Br. J. Pharmac (1987), **92**, 615–625

Action of the 1,4-dihydropyridine derivative, KW-3049, on the smooth muscle membrane of the rabbit mesenteric artery

Kazumasa Terada, Kazuhisa Nakao, Kohji Okabe, [1]Kenji Kitamura & Hirosi Kuriyama

Department of Pharmacology, Faculty of Medicine, Kyushu University, 60, Fukuoka 812, Japan

1 The effect of 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid methyl 1-(phenylmethyl)-3-piperidinyl ester hydrochloride (KW-3049) on the smooth muscle membrane of the rabbit mesenteric artery was investigated using microelectrode and single electrode voltage clamp methods.

2 In whole tissue preparations KW-3049 did not alter the resting membrane potential of the artery, but did inhibit the action potential evoked by a depolarizing current in the presence of 10 mM tetraethylammonium (TEA).

3 Using the voltage clamp technique, the effects of KW-3049 on the inward current evoked in solution containing 100 mM $BaCl_2$ were observed. When the membrane was held at −60 mV, KW-3049 inhibited the inward current in a concentration-dependent manner. The inward current evoked by a larger depolarizing pulse was inhibited to a larger extent than that evoked by a smaller one.

4 When the membrane was held at −80 mV, the inward current evoked at test potentials of −10 and 0 mV was enhanced by low concentrations of KW-3049 (below 100 nM).

5 KW-3049 accelerated the rate of inactivation of the inward current and shifted the voltage-dependent inactivation curve to the left.

6 KW-3049 has a long-lasting inhibitory action on smooth muscle cells, since the inhibition of the inward current persisted for over 1 h after the removal of KW-3049.

7 Our results suggest that KW-3049 has a selective and long-lasting action on the Ca channels of the smooth muscle cell membrane of the rabbit mesenteric artery. This agent has both facilitatory and inhibitory actions on the Ca channel, depending on the values of the holding and command potentials.

Introduction

KW-3049, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid methyl 1-(phenylmethyl)-3-piperidinyl ester hydrochloride, is a newly synthesized dihydropyridine derivative which seems to have anti-hypertensive and anti-anginal actions (Karasawa *et al.*, 1985; Kubo *et al.*, 1985; Yoshitake *et al.*, 1986). In cardiac cells of the rabbit and guinea-pig, Yoshitake *et al.* (1986) found that KW-3049 reduced the excitability of cardiac cells with a slightly weaker potency than that of nifedipine and had a potent and selective inhibitory action on the slow Ca channels in cardiac muscle. No information was obtained on the action of KW-3049 on the properties of vascular smooth muscle cells.

Terada *et al.* (1987a,b,d) investigated the effects of several Ca antagonists, including nicardipine, a water soluble and light insensitive dihydropyridine derivative, on membrane currents of the rabbit intestinal smooth muscle cells. They concluded that nicardipine inhibits the Ca current in a voltage-dependent manner, and possesses a highly selective inhibitory action on the Ca current, in comparison to other Ca antagonists, such as verapamil, diltiazem or flunarizine.

The present experiments were designed to investigate the actions of KW-3049 on arterial smooth muscle cells using microelectrode recording in whole tissue and voltage clamp methods in single isolated cells. Part of this work was presented at the 60th

[1] Author for correspondence.

general meeting of Japanese Pharmacological Society (Terada *et al.*, 1987c).

Preparations for microelectrode and voltage clamp experiments

Albino rabbits (Nippon White; 1.8–2.2 kg) were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.v.) and exsanguinated. Second and third branches of the superior mesenteric artery were isolated using fine forceps and a binocular microscope. For the microelectrode experiments, the artery (3 cm long) was pinned on a rubber plate in a chamber (1.5 ml in volume) and perfused with a modified Krebs solution (35°C). For voltage clamp experiments, cells were enzymatically dispersed using procedures similar to those described by Okabe *et al.* (1987). Briefly, blood in the mesenteric artery was flushed out with fresh physiological salt solution (PSS). The isolated artery (3 cm in length) was cut into small pieces (3–4 mm in length) after the adventitial connective tissue had been carefully removed. These preparations were then transferred into a plastic tube (2 ml in volume) and incubated in Ca^{2+}-free PSS containing 0.3% collagenase (clostridio peptidase A; Boehringer-Mannheim GmbH, FRG) for 60–70 min at 36°C. After completion of digestion, single cells were dispersed by gentle agitation using a blunt-tipped glass pipette. Cells were resuspended in fresh PSS and stored at 10°C. The diameter of dispersed single smooth muscle cells was 10–15 μm and the length 100–150 μm. All voltage clamp experiments were carried out within 1 h after cell harvest at a room temperature of 22–25°C.

Electrical recording

Microelectrode experiments Electrical responses of the membrane were recorded following impalement of the microelectrode (40–60 MΩ) filled with 3 M KCl and the responses were monitored with a high gain oscilloscope and a conventional ink-writing pen recorder (VC-9 and RJG-4024; Nihon Kohden Co., Tokyo, Japan). Electrotonic potentials were produced using the partition chamber stimulating method, described by Abe & Tomita (1968), to estimate the relative change in the membrane resistance of the longitudinal axis of the artery.

