Effect of UK-84149 on voltage-activated calcium currents of single smooth muscle cells from guinea-pig and rabbit jejunum and rabbit coronary artery

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1 Smooth muscle cells of the longitudinal muscle of the guinea-pig and the rabbit jejunum and rabbit coronary artery were dispersed by enzyme treatment and recordings of membrane current were made in the standard whole cell voltage-clamp mode and by perforated patch technique. The effect of UK-84149 on the voltage-dependent calcium inward current of single isolated smooth muscle was studied at room temperature.

2 When inward and outward currents were evoked by stepping to 0 mV or +10 mV from –80 mV every 20 s, UK-84149 reduced the voltage-dependent inward current in guinea-pig jejunum smooth muscle cells with a ID_{50} of 2.3 μ M, and also reduced voltage-dependent outward current with a ID_{50} of 7.2 μ M. The effect of UK-84149 was difficult to wash out.

3 The inactivation of the inward current was studied by holding at various potentials for 10 s before evoking inward current at a test potential of 0 mV. The voltage-inactivation curve was shifted to the left by 3 μ M UK-84149. The voltage at which current was 50% available (V_h) was changed from -37 ± 2 mV to -50 mV ± 2.2 ($n = 6$, means $\pm$ s.e.mean).

4 When inward current was evoked by stepping to 0 mV from –80 mV for 50 ms, every 20 s, nicardipine immediately, and verapamil and UK-84149 slowly, reduced the peak amplitude. Inhibition by the latter two drugs depended on the number of stimulations applied. So, like verapamil, UK-84149 had a use-dependent action.

5 The effect of UK-84149 on inward current from rabbit jejunum and coronary artery smooth muscle cells was compared with the effect of nicardipine and D600. When inward current was evoked by stepping to 0 mV from –80 mV every 20 s, UK-84149 inhibited inward currents from both types of smooth cell with an ID_{50} of 1.7 μ M for jejunum and an ID_{50} of 3.2 μ M for coronary artery. ID_{50} values of D600 were 8.1 μ M for jejunum and 3.9 μ M for coronary artery. ID_{50} values of nicardipine were 24 nM for jejunum and 12 nM for coronary artery.

6 The results of the present studies indicate that UK-84149 has a calcium-antagonist action that can explain its inhibitory action on bowel motility. Its bowel selectivity may arise due to its use-dependent, and persistent, action.

Keywords: UK-84149; nicardipine; D600; calcium channel; smooth muscle

Introduction

UK-84149 (S-5,11-dihydro-5-[1-(4-methoxyphenethyl)-2-pyrrolidinylmethyl]-dibenzo [b,e] [1,4] thiazepinemaleate), a newly developed calcium antagonist drug, showed a significant tissue selectivity for intestine smooth muscle in preliminary experiments (Quinn & Wallis, 1994; Wallis *et al.*, 1994). UK-84149 potently inhibited small and large bowel motility stimulated by cholestylin octapeptide, with cardiovascular effects being evident only at doses 10 times higher. This contrasted with calcium channel blockers such as nifedipine and diltiazem which produced dose-related cardiovascular effects over the same dose-range as those required to inhibit motility (Quinn & Wallis 1994). UK-84149 was able to inhibit calcium-induced contracture of depolarized guinea-pig ileum which could be reversed by the addition of excess calcium, indicating that a calcium antagonist effect might be involved in the effects of UK-84149 (Wallis *et al.*, 1994). The purpose of the present experiments was to investigate the action of UK-84149 on calcium currents from the guinea-pig jejunum, rabbit jejunum, and coronary artery smooth muscle cells, and to compare it with verapamil, nicardipine and

D600 by using the whole-cell voltage clamp recording technique.

