ELSEVIER

European Journal of Pharmacology 383 (1999) 361–371

ejp

www.elsevier.nl/locate/ejphar

Electropharmacological effects of UK-1745, a novel cardiotonic drug, in guinea-pig ventricular myocytes

Hiroko Uemura, Naoya Sakamoto, Haruaki Nakaya *

Department of Pharmacology, Chiba University School of Medicine, 1-8-1 Inohana, Chuo-Ku, Chiba 260-8670, Japan

Received 6 May 1999; received in revised form 3 August 1999; accepted 9 September 1999

Abstract

Effects of (2*RS*, 3*SR*)-2-aminomethyl-2,3,7,8-tetrahydro-2,3,5,8,8-pentamethyl-6*H*-furo-[2,3-e] indol-7-one hydrochloride (UK-1745), a novel cardiotonic drug with β-adrenoceptor blocking property and antiarrhythmic activity, on the action potentials of isolated papillary muscles and the membrane currents of single ventricular myocytes of guinea pigs were examined and compared with those of milrinone using conventional microelectrode and patch–clamp techniques. In papillary muscles, UK-1745 (3–100 μM) produced a mild positive inotropic response and depressed the maximum upstroke velocity of the action potential ($\dot{V}_{max}$) at 100 μM. Milrinone, a phosphodiesterase III inhibitor, markedly shortened the action potential duration with a significant increase in developed tension. In enzymatically-dissociated ventricular myocytes, UK-1745 failed to increase the L-type Ca^{2+} current (I_{Ca}) at 10 and 30 μM and rather decreased I_{Ca} at 100 μM. The high concentration of UK-1745 slightly inhibited the delayed rectifier K^+ current (I_K). Although UK-1745 antagonized the isoproterenol-induced increase in I_{Ca}, it potentiated the histamine-induced increase in I_{Ca}. On the other hand, milrinone per se significantly increased I_{Ca} and markedly enhanced the isoproterenol-induced increase in I_{Ca}. It can be concluded that UK-1745 is a unique cardiotonic drug possessing β-adrenoceptor blocking and weak phosphodiesterase-inhibitory actions in addition to direct inhibitory actions on the Na^+, Ca^{2+} and K^+ channels with its high concentrations.

Keywords: UK-1745; Milrinone; Cardiotonic drug; Ca^{2+} channel

1. Introduction

For more than 200 years cardiac glycosides have been used for the treatment of congestive heart failure. Since cardiac glycosides cause serious side effects, great efforts have been made to develop new positive inotropic agents with various mechanism of action. The majority of the novel inotropic agents inhibit phosphodiesterase III, which results in increases in intracellular cyclic AMP (cAMP) and transmembrane Ca^{2+} influx. Although acute administration of the phosphodiesterase III inhibitors such as amrinone, milrinone and enoximone produces dramatic hemodynamic improvement, long-term use of these agents has been shown to lead to increased mortality in many controlled clinical trials (Packer et al., 1984, 1991; Uretsky et al., 1990). Although the underlying mechanisms of the increased mortality are not well-understood, arrhythmogenesis resulting from increases in cAMP and transmembrane Ca^{2+} influx may play an important role.

UK-1745 {(2*RS*, 3*SR*)-2-aminomethyl-2,3,7,8-tetrahydro-2,3,5,8,8-pentamethyl-6*H*-furo-[2,3-e] indol-7-one hydrochloride} is a newly-synthesized cardiotonic drug which possesses not only phosphodiesterase III-inhibitory action but also β-adrenoceptor blocking action as well as antiarrhythmic action (Kawada et al., 1996; Uchida and Sonoki, 1996; Uchida et al., 1998; Sawada and Endoh, 1999). However, effects of UK-1745 on the action potential and the membrane current system have not been evaluated. This study was undertaken to examine the electropharmacological effect of UK-1745 and compare it with that of milrinone, a prototype cardiotonic drug possessing a phosphodiesterase III-inhibitory action.

2. Materials and methods

2.1. Action potential study

All experiments were performed under the regulations of the Animal Research Committee of School of Medicine,

* Corresponding author. Tel.: +81-43-226-2050; fax: +81-43-226-2052.

E-mail address: nakaya@med.m.chiba-u.ac.jp (H. Nakaya)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00651-2