Voltage clamp experiments Whole cell voltage clamp recording was carried out with a suction electrode (2–5 MΩ) using a single voltage clamp amplifier (Brennecke & Lindemann, 1974; Finkel & Redman, 1985) as described by Ohya *et al.* (1986). The switching frequency was fixed at 10 kHz, and duty cycle was set at 0.05. A 100 MΩ resistor was used in the voltage-current converter. The output of the maximal current was 2.5 nA, since the voltage source for the passing current was 5 V. The cells were placed in the chamber (0.2 ml) on the stage of an inverted microscope (TMD-Diaphoto, Nihon Kogaku Co., Tokyo, Japan). The electrode was made from a Pyrex glass capillary, using a patch electrode puller and a heat polisher (PP-83 and MF-83, Narishige Sci. Inst. Lab., Tokyo, Japan). After preparing a high resistance seal (5–10 GΩ), the patch membrane was disrupted by negative pressure (10–20 mmH_2O) as described by Hamill *et al.* (1981). Command pulses were applied through the voltage clamp amplifier and the evoked membrane currents were monitored by a high gain digital storage oscilloscope (VC-10, Nihon Kohden Co., Tokyo, Japan). Electrical responses were stored on video cassette tape through a PCM data recording system (NV-785H, National, Tokyo, Japan and PCM-501ES, SONY, Tokyo, Japan) and hard copies were obtained using a conventional thermo-writing recticoder (RJG-4124, Nihon Kohden Co., Tokyo, Japan) or an X-Y plotter (HP-7440, Hewlett-Packard CTo., San Diego, CA, U.S.A.).

Solutions and drugs

For microelectrode experiments modified Krebs solution of the following ionic composition was used (mM): Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 134.0, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, glucose 11.5. The solution was aerated with 97% O_2 plus 3% CO_2 and the pH was kept at 7.2–7.3. For the voltage clamp experiments the following ionic compositions were used in the bath (PSS and Ba^{2+} solutions) and pipette (high-K^+ and Cs^+ solutions), respectively. PSS (mM): Na^+ 134.1, K^+ 6.2, Ca^{2+} 2.5, Cl^- 145.9, glucose 12.1. Ba^{2+} solution (mM): Ba^{2+} 100, Cl^- 200, glucose 12.1. High-K^+ solution (mM): K^+ 145, Na^+ 10, Mg^{2+} 5, Cl^- 155, ATP^{2-} (Kohjin Co. Tokyo, Japan) 5, ethylene glycol *bis* (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA; Dojin Kagaku, Kumamoto, Japan) 4. Cs^+ solution (mM); Cs^+ 142.2, Na^+ 10, Mg^{2+} 5, Cl^- 152.2, ATP^{2-} 5, EGTA 10. The pH of the solutions

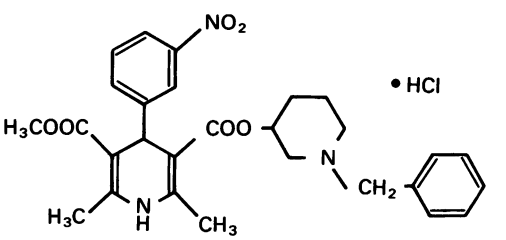

Figure 1 Chemical structure of KW-3049, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid methyl 1-(phenylmethyl)-3-piperidinyl ester hydrochloride. The molecular weight is 542.03.

was kept at 7.3 by 10 mM N-(2-hydroxyethyl)-1-piperazine-N'-2-ethansulphonic acid (HEPES; Dojin Kagaku, Kumamoto, Japan) titrated with tris-hydroxyaminomethane.

The following drugs were used: KW-3049 (Kyowa Hakko Kogyo Co., Tokyo, Japan) and TEA (Tokyo Kasei, Tokyo, Japan). Collagenase (Boehringer-Manheim GmbH, FRG) was used for cell dispersion. Ten mM KW-3049 in 0.1 N HCl was prepared and diluted with PSS to the final concentration given in text. The chemical structure of KW-3049 is shown in Figure 1.

Statistical significances were determined by use of Student's t test and probabilities of less than 5% ($P < 0.05$) were considered to be significant.

Results

Effects of KW-3049 on the membrane potential and action potential

The resting membrane potential of smooth muscle cells of the rabbit mesenteric artery was -68.9 ± 2.9 mV ($n = 45$, 6 preparations) and spontaneous fluctuation was not observed. Application of KW-3049, in concentrations of up to 1 μM, did not alter the resting membrane potential (1 nM, -69.8 ± 2.5, $n = 15$; 10 nM, -69.0 ± 2.7, $n = 16$; 100 nM, -69.5 ± 2.6, $n = 12$; 1 μM, -69.1 ± 2.7, $n = 15$, 6 preparations). Figure 2 shows the effects of KW-3049 on the electrotonic potential and action potential evoked respectively by hyperpolarizing and depolarizing currents (a), and the current-voltage relationship is illustrated in Figure 2b. To provoke the action potential by outward current (a), 10 mM TEA was added to the bathing solution. TEA depolarized the membrane to -60 ± 2.8 mV ($n = 30$, 7 preparations) and there was an increase in the amplitude of electrotonic potential. With the addition of 100 nM KW-3049 to the bathing solution, neither the resting membrane potential (-59 ± 2.6 mV, $n = 18$, 5 preparations) nor the amplitude of the electrotonic potential evoked by the inward current pulse was affected, but there was inhibition of the evoked action potential (Figure 2a). One hundred nM KW-3049 had no effect on the passive membrane electrical properties (Figure 2b).