Methods

Single cell dispersion

Rabbits were killed by overdose of pentobarbitone and guinea-pigs by cervical dislocation and exsanguination. Strips of the longitudinal smooth muscle layer of the guinea-pig or rabbit jejunum were cut into small pieces (3–4 mm) and incubated for 10 min in low Ca (10 μ M) physiological salt solution (PSS) and then transferred to a low-Ca medium containing 0.4 mg ml⁻¹ collagenase (Sigma, type XI) and 0.4 mg ml⁻¹ protease (Sigma, type E) for 20 min at 37°C. At the end of digestion, the tissue was washed with low-Ca PSS without enzyme and agitated mildly by sucking in and out of a wide-bore pipette. The suspension of cells was then centrifuged and the pellet re-suspended in PSS containing about 0.4 mM Ca. Cells were maintained at 4°C and used within 6 h. All experiments were at room temperature (20–26°C). The left descending coronary artery of rabbit was identified under the binocular microscope and was isolated carefully. The connective tissue was removed and the artery was cut into small pieces (2–3 mm). The procedure for dispersion

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was similar to that for jejunum except higher levels of enzymes (collagenase 0.6 mg ml^{-1} , protease 0.6 mg ml^{-1}) and longer incubation time (30 min) were used.

Electrical recordings

Cells were voltage-clamped by use of the whole-cell configuration of the patch-clamp technique (Hamill *et al.*, 1981) or perforated patch recording technique in which nystatin ($100 \mu\text{g ml}^{-1}$) was added to the pipette solution (Horn & Marty, 1988). The current-voltage converter was a patch-clamp amplifier (Biologic). Analogue signals were recorded on FM-tape (Racal) at a tape speed of 3.75 in s^{-1} . Fire-polished pipettes were prepared from borosilicate glass using a patch electrode puller (Narishige Sci. Inst. Lab.). The resistances of the pipettes were $2\text{--}4 \text{ M}\Omega$ for jejunum smooth muscle cell recordings and $3\text{--}5 \text{ M}\Omega$ for rabbit coronary artery smooth muscle cell recordings. Cells were placed in the chamber (0.3 ml) on the stage of an inverted microscope,

which was perfused continuously with solution at a rate of about 2 ml min^{-1} . Command pulses were applied and signals were digitized at 2 kHz using a CED 1401 interface in conjunction with a 386 PC or a BBC microcomputer.

Solutions

The normal PSS (physiological salt solution) in the bath had the following ionic composition (mM): NaCl 130, KCl 5, MgCl_2 1.2, CaCl_2 1.7 or 2.5, glucose 10, HEPES 10, and was titrated to pH 7.4 with NaOH. The pipette solutions had the following ionic composition (mM): (1) Cs solution; CsCl 135, MgCl_2 5, EGTA 5, HEPES 5. To make an ATP-generating solution ATP (3 mM), GTP (0.1 mM), creatine phosphate (15 mM), and creatine phosphokinase (50 u ml^{-1}) were added or nystatin ($100 \mu\text{g ml}^{-1}$) was added for perforated patch recording. (2) K solution: KCl 130, MgCl_2 2, EGTA 5, HEPES 20. The solutions were titrated to pH 7.4 with CsOH or KOH. In some cases, 20 mM TEA chloride was included in the pipette and bath solution.

Figure 1 Time course of changes in size of peak inward current in guinea-pig jejunum smooth muscle cell recorded using three types of Cs-containing pipette solution. (a) Conventional whole cell recording with ATP-free pipette solution; (b) conventional whole cell recording with 3 mM ATP, 15 mM creatine phosphate and 50 u ml^{-1} creatine phosphokinase in the pipette solution; (c) 'perforated patch' whole cell recording with $100 \mu\text{g ml}^{-1}$ nystatin in the pipette solution. The times after the cell membrane was ruptured are indicated. Cells were held at -80 mV and stepped to 0 mV for 50 ms every 20 s as shown below. (d) Relative amplitude of peak inward current recorded with the three types of solution. The inward current amplitude evoked by the first pulse after rupture of the cell membrane was normalized as 1.0. ATP-generating pipette solution (▲), nystatin pipette solution (■), and ATP-free pipette solution (●). Means and 1 s.e.mean from 4–5 cells.

