with Krebs solution. A glass capillary microelectrode filled with 3 M KCl (resistance about 40–60 M\( \Omega \)) was inserted into a smooth muscle cell through the overlying mesenteric membrane and adventitial layers. Electrical responses thus recorded were displayed on a cathode-ray oscilloscope (Nihon Kohden VC-9) and also on a penrecorder (Recticorder, Nihon Kohden RJG4024).

A ring preparation was made to measure the mechanical responses of the smooth muscles of the mesenteric artery. The ring segment (about 1 mm width) was mounted between a pair of L-shaped steel wires, one of which was fixed at the bottom of the chamber and the other connected to a mechanotransducer (FD pick-up, TB612T, Nihon Kohden), and the forces produced by the ring were measured isometrically. The tissues were perfused with warmed (35.5°C) Krebs solution at a constant flow rate of 3 ml/min using a peristaltic pump (P-2, Tokyo Rikakikai). The mechanical
responses were displayed on a pen-recorder (National VP-6521A).

The ionic composition of the Krebs solution was as follows: 137.4 mM Na⁺, 5.9 mM K⁺, 2.5 mM Ca²⁺, 1.2 mM Mg²⁺, 15.5 mM HCO₃⁻, 1.2 mM H₂PO₄⁻, 134 mM Cl⁻, 11.5 mM glucose. The solution was aerated with O₂ containing 5% CO₂, and the pH of the solution was maintained at 7.2–7.4. For the measurement of Ca-contractions, the tissues were perfused with a Ca-free, 39.2 mM [K⁺]₀ solution (i.e., K⁺ was increased by replacement of Na⁺, and Ca²⁺ was omitted). Ca-contractions were produced using a 39.2 mM [K⁺]₀ solution containing 2.5 mM [Ca²⁺]₀. Drugs used were noradrenaline HCl (Sigma), nicardipine (Yamanouchi), adenosine 5'-triphosphate (ATP, Sigma), guanethidine (Tokyo Kasei), phentolamine maleate (Ciba Geigy), procaine hydrochloride (Sigma) and FRC-8653 (Fujirebio, Inc.). FRC-8653 was dissolved in a mixture composed of equal volumes of ethyl alcohol and polyethylene glycol so that its concentration was 10 mM, and this was diluted to the desired concentration using Krebs solution. The pH of the Krebs solution was not detectably changed by the preparation of solutions containing 10⁻⁸–10⁻⁵ M FRC-8653.

Experimental values were expressed by the mean ± standard deviation (S.D.). Statistical significances were determined using Student's t-test, and probabilities of less than 5% (P < 0.05) were considered significant.

RESULTS

Effects of FRC-8653 on mechanical responses

In ring preparations of the rabbit mesenteric artery, the effects of FRC-8653 on "Ca-contractions" (produced by 2.5 mM Ca²⁺ in Ca²⁺-free, 39.2 mM [K⁺]₀ solution) and contractions induced by 10⁻⁶ M noradrenaline (NA) were observed. Two types of Ca-contractions were observed: one consisted of an initial transient component followed by a sustained one (Fig. 1A), and the other developed slowly, requiring about 3 min to reach the sus-

---

Fig. 1. Inhibitory actions of FRC-8653 and nicardipine on Ca-contractions. Smooth muscle tissues of the rabbit mesenteric artery were perfused with a solution containing 39.2 mM [K⁺]₀ and 0 mM [Ca²⁺]₀, and then with a Ca-containing solution ([K⁺]₀ = 39.2 mM, [Ca²⁺]₀ = 2.5 mM) where indicated by a bar. In B and C, contractions were produced in the presence of 10⁻⁷ M FRC-8653 for 196 min (B) or 10⁻⁸ M nicardipine for 52 min (D). A and C show control responses obtained before application of FRC-8653 and nicardipine, respectively. A, B and C, D were obtained from different tissues.
tained level (Fig. 1C). No causal relationship between the type of contraction and the methods of preparation or any feature of the tissues was detected. A Ca-contraction of reproducible amplitude was observed when the Ca\(^{2+}\) concentration was elevated to 2.5 mM at intervals over 15 min, and therefore the following experiments were carried out by applying Ca\(^{2+}\) at intervals of over 15 min (usually over 20 min).