Effects of KW-3049 on the inward current induced in the Ba^{2+} solution

To investigate the effect of KW-3049 on the inward current in smooth muscle cells of the rabbit mesenteric artery, voltage clamp experiments were done. When the pipette contained high-K^+ solution and when the bathing solution was normal PSS, depolarizing command pulses to -10–$+50$ mV from the holding

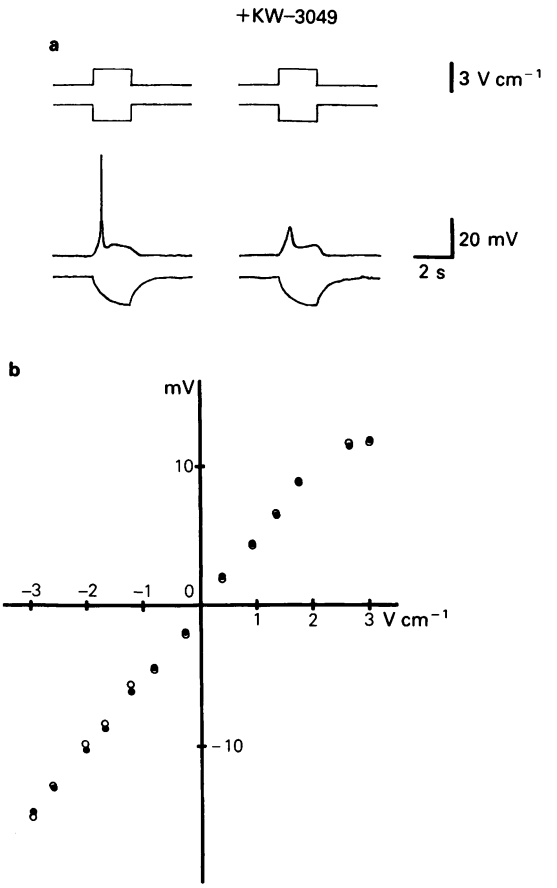

Figure 2 Effect of 100 nM KW-3049 on electrical responses (a) and the current-voltage relationship (b) evoked by depolarizing or hyperpolarizing current in whole tissue using a microelectrode. (a) Examples of the electrical responses evoked by depolarizing or hyperpolarizing current (± 1.5 V cm^{-1} intensity; 2 s in duration) before and during application of KW-3049 100 nM in the presence of 10 mM tetraethylammonium (TEA) and tetrodotoxin 0.3 μM. Traces obtained in the presence of 100 nM KW-3049 were recorded 10 min after application of KW-3049. These traces were all from the same cell. Resting membrane potential was −60 mV. (b) The current-voltage relationship observed before (●) and during (○) application of KW-3049 100 nM. The resting membrane potential of this cell was −70 mV in the absence and presence of 100 nM KW-3049. The current-voltage curve was obtained by applications of various intensities of inward and outward current pulses. KW-3049 was applied 10 min before any measurements were recorded. The microelectrode was inserted into the cell within 100 μm from the stimulating electrode.

potential of −60 mV produced only an outward current (Figure 3a). To block the generation of the outward current, the high-K^+ solution in the pipette was replaced with a solution containing isotonic CsCl.

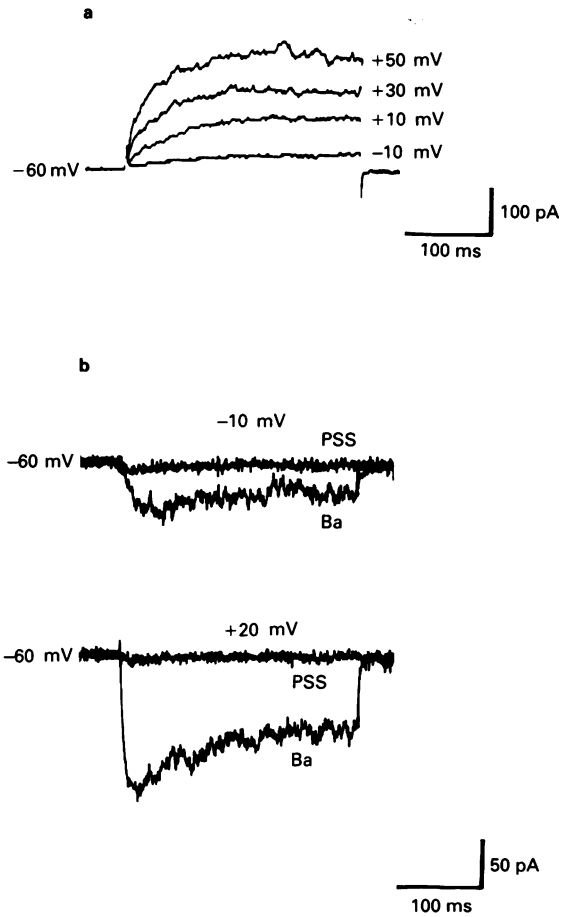

Figure 3 Membrane currents in smooth muscle cells of the rabbit mesenteric artery observed under various ionic conditions. (a) Membrane currents evoked by various amplitudes of depolarizing pulses (command potentials of −10, +10, +30 and +50 mV; 300 ms in duration) from a holding potential of −60 mV. PSS was superfused in the bath and high-K^+ solution in the pipette. (b) Examples of inward currents obtained by depolarizing command pulses in the pipette solutions containing Cs^+. PSS (2.5 mM Ca^{2+}) and the Ba^{2+} (100 mM) solution were superfused in the bath and the recorded inward currents were superimposed. Inward currents evoked by two different command pulses (−10 and +20 mV; 300 ms in duration) from the holding potential of −60 mV are shown. Capacitative and leak currents of all traces were subtracted.

With normal PSS in the bath, a small inward current was evoked by depolarizing pulses to −10 or +20 mV from the holding potential of −60 mV (Figure 3b). When 2.5 mM Ca^{2+} in PSS was replaced with 2.5 mM Ba^{2+}, the amplitude of the inward current increased slightly (data not shown), but there was a marked increase with a 100 mM Ba^{2+} solution (Figure 3b). Hence, hereafter, the effects of KW-3049 on the inward current were investigated in the presence of 100 mM Ba^{2+} in the bath.