Chiba University. Guinea pigs weighing 200–350 g were stunned with a blow on the head, and the hearts were rapidly removed. The hearts were immersed in an oxygenated modified Tyrode solution, and the papillary muscles having a diameter less than 1 mm were dissected from the right ventricle. The preparations were placed in a dissection bath filled with a modified Tyrode's solution equilibrated with 95% O_2 and 5% CO_2, as described previously (Nakaya et al., 1993). The composition of solution was (in mM): NaCl, 125; KCl, 4; NaH_2PO_4, 1.8; $MgCl_2$, 0.5; $CaCl_2$, 2.7; $NaHCO_3$, 25 and glucose, 5.5. The pH of the bubbled Tyrode solution was 7.35 ± 0.05. The papillary muscle was pinned to the bottom of a 5-ml tissue chamber and superfused at a rate of 10 ml/min with the modified Tyrode solution, aerated with 95% O_2 and 5% CO_2. The temperature of the bath solution was maintained at 36 ± 1.0°C. One end of the muscle was hooked to the lever arm of a force transducer (Nihon Kohden TB 651T, Tokyo, Japan) mounted on a micromanipulator and the other was pinned to the bottom of the tissue chamber. The resting tension was progressively increased to 2 mN. The preparation was electrically stimulated at 0.5 Hz with pulses of 1–1.5 ms duration at twice the diastolic threshold through platinum field electrodes. Stimuli were delivered from an electronic stimulator (Nihon Kohden S-7272B). Transmembrane action potentials were recorded with a glass microelectrode filled with 3 M KCl, which had a tip resistance of 10 to 30 MΩ. The microelectrode was connected to the input stage of a high-impedance amplifier with capacitance neutralization (Nihon Kohden MEZ-7200). The amplified signals were displayed on a dual-beam oscilloscope (Nihon Kohden VC-10) and photographed with a Polaroid camera. After an equilibration period of 1 to 2 h, a stable impalement was obtained and control recordings were made. The preparations were then exposed to solutions containing various concentrations of UK-1745 or milrinone. The concentration was increased in a stepwise fashion at intervals of 30 min and the recordings were made when the changes of the action potential and the developed tension (DT) reached a steady state. One preparation was exposed to one or two concentrations of a drug. Only experiments in which a stable impalement was maintained were used for data analysis.

2.2. Patch–clamp study

Single ventricular cells of the guinea pig heart were isolated by an enzymatic dissociation method, as described previously (Tohse et al., 1992). The heart was removed from open-chest guinea pigs (250–350 g) anesthetized with pentobarbital sodium and ventilated with an artificial respirator, and mounted on a modified Langendorff perfusion system for retrograde perfusion of the coronary circulation with a normal HEPES–Tyrode's solution (37°C). The perfused medium was then changed to a nominally Ca^{2+}-free Tyrode's solution and then to the solution containing 0.02% w/v collagenase (Wako, Osaka, Japan). After digestion, the heart was perfused with a high-K^+ and low-Cl^- solution, modified Kraftbrühe (KB) solution (Isenberg and Klockner, 1982; Nakaya et al., 1993). Ventricular tissue was cut into small pieces in the modified KB solution and was gently shaken to isolate cells. The cell suspension was filtered through a 100-μm pore stainless-steel mesh and stored in a refrigerator (4°C) for later use. The composition of the normal HEPES–Tyrode's solution was (in mM): NaCl, 143; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; NaH_2PO_4, 0.33; glucose, 5.5 and HEPES–NaOH buffer (pH 7.4), 5.0. The nominally Ca^{2+}-free Tyrode solution was prepared by omitting $CaCl_2$ from the normal Tyrode solution. The composition of the modified KB solution was (in mM): KOH, 70; L-glutamic acid, 50; KCl, 40; taurine, 20; KH_2PO_4, 20; $MgCl_2$, 3; glucose, 10; ethylene glycol-bis-(β-aminoethylether)-*N,N,N′,N′*-tetra-acetic acid (EGTA), 1.0 and HEPES–KOH buffer (pH 7.4), 10.

Whole-cell membrane currents were recorded by the patch–clamp method (Hamill et al., 1981). Single ventricular myocytes were placed in a recording chamber (1 ml volume) attached to an inverted microscope (Olympus IMT-2, Tokyo, Japan) and superfused with the HEPES–Tyrode's solution at a rate of 3 ml/min. Patch pipettes were made from glass capillaries with a diameter of 1.5 mm by use of a vertical microelectrode puller (Narishige PB-7, Tokyo, Japan). They were filled with an internal solution, and the resistance was 1 to 2 MΩ. The composition of the pipette solution was (in mM): potassium aspartate, 110; KCl, 20; $MgCl_2$, 1.0; potassium ATP, 5.0; potassium phosphocreatine, 5.0; EGTA, 10 and HEPES–KOH buffer (pH 7.4), 5.0. The free Ca^{2+} concentration in the pipette solution was adjusted to pCa 8 according to the calculation by Fabiato and Fabiato (1979) with the correction of Tsien and Rink (1980). After the gigaohm seal between the tip of the electrode and the cell membrane was established, the membrane patch was disrupted by a more negative pressure to make the whole-cell voltage-clamp mode. The electrode was connected to a patch–clamp amplifier (Nihon Kohden CEZ-2300). Voltage command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments, Foster City, CA, USA). Current signals were digitized and stored on the hard disc of an IBM-compatible computer (Compaq Prolinea 4/50 with a 200-M byte hard disc, Houston, TX, USA). A liquid junction potential between the internal solution and the bath solution of -8 mV was corrected. The capacitance of the membrane was calculated from the steady-state current in response to a ramp (-5 mV/2.5 ms) from 0 mV and was 120.9 ± 6.9 pF ($n = 40$). The membrane capacitance was almost compensated for up to 100 pF. The series resistance (R_s) was 2–5 MΩ and was usually compensated by 70%. Since the peak current measured in this study was < 7 nA, the voltage errors would be < 10 mV. These voltage errors

might change the amplitude of membrane currents, but would not seriously affect the qualitative changes of the membrane currents after drug application.