Application of FRC-8653 (up to 10\(^{-5}\) M) produced no detectable change in muscle tension either in the normal Krebs solution or in the Ca\(^{2+}\)-free, high [K\(^+\)]\(_o\) solution. However, in the presence of FRC-8653, the amplitude of the Ca-contraction was decreased, and the latency to its onset was prolonged. The latter varied in different preparations between 120 and 200 sec. In the tissue shown in Fig. 1A, the time to the start of the Ca-contraction after switching to the Ca-containing solution was increased from 39 ± 6 sec (n = 10) in the control to 138 ± 22 sec (n = 3) in the presence of 10\(^{-7}\) M FRC-8653 for 60–250 min, with the amplitude of the Ca-contraction decreased to 75 ± 5% of the control. In the present experiments, most of the delay in the control condition was required for replacement of the solution in the tubing of the recording apparatus, and therefore the real latency in the presence of FRC-8653 was about 100 sec. Nicardipine also caused a potent inhibition of the Ca-contraction in the rabbit mesenteric artery; and in the tissue shown in Fig. 1D, application of 10\(^{-8}\) M nicardipine for 52 min reduced the contraction to 26% of the control and delayed the onset of contraction by about 100 sec.

Figure 2 shows the decay in the amplitude of the Ca-contraction in the presence of 10\(^{-6}\) M FRC-8653 or 10\(^{-7}\) M nicardipine. With application of FRC-8653, the amplitude of the Ca-contraction decreased with time, and it took over 100 min for it to reach the steady amplitude of about 30% of the control. Nicardipine inhibited the Ca-contraction to a steady amplitude of about 10% of the control, within 15 min.

The effects of different concentrations of FRC-8653 on the amplitude of the Ca-contraction are summarized in Fig. 3, in which the

![Graph](image)

**Fig. 2.** Time course of change in amplitude of the Ca-contraction in the presence of 10\(^{-6}\) M FRC-8653 (•) or 10\(^{-7}\) M nicardipine (○) in the rabbit mesenteric artery. Relative amplitude of Ca-contractions was plotted as a function of time after application of FRC-8653 or nicardipine. Number of tissues was 4 for FRC-8653 and 3 for nicardipine. Lines in the figure were fitted by eye.
The effects of FRC-8653 and nicardipine on the NA-contraction are summarized in Fig. 3B. The NA-contraction was not markedly inhibited by FRC-8653 (up to $10^{-6}$ M), but was reduced by $10^{-7}$ M nicardipine to $79 \pm 13\%$ ($n = 20, P < 0.05$) of the control.

ATP is a vasoconstrictor, and application of $10^{-4}$ M ATP produced a transient contraction in the smooth muscle of the rabbit mesenteric artery. The amplitude of the ATP-induced contraction was reproducible when the intervals of the ATP application were over 30 min, and therefore such a condition was applied to observe the effects of FRC-8653 on the ATP-induced contractions. In the presence of $10^{-6}$ M FRC-8653 for over 100 min, the ATP-induced contractions were inhibited to $75 \pm 8\%$ ($n = 8, P < 0.05$) of the control.

**Effects of FRC-8653 on sympathetic transmission**

The effects of FRC-8653 on sympathetic transmission were estimated from the excitatory junction potential (e.j.p.) recorded from smooth muscle cells of the rabbit mesenteric artery. When electrical stimulation of perivascular nerves was applied 10 times at 1 Hz frequency, the amplitude of the e.j.p.s increased progressively (facilitation phenomenon) and reached a steady value of 2–3 times larger than the first, at the 5th or 6th e.j.p. (Fig. 4A). Application of FRC-8653 inhibited all the e.j.p.s in a train evenly, without a marked change in facilitation phenomenon, and in a reversible manner (Fig. 4, B–D). This effect on the e.j.p.s by FRC-8653 was not accompanied by a significant change in the resting membrane potential (control, $-72.5 \pm 2.3$ mV, $n = 16$; in $10^{-6}$ M FRC-8653, $-74.0 \pm 2.2$ mV, $n = 12$). The inhibition appeared evenly on all the e.j.p.s in a train. That the inhibition was an action of FRC-8653, and not of its vehicle, was confirmed using the solvent used to prepare solution containing $10^{-7}$ M of this drug (Fig. 4E).