In 100 mM Ba^{2+} solution at a holding potential of −60 mV an inward current was evoked by command potentials more positive than −20 mV. The peak inward current was observed at about +30 mV and a full current-voltage curve was obtained (Figure 4a). Application of 30 nM KW-3049 inhibited the inward currents evoked by any test potential (Figure 4a). However, inward currents evoked by large command pulses were inhibited to a greater extent than those evoked by smaller command pulses. Figure 4b shows the relationship between the command potential and the inhibition of the peak inward current induced by 30 nM KW-3049. In Figure 4b the relative amplitude of the peak inward current was calculated by dividing the amplitude of the inward current in the presence of KW-3049 (I_{drug}) by the amplitude of the current in the absence of KW-3049 (I_{cont}). KW-3049 appeared to inhibit the inward current in a voltage-dependent manner (Figure 4b). The ratio of the inward current in the absence and presence of 30 nM KW-3049 (I_{drug}/I_{cont}), decreased as the test potential was made more positive (Figure 4b). Figure 4c demonstrates the inward currents evoked at test potentials of 0, +20 and +40 mV from the holding potential of −60 mV in the absence and presence of 30 nM KW-3049. KW-3049 inhibited both the peak amplitude and the amplitude observed at the end of the depolarizing pulse (300 ms). However, the amplitude of the inward current at the end of the pulse was inhibited to a greater extent than the initial peak amplitude of the inward current (Table 1A).

A similar experiment was performed at a holding potential of −80 mV (Figure 5). The inward current was generated by depolarizing steps to potentials more positive than −20 mV, and the maximum amplitude of the inward current was evoked by a command pulse to +30 mV. KW-3049 inhibited the inward current evoked at potentials more positive than +10 mV. However, at test potentials of 0 and −10 mV, 100 nM KW-3049 enhanced the amplitude of the inward current. The maximum amplitude of the inward current in the presence of KW-3049 was recorded at +10 mV. Figure 5b shows examples of the effects of 100 nM KW-3049 on the inward current evoked by three different depolarizing pulses from the holding potential of −80 mV. Enhancement of the amplitude of the peak inward current (at the start of the pulse)

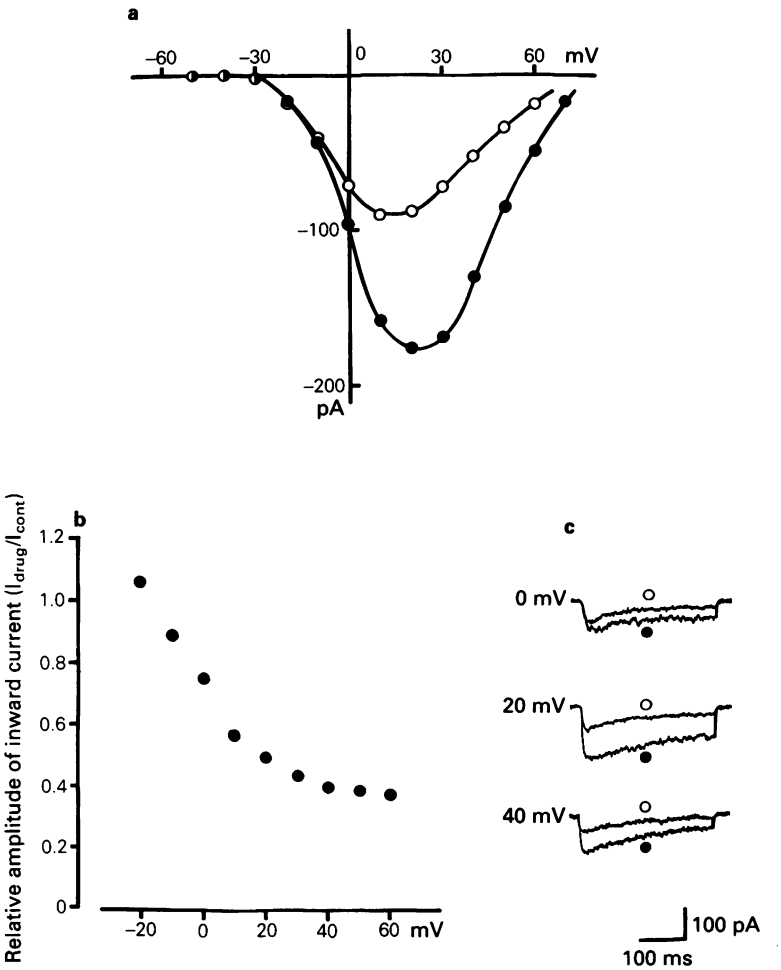

Figure 4 Current-voltage relationship obtained before (●) and after (○) application of 30 nM KW-3049 (a) and the relationship between relative inhibition of the inward current induced by KW-3049 and the command potential (b). (a) Peak amplitude of the inward current was measured. The holding potential was −60 mV. In (b) amplitudes of the peak inward current evoked at any given command potential in the absence of KW-3049 were normalized as 1.0 and those observed at the same amplitude of the command pulse in the presence of 30 nM KW-3049 were expressed in a relative manner. (c) Traces in the absence (●) and presence (○) of 30 nM KW-3049 evoked at test potentials of 0, +20, +40 mV are superimposed. Capacitative and leak currents were subtracted.

occurred at the test potential of 0 mV. With larger command pulses of +20 and +40 mV, the amplitude of the peak inward current was inhibited. On the other hand, the amplitude of the inward current at the end of the test pulse was consistently inhibited. Table 1B shows the relative amplitude of the inward current calculated by dividing the amplitude of the inward current measured at the end of the pulse (I_{end}) by the amplitude of the current at the peak (I_{peak}), in the absence and presence of KW-3049 (100 nM), respectively. The values in the presence of KW-3049 were consistently smaller than those in the absence of drug, suggesting that the decay of the inward current was accelerated byKW-3049, as observed at a holding potential of −60 mV.