In order to obtain the current–voltage (I–V) relationships, hyperpolarizing or depolarizing pulses of 300 ms duration were given to the cells from a holding potential of −40 mV at 0.1 Hz. For the recording of the L-type Ca^{2+} current (I_{Ca}), the cells were depolarized to 0 mV from a holding potential of −40 mV by test pulses of 300 ms at 0.1 Hz. After stabilization of the inward Ca^{2+} current, effect of UK-1745 or milrinone on the current was examined. In a part of the experiments the L-type Ca^{2+} current was isolated from other membrane currents by using Cs^+-rich external and pipette solutions, as previously described (Hara and Nakaya, 1995). The Cs^+-rich external solution was prepared by replacing KCl of the normal HEPES–Tyrode solution with equimolar CsCl. The composition of the Cs^+-rich pipette solution was (in mM): L-aspartate, 110; CsOH, 110; CsCl, 20; $MgCl_2$, 1.0; ATP-K_2, 5.0; EGTA, 10 and HEPES–CsOH-buffer (pH 7.4), 5.0. These experiments were conducted at a temperature of 36.0 ± 1.0°C.

In a part of experiments, the Na^+ current (I_{Na}) was recorded using the following external and pipette solutions at room temperature (22°C–24°C) and the effect of UK-1745 on the Na^+ current was examined. The external solution contained (in mM): NaCl, 30; tetramethylammonium chloride, 110; CsCl, 1; $CaCl_2$, 1.8; $CoCl_2$,1; $MgCl_2$,1.2; glucose, 11; HEPES, 20 (pH 7.4 adjusted with tetramethylammonium hydroxide). The pipette solution was composed of (mM): NaF, 5; CsF, 125; ATP-K_2, 5; phosphocreatinine K_2, 5; EGTA, 5; HEPES, 5 (pH 7.2 adjusted with CsOH). A train of 30 depolarizing pulses of 50 ms duration to −30 mV was applied from a holding potential of −120 mV at 1.0 Hz before and after the application of UK-1745.

2.3. Drugs

Drugs used in this study were as follows: UK-1745 (Kowa, Tokyo, Japan), (−)-isoproterenol hydrochloride (Sigma, St. Louis, USA), histamine dihydrochloride (Wako, Osaka, Japan) and milrinone (Sigma). Milrinone was dissolved in dimethyl sulphoxide (DMSO) as a stock solution of 10 mM. The final concentration of DMSO was less than 1% and the same concentration of DMSO was applied during control period before the introduction of milrinone. Other drugs were dissolved in distilled water.

2.4. Statistics

All data are presented as mean ± S.E.M. Student's t-test was used for statistical analysis of the paired observations and an analysis of variance (ANOVA) was performed to