When membrane potential changes during the generation of an e.j.p. were plotted on a logarithmic scale against time, the potential amplitudes at the steady level were measured. FRC-8653 ($10^{-9}$–$10^{-5}$ M) inhibited the Ca-contraction in a concentration-dependent manner. In addition, the figure shows that nicardipine ($10^{-9}$–$10^{-7}$ M) also inhibited the Ca-contraction in a concentration-dependent manner, with potencies about 50–100 times higher than those of FRC-8653.
decayed on a straight line, i.e., the e.j.p. decayed exponentially. The time constant of the falling phase of the e.j.p. calculated from this relationship was 178 ± 24 msec (n = 11). In the presence of 10^-6 M FRC-8653, the time constant remained unaltered (188 ± 22 msec, n = 10, P < 0.2).

Figure 5A shows the effects of FRC-8653 (10^-6 M) or nicardipine (10^-7 M) on amplitude of the e.j.p.s in the rabbit mesenteric artery. FRC-8653 (10^-6 M) was present throughout. A. Change in amplitude of the last e.j.p. of a train elicited by 10 stimuli at 1 Hz frequency. FRC-8653 (•) or nicardipine (○) was applied for 30 min. All responses were obtained from the same tissue.

B. Concentration-response relationship of the inhibitory actions of FRC-8653 on amplitude of the e.j.p.s elicited by perivascular nerve stimulation in the rabbit mesenteric artery. Amplitude of the last e.j.p. of a train response evoked by 1 Hz stimulation is shown by the mean ± S.D. (n = 5–10).

The inhibitory actions of FRC-8653 on the e.j.p. were detected in concentrations above 10^-7 M.
The effects of FRC-8653 on the facilitation process of the e.j.p.s elicited by repetitive stimuli were observed by expressing the amplitude of individual e.j.p.s relative to that of the first of each train. Although the amplitude of each of the e.j.p.s in a given train was decreased by FRC-8653 (Fig. 6A), the drug did not change significantly their values relative to each other (Fig. 6B). Therefore, the inhibition by FRC-8653 of the e.j.p. is probably not due to prejunctional events, and changes in postjunctional properties must be considered.

Effects of FRC-8653 on electrical responses elicited by current stimuli

In mesenteric arteries from which the surrounding tissues were removed, the resting membrane potential of smooth muscle cells was $-64.6 \pm 3.5$ mV ($n = 12$ from 3 tissues). When current stimuli of 1 sec duration and 1–5 V intensity were applied to the artery using the suction electrode, electrotonic potentials were recorded from cells located close to the edge of the electrode (usually within 500 $\mu$m). The relationship between the intensity of the current and the amplitude of electrotonic potentials measured at their steady value was linear to the inward current and showed strong rectification to the outward current (mostly over 1 V intensity). Outward current stimuli with intensities over 2–2.5 V produced an action potential on the initial part of the electrotonic potential; the amplitude of the potential was about 60 mV and often showed overshoot potentials. However, amplitude of such action potentials was markedly decreased after incubation of the artery with a guanethidine ($5 \times 10^{-6}$ M)-containing solution for 20–30 min; thus, the action potential was probably elicited by transmitters released from perivascular sympathetic nerves.