Drugs

UK-84149 as its maleate salt UK-84149-11 (S-5,11-dihydro-5-[1-(4-methoxyphenethyl)-2-pyrrolidinylmethyl]-dibenzo [b,e] [1,4]thiazepinemaleate) was obtained from Pfizer Central Research, Sandwich, Kent and was dissolved first in dimethylsulphoxide (DMSO) and diluted with PSS to the final concentration given in the text. The final concentration of DMSO was less than 0.3%. Nicardipine, verapamil and methoxyverapamil (D600) were all from Sigma, and were dissolved directly in water to make stock solutions. Collagenase (type XI), protease (type E), EGTA, Na₂ATP, creatine phosphate, creatine phosphokinase, and GTP were from Sigma.

All results are expressed as mean $\pm$ s.e.mean. Statistical significance was evaluated by analysis of variance with repeated measurements or Student's bilateral unpaired *t* tests, when appropriate. Differences with values of $P < 0.05$ were considered to be significant.

Results

Ca current rundown

Figure 1 shows inward Ca current from rabbit jejunum smooth muscle cell evoked by stepping from a holding potential of -80 mV to 0 mV. With caesium chloride in the pipette, outward K current was apparently abolished because no difference was observed when 20 mM TEA was included in the pipette and bath solution (data not shown). In standard whole cell recording mode when no ATP was included in the pipette solution, the peak amplitude of the voltage-activated Ca current gradually decreased with time after a transient increase; the peak inward current decreased to $19 \pm 14\%$ ($n = 5$) of its initial value 20 min after rupture of the membrane patch. ATP (5 mM) markedly delayed the rundown of inward current (data not shown). When the ATP-generating pipette solution (see Methods) was used, the

Figure 2 Effect of UK-84149 on inward currents in guinea-pig jejunal single smooth muscle cells. The cells were bathed in PSS containing 1.7 mM Ca^{2+} and perfused continuously at the rate of 2 ml min^{-1} . (a)(i) Cells were held at -80 mV and stepped to various potentials for 300 ms, every 20 s. Open (O) and closed (●) circles indicated the peak inward currents in the control and with UK-84149 (3 μ M), respectively. (a)(ii) Current-voltage curves in the absence (O) and presence (●) of UK-84149 (3 μ M). Open and closed circles indicated peak inward current. (b)(i) Peak inward currents in the absence and presence of UK-84149 (1 – 10 μ M) plotted against time. Cells were held at -80 mV and stepped to 0 mV for 300 ms, every 20 s. (b)(ii) The relative amplitude of inward current against various concentrations of UK-84149. The peak inward current amplitude in the absence of the drug was normalised as 100% . The ID_{50} was about 2.3 μ M. Mean $\pm$ s.e.mean ($n = 6$).

rundown was delayed further and the initial increase of the peak inward current persisted even as long as 20 min; thereafter the Ca current eventually declined to $58 \pm 17\%$ ($n = 3$) of its initial peak amplitude at 60 min. With $100 \mu\text{g ml}^{-1}$ nystatin in the pipette solution ('perforated patch' recording mode), run down of inward current was usually negligible for 30 min or longer but by 60 min run down was apparent. Thus, run down was very slow compared with standard whole cell recordings. The peak inward current 50 min after gaining electrical access to cell (series resistance

10–30 M Ω) was still $96 \pm 16\%$ of its initial value ($n = 3$). Rundown of inward current is a problem when constructing concentration-response relationships for calcium antagonists, because the block often took a long time to reach steady-state. Thus, nystatin pipette solution was used for constructing concentration-response relationship for the effects of UK-84149, D600 and nifedipine on inward calcium current from rabbit jejunum and coronary artery cells and ATP-generating pipette solution was used for the rest of the experiments which involved observations on inward calcium current.