Figure 6 shows the effects of various concentrations

Table 1 Effects of KW-3049, 30 (A) and 100 (B) nM, on the ratios of inward currents

		I_{drug}/I_{cont}		
A		*0 mV*	*+20 MV*	*+40 mV*
	Peak	0.74 ± 0.13 ($n = 4$)	0.47 ± 0.12 ($n = 4$)	0.35 ± 0.08 ($n = 4$)
	End	0.34 ± 0.16** ($n = 4$)	0.21 ± 0.10* ($n = 4$)	0.20 ± 0.11 ($n = 4$)
		I_{end}/I_{peak}		
B	Control	0.80 ± 0.10 ($n = 4$)	0.68 ± 0.10 ($n = 4$)	0.56 ± 0.06 ($n = 4$)
	Drug	0.37 ± 0.05** ($n = 4$)	0.32 ± 0.06** ($n = 4$)	0.27 ± 0.06** ($n = 4$)

(A) Ratio of the inward current measured in the absence or presence of KW-3049 (I_{drug}/I_{cont}) at the peak and end of the depolarizing pulse (300 ms) at a holding potential of −60 mV. (B) Ratio of the inward currents measured at peak and end of the depolarizing pulse (I_{end}/I_{peak}) was compared in the absence and presence of KW-3049 at a holding potential of −80 mV (*$P < 0.05$, **$P < 0.01$).

of KW-3049 on the amplitude of the peak inward current evoked at three different command potentials (0, +20 and +40 mV) from holding potentials of −60 (a) or −80 mV (b). When the membrane potential was hold at −60 mV, KW-3049 inhibited the peak amplitude of the inward current in a concentration-dependent manner (Figure 6a). The concentration which inhibited the inward current evoked by a depolarization to 0 mV to half of the control (ID_{50}) was 100 nM (Figure 6a). When the inward current was

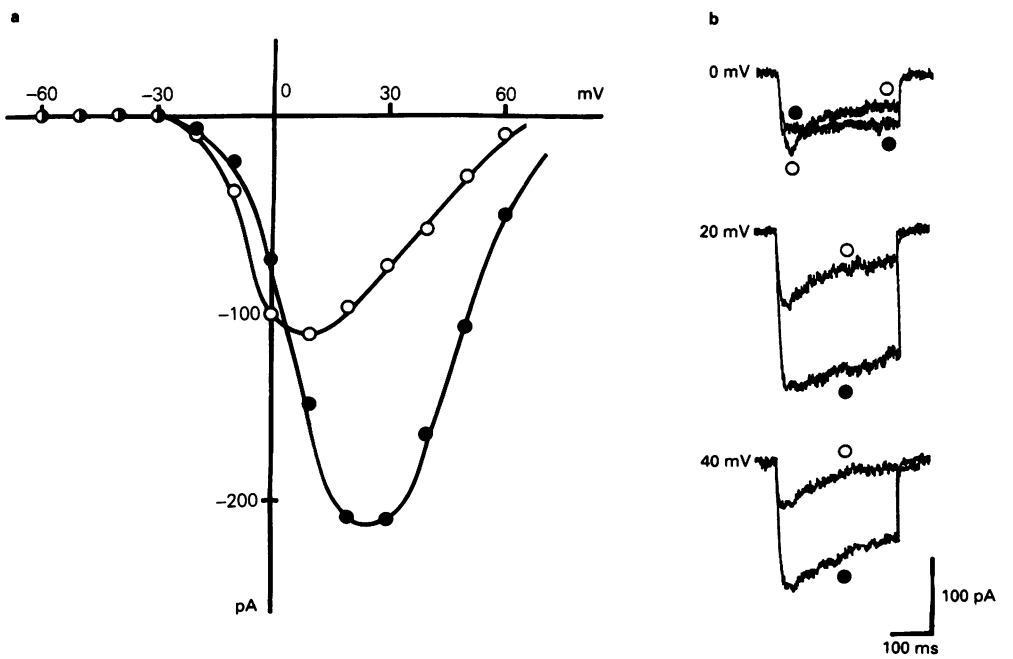

Figure 5 Effects of KW-3049 on the inward current obtained at a holding potential of −80 mV. (a) Current-voltage relationship in the absence (●) and presence (○) of 100 nM KW-3049. Peak amplitude of the inward current was measured. (b) Traces of the inward current evoked at three command potentials (0, +20 and +40 mV) in the absence (●) and presence (○) of 100 nM KW-3049. All traces were obtained from the same cell. Capacitative and leak currents were subtracted.