![Fig. 6](image-url) Effects of FRC-8653 on facilitation process of the e.j.p.s in the rabbit mesenteric artery. Phentolamine ($3 \times 10^{-7}$ M) was present throughout. Perivascular nerves were stimulated for 10 times at 1 Hz frequency (stimuli: 0.05 msec duration, 25 V intensity), and e.j.p.s were recorded before (●, control) and after application of $10^{-7}$ M FRC-8653 (○) for 10–24 min and $3 \times 10^{-6}$ M FRC-8653 (△) for 10–25 min. Mean ± S.D. ($n = 7$ for each condition). All are from the same tissue. Amplitudes of the e.j.p.s elicited by individual stimuli are expressed as actual (A) or relative values (B) where the amplitude of the first e.j.p. of a train has been given the relative value of 1.
In sympathectomized arteries using guanethidine, application of procaine (3 mM) depolarized the membrane by about 5 mV (mean value, \(-60.1 \pm 2.5\) mV, \(n = 15\) from 3 tissues), increased the slope of the current-voltage relationships, inhibited the rectification to outward current stimuli and elicited action potentials (amplitude being 8-15 mV) on the initial part of the electrotonic potentials produced by outward current stimuli (Fig. 7B). Application of \(10^{-6}\) M FRC-8653 for 60 min reduced the amplitude of the action potential to about half the control, with no significant change in the amplitude of the electrotonic potential (Fig. 7D).

**Effects of FRC-8653 on membrane responses elicited by NA and ATP**

In smooth muscle cells of the rabbit mesenteric artery, application of NA (10^{-6} M) produced membrane depolarizations which developed slowly and reached the peak amplitude of 9.3 \(\pm\) 1.3 mV (\(n = 8\)) from the resting potential, within 3-4 min (Fig. 8A). The amplitude of the NA-induced depolarization remained unaltered in the presence of \(10^{-6}\) M FRC-8653 for 30-150 min (9.6 \(\pm\) 1.9 mV, \(n = 8\), Fig. 8B). The smooth muscle membrane of this artery was also depolarized on application of \(10^{-6}\) M ATP; the peak of the depolarization was reached within 20 sec after application of ATP (Fig. 8C). Reproducible amplitude of the ATP-induced depolarization was obtained when the stimulant was applied at an interval of over 30 min, and the mean value was 17.5 \(\pm\) 1.9 mV (\(n = 10\)). In the presence of \(10^{-6}\) M FRC-8653 for over 60 min, the amplitude of the ATP-induced depolarization was...
decreased to 12.5 ± 1.7 mV (n = 6, P < 0.05; Fig. 8D). The depolarization of smooth muscle membrane induced by 39.2 mM [K⁺]₀ solution (-34.7 ± 2.3 mV, n=12) was not changed in the presence of FRC-8653 (10⁶ M) for over 60 min (-34.1 ± 1.3 mV, n = 14, P > 0.5).

Effects of FRC-8653 on endothelium-dependent relaxations

In ring preparations of the rabbit mesenteric artery, application of 29.6 mM [K⁺]₀ solution produced a sustained contraction, and application of 10⁻⁵ M acetylcholine (ACh) in the presence of high-[K⁺]₀ solution produced an endothelium-dependent relaxation of 70 ± 19% (n = 6) of the contraction. In the presence of 10⁻⁶ M FRC-8653 for over 60 min, the relaxation by ACh remained unaltered (72 ± 5.4%, n = 3).

DISCUSSION

The present experiments showed that in the rabbit mesenteric artery, FRC-8653 inhibited the Ca-contraction but not the NA-contraction. In the presence of procaine, evoked action potentials generated by an influx of Ca ions (9) were also sensitive to FRC-8653. Comparison of the actions of FRC-8653 with those of nicardipine, a known dihydropyridine-derivative Ca-antagonist, revealed that FRC-8653 and nicardipine inhibited the Ca-contractions in a similar manner. Thus, FRC-8653 is an inhibitor of Ca influx, and this is in good agreement with the findings reported by Yoshimoto et al. (2) for the rat superior mesenteric artery or by Oike et al. (10) for the rabbit basilar artery. Therefore, the potent vasodilator actions of FRC-8653 in hypertensive rats (1) may be due to an inhibition of Ca entry into smooth muscle cells, possibly through voltage-dependent Ca-chan-
A slow development of the inhibitory actions of flunarizine, a Ca-antagonist, on Ca-action potentials has been reported in smooth muscle cells of the rabbit basilar artery (11). The present experiments revealed that in the rabbit mesenteric artery, the inhibitory actions of FRC-8653 on Ca influx appear very slowly compared with those of nicardipine. Such actions of FRC-8653 may not be specific to the rabbit mesenteric artery, because similar properties have been reported in the rat aorta (1) and superior mesenteric artery (2). It is possible that the inhibitory actions of such Ca-antagonists on Ca-channels is exerted from the inside of the cell membrane (12).