Figure 3 Effect of UK-84149 on inward and outward currents in guinea-pig jejunum smooth muscle cells. (a)(i) Cells were held at -80 mV and stepped to various potentials for 300 ms, every 20 s. Peak inward current (circles), peak (squares) and end of pulse (triangles) outward current in the absence (open symbols) and presence (closed symbols) of 3 μM UK-84149 were measured. (a)(ii) Current-voltage curve obtained in the absence and presence of 3 μM UK-84149. Symbols are as in (a)(i). (b)(i) Inward and outward current in one cell, plotted against time. Cells were held at -80 mV and stepped to $+10$ mV for 300 ms, every 20 s. (b)(ii) The relative amplitude of inward and outward current (evoked by a step to $+10$ mV) against various concentrations of UK-84149 (means $\pm$ s.e. mean, $n = 4$). The amplitude of inward and outward current in the absence of the drug were normalized as 100%. The ID_{50} s were 2.3 μM for peak inward ($\circ$), 7.2 μM and 3.3 μM for peak ($\square$) and end (∇) of pulse outward current, respectively.

Effects of UK-84149 on inward and outward current from guinea-pig jejunum smooth muscle cells

Figure 2(a)(i) and (a)(ii) show the effect of UK-84149 on the current-voltage relationships of the inward Ca current. Cells were held at -80 mV and stepped to various potentials. The inward current was evoked by stepping more positive than -30 mV from -80 mV. Outward current was inhibited by including Cs instead of K in the pipette solution. Figure 2(a)(i) shows inward current before and 10 min after application of UK-84149 ($3 \mu\text{M}$). UK-84149 consistently inhibited the peak inward current amplitude observed at any given potential (from -30 to $+40$ mV) (Figure 2(a)(i) and (ii)). Figure 2(b)(i) and (ii) show the relationship between the amplitude of inward current and concentrations of UK-84149. In these experiments, the inward current was evoked by stepping to 0 mV for 300 ms from a holding potential of -80 mV, every

Figure 4 Effect of UK-84149 on voltage-dependent inactivation of the inward current in a guinea-pig jejunal smooth muscle cell. The duration of the conditioning pulse (pre-pulse) was 10 s. (a) Absolute amplitude of inward current evoked by the test pulse (50 ms in duration, stepped to 0 mV from the appropriate membrane potential set by the conditioning pulse). The interval between conditioning pulses was 20 s. (b) Relative amplitude of inward current. The inward current amplitude evoked by the test pulse, without conditioning (the holding potential was -80 mV) was normalized as 1.0 . (■) Control; (●) $3 \mu\text{M}$ UK. Solid lines were drawn from Boltzmann's equation (see text). The value of V_h in this cell was -39 mV for control and -52 mV in $3 \mu\text{M}$ UK-84149. The slope was 5.5 for control, and 7 in $3 \mu\text{M}$ UK-84149. Average V_h values from another 5 cells were -37 ± 2 mV (mean $\pm$ s.e.mean) for control and -50 ± 2.2 mV in $3 \mu\text{M}$ UK-84149, and for the slope, 6.2 ± 0.4 for control and 7.3 ± 0.1 in $3 \mu\text{M}$ UK-84149.

20 s. Figure 2(b)(i) shows the effect in a cell of different concentrations of UK-84149 on peak inward current. About 5 min or longer was needed for the drug to reach its steady-state effect at the lower concentrations. Figure 2(b)(ii) shows data from 6 cells. The peak inward current before application of UK-84149 was normalized at 100% . The ID_{50} value for the effect of UK-84149 on peak inward current was $2.3 \mu\text{M}$.