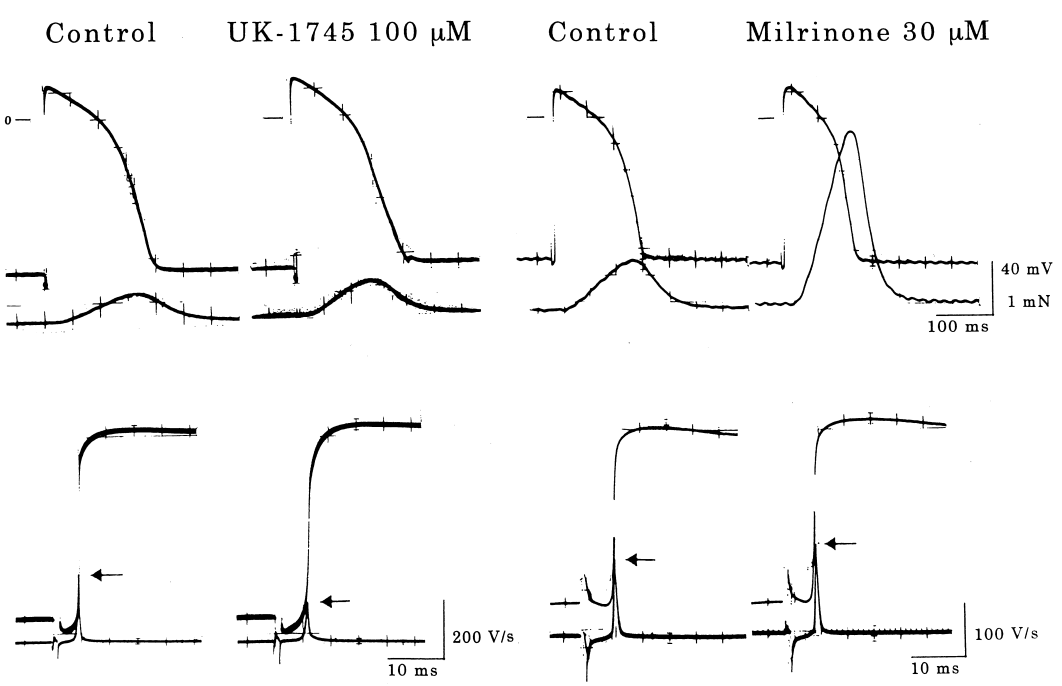

Fig. 1. Effects of UK-1745 and milrinone on the action potential configuration and the DT (upper panels) and the maximum rate of rise of action potential (lower panels) in guinea-pig papillary muscles stimulated at 0.5 Hz. The transmembrane potentials and dV/dt are shown on expanded scales in lower panels. Arrows indicate the peak of dV/dt ($\dot{V}_{max}$). Note that UK-1745 but not milrinone decreased $\dot{V}_{max}$ although both UK-1745 and milrinone produced increases in DT.

Table 1
Effects of UK-1745 on action potential parameters and DT in isolated guinea-pig papillary muscles stimulated at 0.5 Hz. APA, action potential amplitude; APD_{50} and APD_{90}, action potential duration at 50% and 90% repolarization, respectively; $\dot{V}_{max}$, maximum rate of rise; DT, developed tension; $RT_{1/2}$, half relaxation time; *n*, number of experiments. Values are expressed as mean ± S.E.M. of percent of control

	n	APA	APD_{50}	$\dot{V}_{max}$	DT	$RT_{1/2}$
Basal values	33	132.7 ± 0.5 mV	149.3 ± 3.1 ms	205.3 ± 10.9 V/s	33.1 ± 3.5 mg	55.9 ± 2.0 ms
UK-1745 (μM)		Percent of control				
1	9	99.7 ± 0.4	98.8 ± 0.9	100.1 ± 2.0	94.9 ± 3.1	93.7 ± 7.1
3	10	100.6 ± 0.2	101.3 ± 1.5	103.6 ± 2.8	100.6 ± 4.7	98.1 ± 9.4
10	9	100.6 ± 0.2	101.8 ± 2.0	90.6 ± 7.6	105.2 ± 7.3	98.4 ± 6.9
30	12	100.7 ± 0.3	100.2 ± 2.1	92.9 ± 4.3	106.3 ± 8.3	110.1 ± 11.6
100	8	100.0 ± 0.6	96.8 ± 2.2	55.8 ± 4.5 *	138.7 ± 11.9*	100.7 ± 5.4

* Significant change from each control value ($P < 0.05$). Statistical analysis was conducted using absolute values except for DT.

test the difference among the groups; *P*-values of < 0.05 were considered significant.

3. Results

3.1. Effects of UK-1745 and milrinone on action potential configuration and DT

Fig. 1 illustrates representative changes in action potential configuration produced by UK-1745 and milrinone in guinea-pig papillary muscles stimulated at 0.5 Hz. UK-1745 produced a mild positive inotropic response. UK-1745 depressed the maximum rate of rise of the action potential ($\dot{V}_{max}$) in a high concentration (100 μM) (Fig. 1). Milrinone markedly increased the DT although it did not inhibit $\dot{V}_{max}$. Milrinone slightly but significantly shortened the action potential duration (APD).

Changes in action potential parameters and the DT after UK-1745 and milrinone are summarized in Tables 1 and 2, respectively. UK-1745 increased the DT and decreased $\dot{V}_{max}$ in papillary muscles stimulated at 0.5 Hz (Table 1). The increase in the DT and the decrease in $\dot{V}_{max}$ after 100 μM UK-1745 were statistically significant ($P < 0.05$). Milrinone also increased the DT in a concentration-dependent manner (Table 2). The increase in the DT produced by 30 μM milrinone was greater than that produced by 100 μM UK-1745. However, milrinone failed to decrease $\dot{V}_{max}$. Milrinone significantly decreased the APD. Although the half relaxation time of contraction ($RT_{1/2}$) was concentration-dependently decreased by milrinone, it was unchanged by UK-1745 (Tables 1 and 2).

3.2. Effects of UK-1745 and milrinone on membrane currents in isolated ventricular cells

Effects of UK-1745 on the whole-cell membrane currents were evaluated and compared with those of milrinone, a prototype phosphodiesterase III inhibitor (Fujino et al., 1988). Membrane currents were elicited by test pulses to various potentials from a holding potential of −40 mV at 0.1 Hz, as shown in Fig. 2. After rupture of the cell membrane, the peak Ca^{2+} current elicited by depolarizing pulses to 0 mV tended to decrease slightly due to 'run down'. The amplitude of the Ca^{2+} current was −13.6 ± 1.6 pA/pF and −11.5 ± 1.2 pA/pF at 5 min and 15–20 min after the rupture in five untreated cells (14.9 ± 2.9% run down, n.s.). UK-1745 at a concentration of 10 μM hardly affected the inward current elicited by depolarizing pulses and the steady state current elicited by hyperpolarizing pulses. UK-1745 at a concentration of 30 μM also failed to affect the inward and outward currents ($n = 4$). However, a higher concentration (100 μM) of UK-1745 decreased rather than increased the inward Ca^{2+} current, as shown in Fig. 3. The L-type Ca^{2+} current that was elicited by a depolarizing pulse to 0 mV was significantly decreased by 59.2 ± 8.0% after 100 μM UK-1745 ($P < 0.05$). The tail outward current, recorded upon clamp back