Ca-antagonists that are dihydropyridine derivatives do not seem to inhibit Ca channels at autonomic nerve terminals and, as a consequence, the release of transmitter is not modified by these agents (6). The absence of any inhibitory action of nifedipine and its derivatives on the e.j.p. has been reported in the rabbit mesenteric artery (4). The present experiments showed that the e.j.p. is inhibited by FRC-8653 but not by nicardipine. The inhibitory effects of FRC-8653 on the e.j.p. appeared rapidly and reversibly, in contrast to the slow onset and long-lasting actions of this drug on Ca-channels. These results suggest that the inhibition by FRC-8653 of the e.j.p. may not be directly related to its Ca-antagonistic actions.

The lack of any detectable action on the resting membrane potential of vascular smooth muscle is one of the properties of the dihydropyridine Ca-antagonists (4), and FRC-8653 also had such a property. Our finding that FRC-8653 did not change the electrotonic potential or the time constant of the falling phase of the e.j.p. indicates that this drug does not produce any change in the ionic conductance of the membrane.

It would be of interest to know the sites at which FRC-8653 acts to inhibit the e.j.p.s. The present experiments revealed that the inhibition of the e.j.p. is not prejunctional, since the facilitation process of the e.j.p.s elicited by repetitive nerve stimulation remained unaltered despite the depressed amplitude of the e.j.p.s evoked in the presence of FRC-8653. Therefore, we suspect that FRC-8653 inhibited the e.j.p. by acting on the postjunctival events, possibly on receptors or receptor-linked cellular mechanisms related to the transmitter substances. The e.j.p. is not elicited by NA, but possibly by co-transmitter substances released together with NA, and one of the candidates is ATP (13). In our experiments, smooth muscle membranes were depolarized by exogenously applied NA and ATP, and FRC-8653 inhibited the depolarization produced by the latter but not by the former. Thus, our results could be interpreted to indicate that FRC-8653 inhibits the actions of ATP on the muscle membrane, but does not affect those of NA. Detailed comparison of the actions of FRC-8653 on the e.j.p. and on the ATP-responses would be required.

A contraction of arterial smooth muscle produced by perivascular nerve stimulation consists of three components, viz. a depolarization produced by the e.j.p., an action potential triggered on the e.j.p., and an activation of α-adrenoceptors by NA (7). The present experiments showed that FRC-8653 inhibits the former two components. The contractions elicited by these two components are estimated to make up about one-third of the nerve-mediated contractions (7), and therefore the inhibition by FRC-8653 of these components might be involved in the hypotensive actions of this drug.

Endothelial cells produce vasodilator substances such as the endothelium-derived relaxing factor (EDRF, 14) or hyperpolarizing factors (EDHF, 15) or prostacyclin (16); and under physiological conditions, a sustained release of EDRF by chemical and physical stimuli has been considered to occur (17, 18). In contractions produced by high [K+]o solution, the ACh-induced endothelium-dependent relaxation is mainly produced by EDRF (19); and in the rabbit mesenteric artery, FRC-8653 did not alter this relaxation. Thus, the vasodilation induced by FRC-8653 in this artery may
not be due to an enhanced release of EDRF. The absence of any change in the membrane potential in the presence of FRC-8653 suggests that EDHF may not be involved in the vasodilation produced by this drug.

In summary, in the rabbit mesenteric artery, FRC-8653 inhibits voltage-dependent Ca-channels and also the actions of transmitter substances that elicit the e.j.p. (possibly ATP). The former action appears slowly, while the latter is fast and reversible. Both of these actions could induce vasodilation and therefore may contribute to the hypotensive actions of FRC-8653.

Acknowledgments

We thank Dr. R.J. Timms for Language-Editing. FRC-8654 was kindly provided to us from Fujirebio, Inc.