Figure 3 shows the effects of UK-84149 on inward and outward currents from guinea-pig jejunum smooth muscle cells. In these experiments, high K solution was used in the pipette solution. When the cell was stepped more positive than -20 mV from -80 mV, both inward and outward currents were observed (Figure 3(a)(i) and (ii)). Figure 3(a)(i) and (a)(ii) shows the effect of UK-84149 on the current-voltage relationship of inward and outward currents which were inhibited at all potentials (-20 to $+30$ mV) after application for 10 min. Figure 3(b)(i) and (ii) shows the relationship between the amplitude of inward and outward currents and concentrations of UK-84149. Inward and outward currents were evoked by stepping to $+10$ mV for 300 ms from -80 mV, every 20 s. Figure 3(b)(i) shows the effect of UK-84149 on peak inward, peak and end of pulse outward current from one cell. It seems that UK-84149 was much slower to take effect on inward current than on outward currents, with a delayed start and a delayed steady-state (Figure 3(b)(i)). Figure 3(b)(ii) shows the data from 4 cells. UK-84149 was a more potent inhibitor of the end of pulse outward current than the peak outward current and accelerated the decay of the outward current. The ID_{50} s for the effects of UK-84149 on peak inward, peak outward and end of pulse outward current were 2.3 , 7.2 and $3.3 \mu\text{M}$, respectively.

The effect of UK-84149 on the availability of the inward current

The availability of the inward current was tested by means of a double pulse protocol. The peak inward current was evoked by a test potential of 0 mV after holding for 10 s at various conditioning potentials. The holding potential was -80 mV. Double pulses were applied at 20 s intervals. In the control, the inward current amplitude evoked by a test pulse was reduced when conditioning potentials were more positive than -60 mV. Figure 4a shows that the current was half available at $V_h = -30$ mV, and the slope, V_s , was 5.5 , when a Boltzmann equation of the form $I/I_{\max} = (1 + \exp((V - V_h)/V_s))^{-1}$ was fitted to the inactivating component of the current. V is the conditioning potential and $I/I_{\max}$ the fraction of the current available relative to that available from -80 mV. UK-84149 ($3 \mu\text{M}$) reduced the inward current amplitude from all potentials and shifted the steady state inactivation curve to the left (Figure 4b). V_h was shifted to -58 mV, and the slope value to 7 in this cell. Similar changes were observed in another 5 cells, with a mean value of V_h shifted from -37 ± 2 mV (control, $n = 5$) to -50 ± 2.2 mV ($n = 5$), and of V_s from 6.2 ± 0.4 (control, $n = 5$) to 7.3 ± 0.1 ($n = 5$).

Use-dependent actions of UK-84149

Inward current block by verapamil and D600 is known to be use-dependent; UK-84149 seemed to have a similar property. As shown in Figures 2 and 3, it took a long time for UK-84149 to reach its steady-state effect. Figure 5 shows the results of an investigation of the use-dependence of UK-84149, verapamil and nifedipine on inward current in guinea-pig jejunum smooth muscle cells. Inward current was evoked by stepping from -80 mV to 0 mV for 50 ms, every 20 s. The amplitude of the peak inward current was plotted against time. After rupture of the cell membrane the drugs were not applied until the inward current became stable. Figure 5a shows the effect of control PSS and verapamil ($3 \mu\text{M}$) on inward current. The cell had an inward current size

of about 600 pA. When the command pulse was stopped for 3 min in control PSS, which is indicated by the first interruption between the open circles, the peak inward current amplitude was temporarily very slightly increased. When the command pulse was stopped for another 3 min in verapamil ($3 \mu\text{M}$), the inward current amplitude evoked by the first command pulse after rest was not changed, compared with that before rest. Thereafter the current gradually decreased in the continuous presence of $3 \mu\text{M}$ verapamil and reached a steady state after about 3 min. When pulses were again stopped in the presence of verapamil ($3 \mu\text{M}$), inward current evoked by the first pulse after stopping had recovered to about 80% of the control size. Figure 5b shows that a high concentration of verapamil ($10 \mu\text{M}$) also had a use-dependent action. Both cells showed good recovery of inward current