Table 2
Effects of milrinone on action potential parameters and DT isolated guinea-pig papillary muscles stimulated at 0.5 Hz. Abbreviations are the same as in Table 1. Statistical analysis was conducted using absolute values except for DT

	n	APA	APD_{50}	$\dot{V}_{max}$	DT	$RT_{1/2}$
Basal values	11	132.6 ± 0.6 mV	132.0 ± 4.8 ms	155.2 ± 7.1 V/s	41.6 ± 6.8 mg	53.1 ± 2.2 ms
Milrinone (μM)		Percent of control				
1	5	102.1 ± 0.7	98.0 ± 4.5	98.4 ± 1.4	102.6 ± 3.5	96.5 ± 10.2
3	5	100.9 ± 0.8	96.4 ± 2.0	105.8 ± 2.3	127.0 ± 8.8	93.9 ± 6.0
10	5	102.6 ± 0.5	93.3 ± 7.9	95.8 ± 5.0	133.2 ± 13.3	83.3 ± 6.6
30	5	102.3 ± 0.8	85.2 ± 2.9 *	107.7 ± 2.2	272.7 ± 37.6*	75.7 ± 7.0*

* Significant change from each control value ($P < 0.05$).

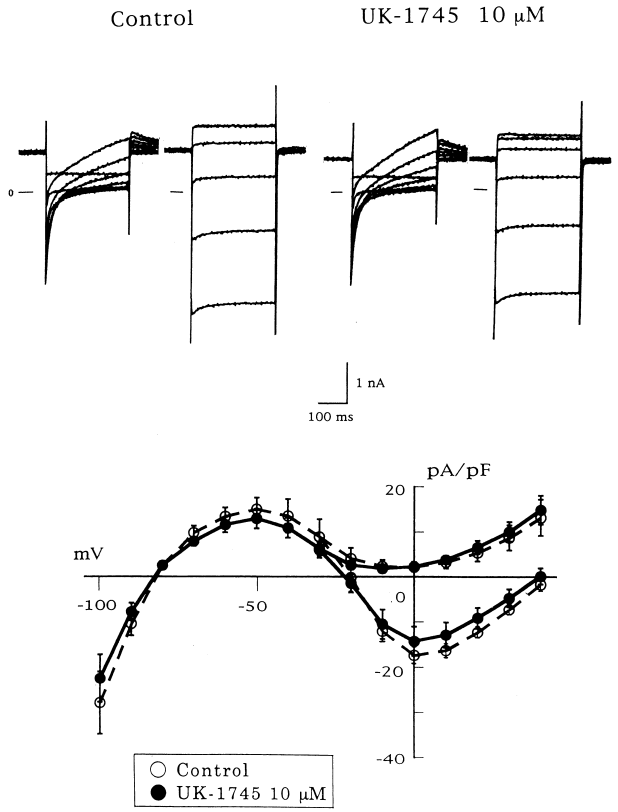

Fig. 2. Effects of a low concentration (10 μM) of UK-1745 on membrane currents of guinea-pig ventricular cells. Actual current traces elicited by depolarizing and hyperpolarizing pulses from a holding potential of −40 mV before and after 10 μM UK-1745 in a single ventricular cell are shown on upper panels. Current–voltage relations for the peak current and the current at the end of 300 ms test pulse in the absence (open circles) and presence (closed circles) of UK-1745, obtained from five cells, are shown on lower panels. Values are expressed as mean ± S.E.M.

to the holding potential after depolarizing pulses, was also decreased by 100 μM UK-1745, indicating the inhibitory effect on the delayed rectifier K^+ current (I_K) (Fig. 3). The high concentration of UK-1745 also slightly inhibited the outward current elicited by hyperpolarizing test pulses without affecting the reversal potential, suggesting that UK-1745 might slightly inhibit the inward rectifier K^+ current (I_{K1}). In contrast with UK-1745, milrinone increased the L-type Ca^{2+} current. Milrinone at a concentration of 30 μM significantly increased the Ca^{2+} current at 0 mV by 192.4 ± 28.3% ($P < 0.05$). Milrinone increased the tail current of the delayed rectifier K^+ current (Fig. 4). The decrease in the Ca^{2+} current after 100 μM UK-1745 or the increase after 30 μM milrinone reverted toward the control on changing to the drug-free solution. In a part of the experiments, the Ca^{2+} current was isolated from other membrane currents using Cs^+-rich external and pipette solutions. Even in such an experimental condition, UK-1745 at a concentration of 100 μM decreased the Ca^{2+} current by 64.1 ± 4.1% ($P < 0.05$, $n = 5$) and milrinone at a concentration of 30 μM increased the current by 184.5 ± 27.5% ($P < 0.05$, $n = 4$). These changes in the Ca^{2+} current were not significantly different from those in the current measured with normal pipette and external solutions after these drugs at the respective concentration.