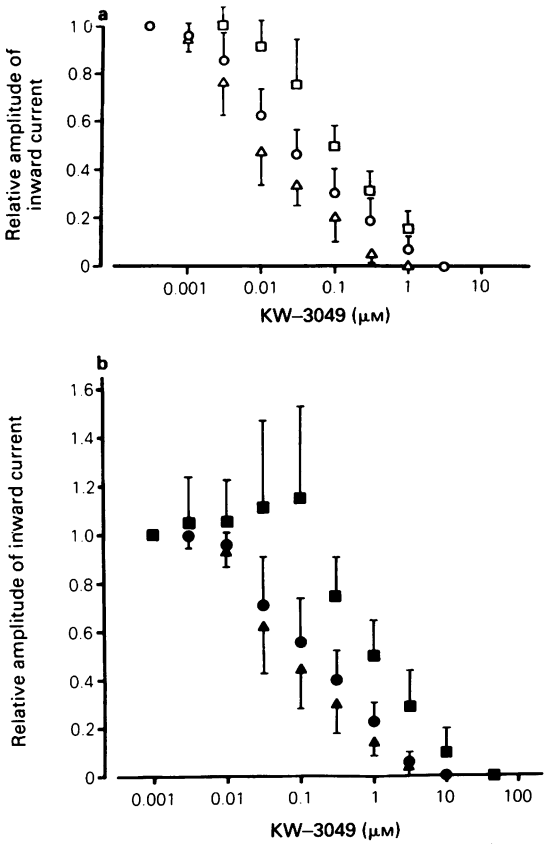

Figure 6 Effect of various concentrations of KW-3049 on the relative amplitude of the peak current evoked at three different command potentials (0, +20 and +40 mV) from the holding potentials of −60 (a) and −80 (b) mV. (□, ■) 0 mV, (○, ●) +20 mV and (△, ▲) +40 mV pulses. Symbols represent mean (n = 5–9) and vertical lines indicate s.d.

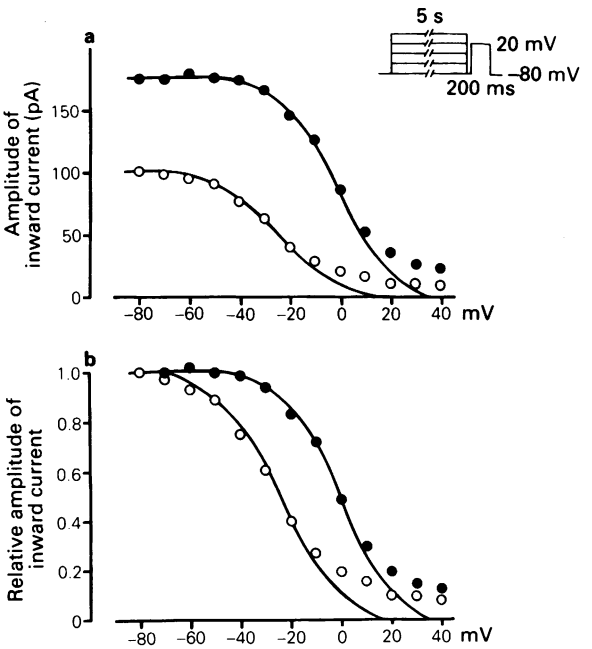

Figure 7 Voltage-dependent inhibition of the inward current obtained by applying two depolarizing pulses. The test pulse, stepped to +20 mV (200 ms), was applied 20 ms after cessation of the conditioning pulse (5 s). The holding potential was −80 mV. Absolute (a) and relative (b) values of amplitude of the inward current were plotted against the value of the membrane potential during the conditioning pulse in the absence (●) and presence (○) of 100 nM KW-3049. (b) The amplitude of the inward current evoked in the absence and presence of KW-3049 with the conditioning pulse of −80 mV was normalized as 1.0. The continuous lines were drawn according to the Boltzmann distribution of the following equation: $I_V/I_{max} = (1+\exp(V-V_h)/k)^{-1}$. For the control, V_h = 0 mV, k = 8.5. with KW-3049 (100 nM), V_h = −23 mV, k = 8.6. The inset illustrates the experimental protocol.

evoked by a greater depolarizing pulse (+20 or +40 mV), application of KW-3049 led to a greater inhibition than that seen with a smaller depolarizing pulse (0 mV) (Figure 6a); the ID_{50} value for KW-3049 measured at +40 mV was 10 nM. When the membrane potential was held at −80 mV, higher concentrations of KW-3049 were required to inhibit the peak amplitude of the inward current to the same extent as that observed with the holding potential at −60 mV. With the application of a low concentration of KW-3049 (30–100 nM), the peak inward current evoked by the depolarizing pulse to 0 mV was slightly enhanced (Figure 6b). However, the amplitude of the inward current evoked by a larger depolarizing pulse (+20 or +40 mV) was not increased with the application of any concentration of KW-3049 (Figure 6b). The ID_{50} values of KW-3049 measured at 0 and +40 mV at the holding potential of −80 mV were 1 μM and 50 nM, respectively (n = 5–9).

To investigate the effects of KW-3409 on the voltage-dependent inactivation of the inward current, various amplitudes of a long conditioning pulse (5 s) were applied before application of a 200 ms test pulse of +20 mV. The relationship between the amplitude of the peak inward current evoked by the test pulse and the membrane potential of the conditioning pulse in the absence and presence of 100 nM KW-3049 are shown in Figure 7. In the control, inhibition of the

peak inward current was seen with application of a conditioning pulse of −30 mV. When the membrane potential was set at 0 mV during the conditioning pulse, the amplitude of the inward current to the test pulse was reduced to half that of the control. On the other hand, in the presence of 100 nM KW-3049, a conditioning pulse of −50 mV was sufficient to inhibit the amplitude of the inward current evoked by the test pulse. In Figure 7b the amplitude of the inward current evoked by the test pulse in the absence and presence of KW-3049 was plotted, in a relative manner, by normalizing the amplitude of the inward current so that when the conditioning pulse was −80 mV the relative amplitude was 1.0. It can be seen that the voltage-dependent inhibition curve was shifted to more negative potentials in the presence of 100 nM KW-3049 (Figure 7b). The conditioning pulse required to inhibit the peak inward current by half was −23 mV in 100 nM KW-3049.