after washout of verapamil. Figure 5c shows the effect of nicardipine on inward current. In contrast to verapamil, nicardipine had little use-dependent action on the inward current. Upon resting in the presence of nicardipine ($0.1 \mu\text{M}$) for 3 min, the inward current evoked by the first pulse was 33% of current size before rest, which was almost the same value as that after 5 min stimulation. UK-84149, more like verapamil, showed similar use-dependent action on inward current (Figure 5d). After resting in the presence of $3 \mu\text{M}$ UK-84149 for 3 min, the amplitude of the inward current evoked by the first pulse did not change, compared with that before rest. Thereafter it decreased gradually and reached a steady state about 10 min later. However unlike verapamil, resting in the continuous presence of UK-84149 ($3 \mu\text{M}$) did not improve inward current, and also it took a long time to

Figure 5 Time courses of inhibition by verapamil (a,b), nicardipine (c) and UK-84149 (d) of inward current in guinea-pig jejunum smooth muscle cells. Inward current was evoked by stepping from -80 mV to 0 mV for 50 ms every 20 s . Peak inward current before and after application of $3 \mu\text{M}$ (a) and $10 \mu\text{M}$ (b) verapamil, $0.1 \mu\text{M}$ nicardipine (c) and $3 \mu\text{M}$ UK-84149 (d) were plotted against time. The interruption between the circles indicates that command pulses were stopped for 3 min. Some recovery of inward current occurred after rest periods in the cases of verapamil (3 and $10 \mu\text{M}$) but not nicardipine or UK-84149.

wash out the effect of UK-84149 on inward current. The poor recovery of the inward current after stopping stimulation in the continuous presence of UK-84148 ($3\text{ }\mu\text{M}$) was probably due to the strong binding of UK-84148 to the receptor. To clarify the use-dependent effect of UK-84148, cells from guinea-pig jejunum were exposed to UK-84148 ($3\text{ }\mu\text{M}$) for 10 min before stimulating, and the block of the inward current was compared with that achieved with continuous repetitive stimulation for 10 min in the presence of UK-84148 ($3\text{ }\mu\text{M}$). Figure 6 shows the results of these experiments. When cells were exposed to UK-84148 ($3\text{ }\mu\text{M}$) for 10 min before stimulating, the inward current was only slightly inhibited, although the inward current gradually decreased after the stimulation was reapplied to the cell in the continuous presence of UK-84148 (Figure 6a). However, when the cell was exposed to UK-84148 ($3\text{ }\mu\text{M}$) for 10 min and was repetitively stimulated without any rest period the inward current was greatly inhibited (Figure 6b). Figure 6c shows the summarized data. In 4 cells, the inhibition of the inward current by UK-84148 ($3\text{ }\mu\text{M}$) without stimulation was $5.3 \pm 3.6\%$ (from $486 \pm 60\text{ pA}$ to $470 \pm 51\text{ pA}$) which was significantly ($P < 0.01$) less than that with repetitive stimulation ($52 \pm 8\%$; from $455 \pm 59\text{ pA}$ to $219 \pm 50\text{ pA}$, $n = 4$).

Effects of UK-84149, D600 and nicardipine on inward currents from rabbit jejunum and coronary artery smooth muscle cell

Figure 7 shows the effect of UK-84149 on inward current from rabbit jejunum and coronary artery smooth muscle cells, compared with the effects of D600 and nicardipine. The nystatin recording method was used in these experiments. The cell was held at -80 mV and stepped to 0 mV for 50 ms , every 20 s . A $10\text{ ms } 10\text{ mV}$ depolarizing pulse was used to monitor the series resistance. The drugs were bath applied and perfusion was continuous at a rate of 2 ml min^{-1} . Cadmium ($100\text{ }\mu\text{M}$) was used to abolish inward current completely at the end of the experiments and create a base line for measurement. Figure 7(a)(i) shows the effect of UK-84149 ($3\text{ }\mu\text{M}$) on inward current in jejunum (left) and coronary artery (right) smooth muscle cells. UK-84149 ($3\text{ }\mu\text{M}$) inhibited inward current from both types of smooth muscle cells and with a similar potency. At this concentration, the effect could be reversed by washing, although it took a long time (about 15 min). Figure 7(a)(ii) shows the