In another series of experiments, effects of UK-1745 on the isoproterenol- and histamine-induced increases in the Ca^{2+} current were examined and compared with those of milrinone. Isoproterenol at a concentration of 0.03 μM markedly increased the Ca^{2+} current, which was readily blocked by 10 μM UK-1745 (Fig. 5). In six cells the peak Ca^{2+} current elicited by depolarizing pulses to 0 mV, was significantly increased from −11.8 ± 1.3 pA/pF to −31.5 ± 4.3 pA/pF ($P < 0.05$) by 0.03 μM isoproterenol, which was then significantly decreased to −22.4 ± 3.9 pA/pF ($P < 0.05$) after the addition of 10 μM UK-1745. The blocking effects of UK-1745 on the isoproterenol-induced increase in the Ca^{2+} current readily disappeared after the washout of the drug. Histamine also increased the Ca^{2+} current in guinea-pig ventricular cells probably via the stimulation of histamine–H_2 receptors, as

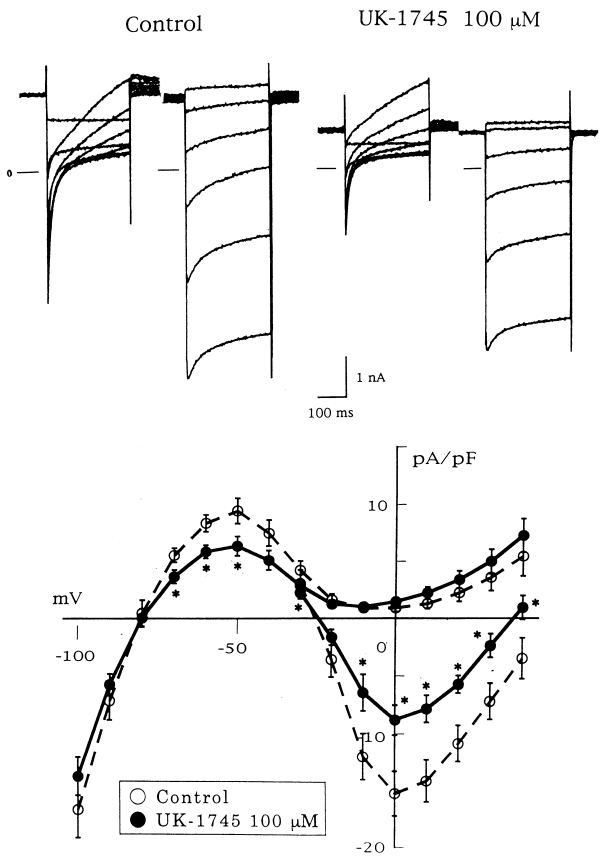

Fig. 3. Effects of a high concentration (100 μM) of UK-1745 on membrane currents of guinea-pig ventricular cells. Actual current traces induced by depolarizing and hyperpolarizing pulses from a holding potential of −40 mV before and after 100 μM UK-1745 in a single ventricular cell are shown on upper panels. Current–voltage relations for the peak current and the current at the end of 300 ms test pulse in the absence (open circles) and presence (closed circles) of UK-1745, obtained from six cells, are shown on lower panel. Values are expressed as mean ± S.E.M. * Represents a significant change from the control value at $P < 0.05$.

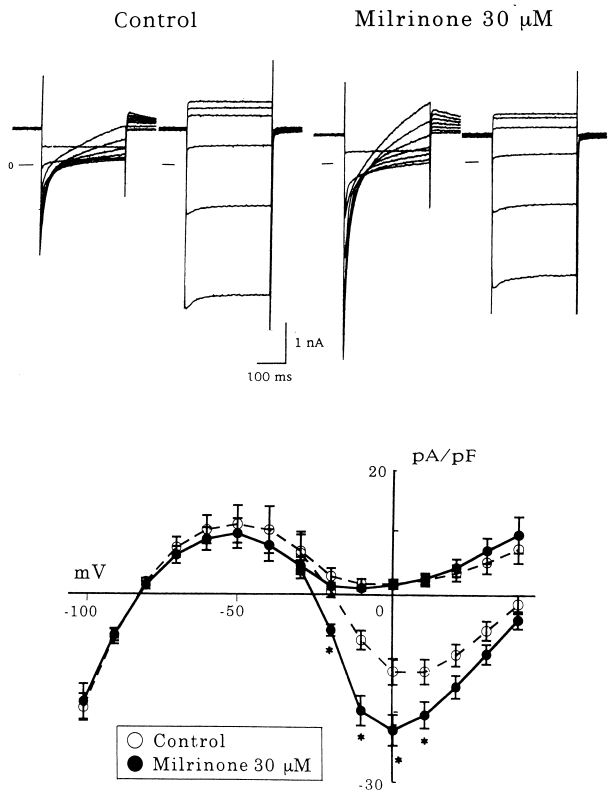

Fig. 4. Effects of 30 μM milrinone on membrane currents of guinea-pig ventricular cells. Actual current traces induced by depolarizing and hyperpolarizing pulses from a holding potential of −40 mV before and after 30 μM milrinone in a single ventricular cell are shown on upper panels. Current–voltage relations for the peak current and the current at the end of 300 ms test pulse in the absence (open circles) and presence (closed circles) of milrinone, obtained from five cells, are shown on lower panel. Values are expressed as mean ± S.E.M. *Represents a significant change from the control value at $P < 0.05$.