Recovery of the inward current following washout of 100 nM KW-3049 was investigated. At a holding potential of −60 mV, there appeared to be no recovery of the inward current from inhibition after removal of 100 nM KW-3049 from the bath even after 1 h. To determine whether the recovery of the amplitude of the inward current from the inhibition of KW-3049 depended on the holding potential, depolarizing pulses of +20 mV were applied every 20 s (100 ms in duration) from the holding potential of −80 mV. Under these conditions, the amplitude of the inward current remained unchanged in the absence of KW-3049 for 60 min. Figure 8 shows a typical result observed after the application of 100 nM KW-3049. Three min after the application of KW-3049, the amplitude of the inward current was inhibited to 0.6 times control. Ten min after the application of KW-3049, the inward current was inhibited by half. Removal of KW-3049 from the bath did not restore the amplitude of the inward current for over 20 min, but there was a slow recovery to a limited level, i.e., 47 min after removal of KW-3049, the amplitude was 0.6 times that of the initial control value. As shown in Figure 8A (b–d), the peak amplitude of the inward current was not restored 20 min after the removal of KW-3049, but the increased decay of the inward current during the pulse induced by the drug appeared to be reversed.

Discussion

The main finding of the present experiments was that KW-3049 inhibited the generation of the action potential and the inward current evoked in smooth muscle cells of the rabbit mesenteric artery. The degree of inhibition appeared to be potential-dependent and subsequent recovery of the amplitude of the inward current following removal of KW-3049 from the bath was slow.

In whole tissue preparations of the rabbit mesenteric artery, depolarization of the membrane evoked by current injection did not generate an action potential. However, after suppression of the K current with TEA (10 mM), a graded response or action potential was evoked (Kanmura *et al.*, 1983 and the present experiments). With voltage clamp procedures and using a high-K^+ solution in the recording pipette depolarizing steps did not evoke an inward current. A small inward current was obtained after total replacement of the high-K^+ pipette solution with Cs^+ solution. These results support the findings obtained using the microelectrode method. Inclusion of 2.5 mM Ba^{2+} solution in the bath increased the amplitude of the inward current, but this amplitude was too small to analyse the inhibitory action of KW-3049. Therefore, in the present experiments a 100 mM Ba^{2+} solution was used in the bath to obtain an inward current of adequate amplitude.

KW-3049 (1 nM–1 μM) neither modified the membrane resistance, estimated from the amplitude of the electrotonic potential, nor the resting membrane potential. One hundred nM KW-3049 did block the action potential evoked by depolarization in the presence of 10 mM TEA in whole tissue and the evoked inward current in isolated cells when 100 mM $BaCl_2$ was used in the bath solution and isotonic CsCl in the pipette solution. While the effects of KW-3049 on the outward current were not investigated, preliminary results indicated that 1 μM KW-3049 slightly reduced the amplitude of the outward current (unpublished observations). These results suggest that KW-3049 selectively inhibits the voltage-dependent inward current, as observed in smooth muscle cells using other dihydropyridine derivatives. In smooth muscle cells of the longitudinal muscle layer of the rabbit ileum, high selectivity of nicardipine, a dihydropyridine derivative, against the inward current rather than the outward current was noted, in comparison to other Ca antagonists, namely verapamil, diltiazem or flunarizine (Terada *et al.*, 1987b,d). The highly selective inhibitory action of the dihydropyridine derivatives was also evident in cardiac cells (Hume, 1985).

In cardiac muscle cells, nitrendipine enhanced the inward current when the membrane was held at potential levels hyperpolarized to −80 mV (Brown *et al.*, 1986), and it is well known that Bay K 8644 augments the amplitude of the inward current in skeletal, cardiac and smooth muscle cells (Brown *et al.*, 1984; 1986; Affolter & Coronado, 1985; Sanguinetti *et al.*, 1986; Reuter *et al.*, 1986; Bean *et al.*, 1986). In the present experiments, facilitatory actions of KW-3049 were observed at concentrations below 100 nM at a holding potential of −80 mV (which was lower than the resting membrane potential of about −70 mV in