concentration-effect relationship for the effect of UK-84149 on inward current from the two types of smooth muscle cells. The amplitude of inward current before application was normalised as 100% . The ID_{50} values were not significantly different (analysis of variance, $P > 0.05$) ($1.7\text{ }\mu\text{M}$ for jejunum (squares) and $3.2\text{ }\mu\text{M}$ for coronary artery (circles) smooth muscle cells). Figure 7(b)(i) shows the effect of D600, and Figure 7(c)(i) shows the effect of nicardipine on the two types of smooth muscle cell. Again, no significant differences were found. The ID_{50} values for the effect of D600 on inward current were $8.1\text{ }\mu\text{M}$ for jejunum and $3.9\text{ }\mu\text{M}$ for coronary artery smooth muscle cells (Figure 7(b)(ii)). The ID_{50} values for the effect of nicardipine on inward current were 24 nM for jejunum and 12 nM for coronary artery smooth muscle cells. Among the three Ca antagonists, nicardipine was most potent in both jejunum and coronary artery cells. UK-84149 was 2–5 times more potent than D600 but 200–300 times less potent than nicardipine.

Discussion

The main finding of the present experiments was that UK-84149 inhibited the Ca inward current evoked in smooth muscle cells from the guinea-pig and rabbit jejunum and rabbit coronary artery cell with a potency similar to that of D600 although it was less potent than nicardipine. The effect of UK-84149 was long-lasting and voltage-dependent. Like verapamil, UK-84149 showed use-dependent block. UK-84149 inhibited voltage-dependent outward current as well as inward current. This was not surprising because many Ca antagonists, including nicardipine, verapamil and diltiazem have been shown to be blockers of voltage-dependent outward currents in smooth muscle cells (Terada *et al.*, 1987a). Since 5 mM EGTA was included in the pipette in the present experiments and the outward current was evoked by stepping only to $+10\text{ mV}$, there should be little activation of large-conductance, Ca -activated potassium channels. Therefore the effect of UK-84149 on outward current is unlikely to be due to the inhibition of Ca^{2+} influx via voltage-dependent Ca channels. The ratio of ID_{50} values for the inhibition of UK-84149 of peak outward and inward current was about 3 ($7.2\text{ }\mu\text{M}/2.3\text{ }\mu\text{M}$) which was much lower than that of nicardipine on rabbit jejunum cell which had a similar ratio to D600 (ratio of ID_{50} values was 11, Terada *et al.*, 1987a). Like the effect of verapamil and nicardipine on rabbit ileum smooth muscle cells (Terada *et al.*, 1987a), UK-84149 accelerated the outward current decay. In general, the selectivity of the Ca antagonists for the Ca inward current in smooth muscle cells was low in comparison with that seen in cardiac cells (Hume, 1985). However, the ID_{50} values for the inhibition of outward current by different Ca antagonists in different tissues (smooth muscle, cardiac and neurone) were quite similar, ranging from $1\text{--}16\text{ }\mu\text{M}$ (Nishi *et al.*, 1983; Hume, 1985; Terada *et al.*, 1987a; Figure 3), but the ID_{50} values for the inhibition of inward current by different Ca antagonists in different tissues were dramatically different, ranging from 16 nM to $9\text{ }\mu\text{M}$ (Nishi *et al.*, 1983; Hume, 1985; Terada *et al.*, 1987b,c; Figure 6). Hence, inhibition of voltage-dependent outward currents by Ca antagonists seems to be due to a non-specific blocking action of higher concentrations.