previously reported (Hescheler et al., 1987; Tanaka et al., 1991; Yazawa and Abiko, 1993). The peak Ca^{2+} current was significantly increased from -14.7 ± 1.9 to -22.7 ± 3.5 pA/pF ($n = 5$, $P < 0.05$) by 1 μM histamine, which was further increased to -41.0 ± 4.9 pA/pF ($P < 0.05$) after the addition of 10 μM UK-1745, as shown in Fig. 5.

As already mentioned, milrinone at a concentration 30 μM per se increased the Ca^{2+} current, as shown in Fig. 6. Milrinone at concentrations of 10 and 30 μM increased the current by $119.1 \pm 4.0\%$ ($n = 5$, $P < 0.05$) and $162.4 \pm 18.1\%$ ($n = 6$, $P < 0.05$), respectively. The influence of milrinone on the isoproterenol-induced increase in the Ca^{2+} current was quiet different from that of UK-1745. Milrinone at a concentration of 10 μM potentiated the isoproterenol-induced increase in the Ca^{2+} current, as shown in Fig. 6. In five cells the peak Ca^{2+} current was increased from -12.2 ± 1.0 to -28.5 ± 3.9 pA/pF ($P < 0.05$) after 0.03 μM isoproterenol, and it was further increased to -39.5 ± 4.7 pA/pF ($P < 0.05$) after the addition of 10 μM milrinone. Milrinone also potentiated the histamine-induced increase in the Ca^{2+} current. Histamine (1 μM) increased the peak of Ca^{2+} current from -16.0 ± 1.5 to -30.2 ± 6.7 pA/pF ($n = 5$, P = 0.066), and milrinone at a concentration of 10 μM further increased it to -50.8 ± 5.5 pA/pF, ($n = 5$, $P < 0.05$). Thus, both the increases in the L-type Ca^{2+} current after isoproterenol and histamine were potentiated by milrinone.

Since UK-1745 decreased V_{max} in guinea-pig papillary muscles, effects of the drug on the Na^+ current (I_{Na}) were examined in isolated ventricular myocytes. The Na^+ current was recorded by applying 50-ms depolarizing pulses to −30 mV from a holding potential of −120 mV at 1.0 Hz. During a train of 30 pulses after a 5-min quiescent period, the peak Na^+ current showed little change in the absence of the drug. After the train of 30 pulses, the cells were exposed to the external solution containing 10 or 100 μM UK-1745 without delivering any test pulses. After 3 min superfusion of the drug-containing solution, a train of 30 pulses were delivered. The tonic block, which was

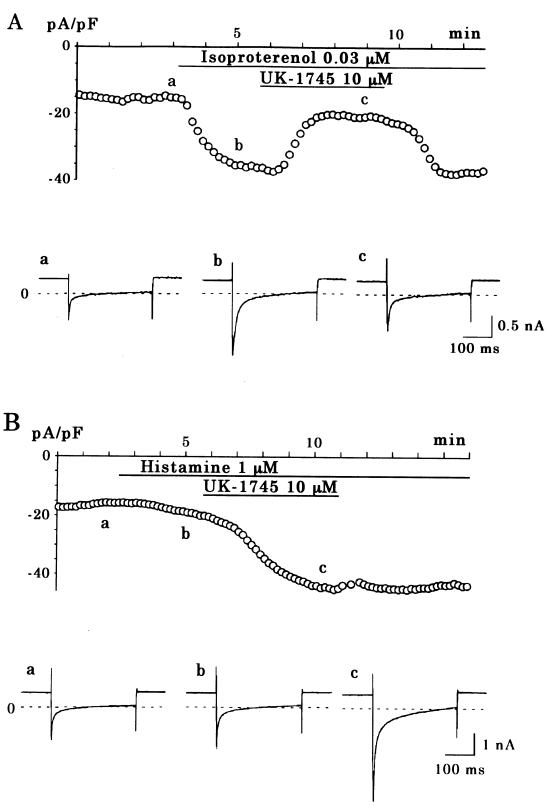

Fig. 5. Effects of UK-1745 on the L-type calcium current (I_{Ca}) in guinea-pig ventricular cells. Time course changes of I_{Ca}, which was elicited by a 300-ms depolarizing test pulse to 0 mV from a holding potential of −40 mV, are shown in the upper part of each panel. (A) Effects of UK-1745 (10 μM) on the isoproterenol (0.03 μM)-induced increase in I_{Ca}. Actual current traces obtained at the time point of a, b and c are shown in lower part. (B) Effects of UK-1745 (10 μM) on the histamine (1 μM)-induced increase in I_{Ca}. Actual current traces obtained at the time point of a, b and c are shown in lower part. The dotted lines indicate the zero current level.