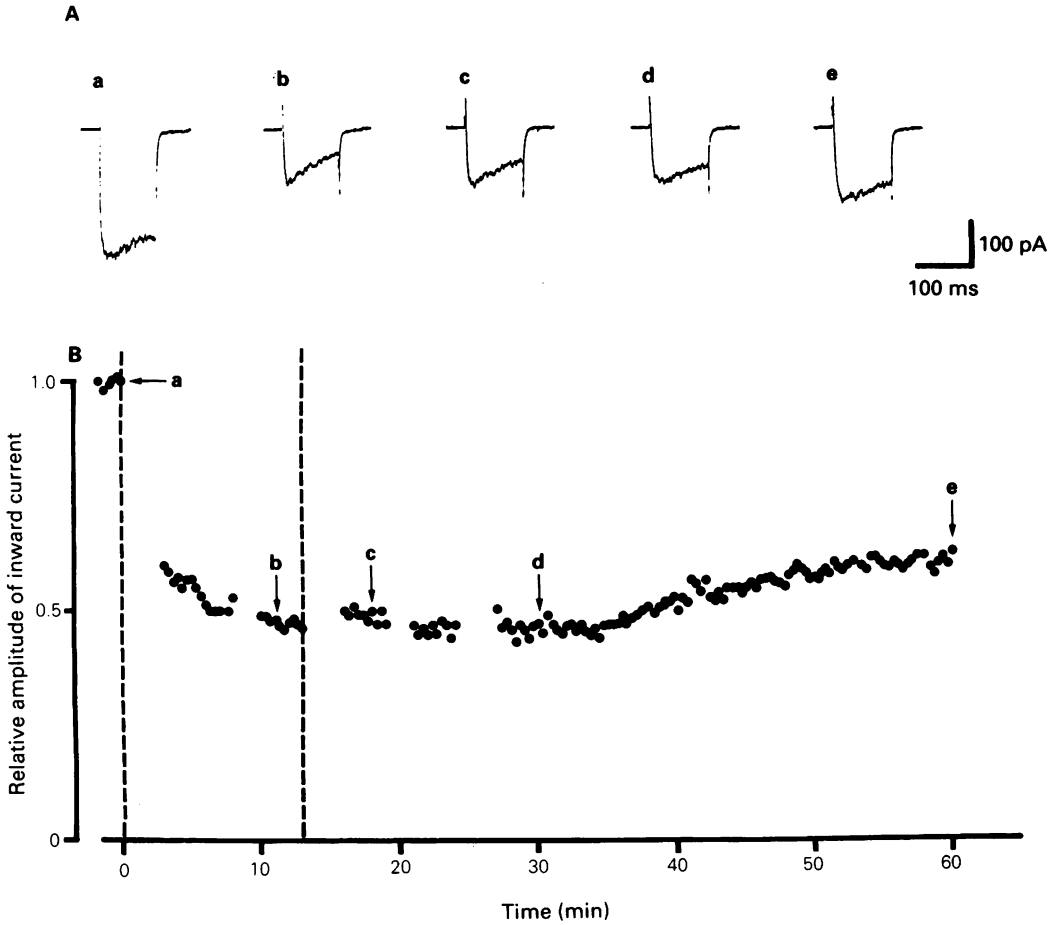

Figure 8 Time course of recovery of the inward current after removal of KW-3049.(A) Traces of the inward current observed before (control, a; 0 min), during (b; 11 min) and after (c, d, e; 18 min, 30 min, 60 min, respectively) application of 100 nM KW-3049. (B) Relative amplitude of the peak inward current was plotted against time. The amplitude of the peak inward current obtained just before application of KW-3049 was normalized as 1.0. Zero min indicates the time when KW-3049 was applied. KW-3049 was applied for 13 min (between two broken lines). Inward currents obtained at (a–e) are shown in (A). Command pulses to +20 mV from the holding potential of −80 mV (100 ms in duration) were applied every 20 s.

whole tissue, as determined using the microelectrode method). In microelectrode experiments, KW-3049 (1–100 nM) did not enhance the amplitude of the action potential evoked by the depolarizing current in the presence of 10 mM TEA. One reason for the lack of augmentation of the action potential may be due to the depolarization induced by TEA which was used for the study of action potentials in whole tissue.

KW-3049 shifted the voltage-dependent inactivation curve to the left. As KW-3049 enhanced the amplitude of the inward current at a negative holding potential (−80 mV), both the inhibitory and stimulatory actions of KW-3049 may be involved in the effect of KW-3049 on the voltage-dependent inactivation curve. However, the contribution of the facilitatory action of KW-3049 was probably weak because a relatively higher command potential (+20 mV) was used in these experiments. In intestinal smooth muscle cells, nicardipine and flunarizine shifted the voltage-dependent inactivation curve to the left when 5 s conditioning pulses were used (Terada *et al.*, 1987a,d). Similar shifts to the left of the voltage-dependent inactivation curve by dihydropyridine derivatives were also observed in cardiac cells (San-

guinetti & Kass, 1984; Uehara & Hume, 1985). Therefore, presumably this action of KW-3049 is one of the features of dihydropyridine derivatives.

KW-3049 accelerated the decay of the inward current observed in Ba^{2+} solution. Increase in the rate of the decay of the inward current in cardiac cells with nisoldipine was also noted when Ba^{2+} or Sr^{2+} was present in the bathing solution (Lee & Tsien, 1983; Sanguinetti & Kass, 1984). On the other hand, Brown *et al.* (1986) found that nitrendipine did not accelerate decay of the inward Ca current. Similarly, no significant effect on the decay of the inward current by nicardipine was seen in intestinal smooth muscle cells by Terada *et al.* (1987a). These differences may not be due to the drug moiety but rather to different solutions used to generate the inward current, since KW-3049 did not modify the decay of the inward current evoked in solution containing Ca^{2+}. Moreover, nicardipine accelerated the decay of the inward Ba current but not the inward Ca current in the rabbit pulmonary artery (unpublished observations).

A slight increase in the amplitude of the inward current was observed 1 h after removal of KW-3049 when using a holding potential of −80 mV. However, restoration of the inward current did not appear immediately after the removal of KW-3049 but rather 20 min after removal of KW-3049 with a holding potential of −80 mV. Thus, the inhibitory action of KW-3049 lasts a long time.

In conclusion, KW-3049 inhibits the action potential without affecting the membrane potential or resistance of smooth muscle cells of the rabbit mesenteric artery and such actions of KW-3049 may be attributed to a selective inhibitory action on the inward current carried by divalent cations. These inhibitory actions of KW-3049 on the inward current appeared to depend on the membrane potential level.

The authors thank M. Ohara for reading the manuscript. KW-3049 was kindly provided by Kyowa Hakko Kogyo Co. This work was supported in part by grants in aid for scientific research from The Ministry of Education, Science and Culture, and The Ministry of Health and Welfare, Japan.

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(Received May 5, 1987.
Revised June 19, 1987.
Accepted June 30, 1987.)