UK-84149 shifted the voltage-dependent inactivation curve to the left. Nicardipine and other dihydropyridines have been shown to shift the voltage-dependent inactivation curve to the left when the conditioning pulses were 10 s (the same condition as used in the present experiment) (Terada *et al.*, 1987b,c). Therefore, shifting the voltage-dependent inactivation curve to the left seems to be one of the common features of calcium channel blockers.

In most smooth muscles the Ca current can be dissected into components on the basis of its voltage- and time-dependent properties (Aaronson *et al.*, 1986; Caffrey *et al.*,

Figure 6 Use-dependency of inhibition of UK-84149 of inward current in guinea-pig jejunum smooth muscle cells. Inward current was evoked by stepping from -80 mV to 0 mV for 50 ms every 20 s . (a) Stimulation was stopped (as indicated by interruption between the circle symbols) for 10 min in the presence of UK-84149 ($3\text{ }\mu\text{M}$). (b) UK-84149 ($3\text{ }\mu\text{M}$) was applied to a repetitively stimulated cell for 10 min . (c) The peak inward current amplitude is shown (mean $\pm$ s.e.mean) in the absence (solid columns) and presence (stippled columns) of $3\text{ }\mu\text{M}$ UK-84149 with and without stimulation.

Figure 7 Effect of UK-84149, D600 and nicardipine on inward current of rabbit coronary artery and jejunum smooth muscle cells. Cell was held at -80 mV and stepped to 0 mV for 50 ms, every 20 s. Nystatin ($100 \mu\text{g ml}^{-1}$) was included in CsCl_2 pipette solution to make perforated patch whole cell recordings. (a)(i), (b)(i), (c)(i): Effect of $3 \mu\text{M}$ UK-84149 (UK), $3 \mu\text{M}$ D600 (D) and $0.03 \mu\text{M}$ nicardipine (N) on inward current from jejunum (left panel) and coronary artery (right panel) smooth muscle cells. Cadmium (Cd) $100 \mu\text{M}$ was applied at the end of the experiments. C control, W after washout. (a)(ii), (b)(ii), (c)(ii): Concentration-effect relationship for the effect of UK-84149, D600 and nicardipine on inward current from jejunum ($\square$) and coronary artery ($\circ$) smooth muscle cell. No significant differences were found for all three drugs among jejunum and coronary artery smooth muscle cells. The ID_{50} s: UK-84149, $1.7 \mu\text{M}$ for jejunum cells, and $3.2 \mu\text{M}$ for coronary artery cells (analysis of variance, $P > 0.05$, 3–8 cells); D600, $8.1 \mu\text{M}$ for jejunum cells, and $3.9 \mu\text{M}$ for coronary artery cells ($P > 0.05$, 3–6 cells); nicardipine, $0.024 \mu\text{M}$ for jejunal cell, and $0.012 \mu\text{M}$ for coronary artery cells ($P > 0.05$, 2–7 cells).

1986). The diversity of Ca channels might contribute to the diversity of responses of Ca current to Ca antagonists. The effect of calcium antagonists on Ca current may be tissue-dependent, voltage-dependent and use or frequency-dependent. Nicardipine and other dihydropyridines are the most potent calcium antagonists and their effects are voltage-dependent. Verapamil and D600 have a use-dependent action (Terada *et al.*, 1987b; present data). In the present experiments, UK-84149 showed a use-dependent block which may explain, at least partially, why UK-84149 was more potent on the bowel than on blood vessel (Quinn & Wallis, 1994; Wallis *et al.*, 1994), since bowel has a high rate of spontaneous activity and artery is quiescent under normal condi-

tions. Any blockade arising during spontaneous activity would be only slowly dissipated during a rest period; during hypermotility its blocking action would be expected to develop particularly strongly.

In conclusion, our experiments indicate that UK-84149 is a calcium antagonist and is active in blocking voltage-activated calcium current. UK-84149 might be expected to be useful in conditions such as irritable bowel syndrome which are characterized by hypermotility of the smooth muscle.

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