REFERENCES

1 Iida, H., Fujiyoshi, T., Ikeda, K., Hosono, M., Yamura, T., Kase, N., Sekine, A. and Uematsu, T.: Antihypertensive and cardiovascular effects of a new dihydropyridine derivative, 2-methoxyethyl (E)-3-phenyl-2-propen-1-yI-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate (FRC-8653). Japan J. Pharmacol. 43, Supp. 296P (1987)
2 Yoshimoto, R., Dohmoto, H., Yamada, K. and Goto, A.: Prolonged inhibition of vasodilation and calcium influx by novel 1,4-dihydropyridine calcium antagonist FRC-8653. Japan. J. Pharmacol. 56, 225-229 (1991)
3 Fleckenstein, A.: Calcium Antagonism in Heart and Smooth Muscle. John Willey and Sons, New York (1983)
4 Makita, Y., Kannura, Y., Itoh, T., Suzuki, H. and Kuriyama, H.: Effects of nifedipine derivatives on smooth muscle cells and neuromuscular transmission in the rabbit mesenteric artery. Naunyn Schmiedebergs Arch. Pharmacol. 342, 302-312 (1983)
5 Kuriyama, H., Ito, Y., Suzuki H., Kitamura, K. and Itoh, T.: Factors modifying contraction-relaxation cycle in vascular smooth muscles. Am. J. Physiol. 243, H641-H662 (1982)
6 Godfraind, T., Miller, R. and WiJo, M.: Calcium antagonism and calcium entry blockade. Pharmacol. Rev. 38, 321-420 (1986)
7 Suzuki, H. and Kou, K.: Electrical components contributing to the nerve-mediated contractions in smooth muscles of the rabbit ear artery. Japan. J. Physiol. 33, 745-758 (1983)
8 Chen, G. and Suzuki, H.: Direct and indirect actions of acetylcholine and histamine on intrapulmonary artery and vein muscles of the rat. Japan. J. Physiol. 39, 51-65 (1989)
9 Itoh, T., Kuriyama, H. and Suzuki, H.: Excitation-contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. J. Physiol. (Lond.) 321, 513-535 (1981)
10 Oike, M., Inoue, Y., Kitamura, K. and Kuriyama, H.: Dual action of FRC-8653, a novel dihydropyridine derivative, on the Ba""'-current recorded from the rabbit basilar artery. Circ. Res. 67, 930-1006 (1990)
11 Nagao, T., Suzuki, H. and Kuriyama, H.: Effects of flunarizine on smooth muscle cells and on neuromuscular transmission in the rabbit basilar and ear arteries. Naunyn Schmiedebergs Arch. Pharmacol. 333, 431-438 (1986)
12 Pang, D.C. and Sperelakis, N.: Nifedipine, dihydrazem, bepridil, and verapamil uptakes into cardiac and smooth muscles. Eur. J. Pharmacol. 87, 199-207 (1986)
13 Burnstock, G. and Kennedy, C.: A dual function for adenosine 5'-triphosphate in the regulation of vascular tone. Circ. Res. 58, 319-330 (1986)
14 Furchgott, R.F. and Zawadzki, J.V.: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288, 373-376 (1980)
15 Suzuki, H. and Chen, G.: Endothelium-derived hyperpolarizing factor (EDHF): an endogenous potassium channel activator. NIPS 5, 212-215 (1990)
16 Moncada, S.: Biological importance of prostacyclin. Br. J. Pharmacol. 76, 3-31 (1982)
17 Furchgott, R.F.: Role of endothelium in responses of vascular smooth muscle. Circ. Res. 53, 557-573 (1983)
18 Vanhoutte, P.M., Rubanyi, G.M., Miller, V.M. and Houston, D.S.: Modulation of vascular smooth muscle contraction by the endothelium. Annu. Rev. Pharmacol. Toxicol. 28, 307-320 (1988)
19 Suzuki, H., Chen, G. and Hashitani, H.: Electrophysiological properties of acetylcholine-induced hyperpolarization in arterial smooth muscles. In Endothelium-Derived Relaxing Factors, Edited by Rubanyi, G.M. and Vanhoutte, P.M., p. 166-173, Karger, Basel (1990)