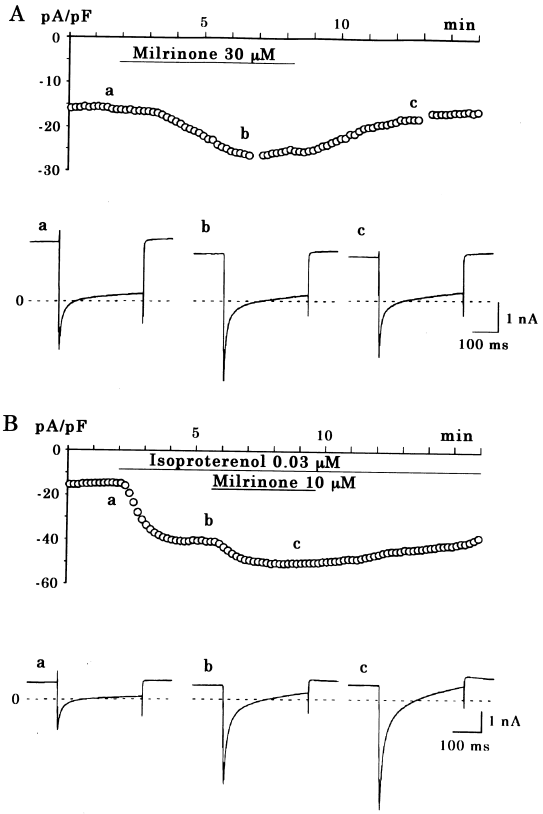

Fig. 6. Effects of milrinone on the L-type calcium current (I_{Ca}) in guinea-pig ventricular cells. Time course changes of I_{Ca}, which was elicited by a 300-ms depolarizing test pulse to 0 mV from a holding potential of −40 mV, are shown in the upper part of each panel. (A) Effects of milrinone (30 μM) on I_{Ca}. Actual current traces obtained at the time point of a, b and c are shown in lower part. (B) Effects of milrinone (10 μM) on the isoproterenol (0.03 μM)-induced increase in I_{Ca}. Actual current traces obtained at the time point of a, b and c are shown in lower part. The dotted lines indicate the zero current level.

designated as a percent decrease in the Na^+ current of the first depolarizing pulse of the train after a pulse-free period, was $9.8 \pm 9.4\%$ ($n = 6$, n.s.) and $55.3 \pm 7.9\%$ ($P < 0.05$, $n = 5$) with 10 and 100 μM UK-1745, respectively (Fig. 7). The peak Na^+ current was slightly and gradually decreased with successive pulses, indicating that use-dependent block with this drug would be small. Total decrease in the peak Na^+ current by 10 and 100 μM UK-1745 was $13.0 \pm 10.2\%$ ($n = 6$, n.s.) and $65.7 \pm 8.1\%$ ($P < 0.05$, $n = 5$), respectively. Such a large tonic block and a small use-dependent block with UK-1745 were confirmed in the measurement of $\dot{V}_{max}$ of the action potential in guinea-pig papillary muscles (data not shown).

4. Discussion

As substitute for digitalis glycoside, a number of new cardiotonic drugs with diverse mechanisms of action have emerged. However, most of them failed to give consistent clinical benefits with long term therapy (Reddy et al., 1997). The goals of the treatment of congestive heart failure may be improvement of survival rate and relief of symptoms. Such a consideration led to the search for a cardiotonic drug having mild positive inotropic action, vasodilating action, neutral chronotropic action and antiarrhythmic action. Consequently, UK-1745 was developed as a cardiotonic drug having these characteristics (Uchida et al., 1998).

UK-1745 was shown to produce a positive inotropic response, which was comparable to that obtained with vesnarinone, in isolated atrial preparations of the guinea pig and blood-perfused papillary muscle preparations of the dog (Uchida et al., 1998). The drug has also been reported to produce a weak but definite concentration-dependent positive inotropic effect in isolated canine right ventricular trabeculae (Sawada and Endoh, 1999). In this study, UK-1745 produced a mild positive inotropic response, which was smaller than that produced by milrinone, in guinea-pig papillary muscles stimulated at 0.5 Hz. In terms of chronotropic action, UK-1745 was shown to produce a slight negative chronotropic effect in its high concentrations in isolated right atrial preparations of guinea pigs (Uchida et al., 1998). Such a neutral chronotropic effect has been observed with several cardiotonic drugs such as vesnarinone (Satoh and Hashimoto, 1984), OPC-18970 (Hosokawa et al., 1992), MCI-154 (Eto et al., 1998) and (+)-EMD 60263 (Ravens et al., 1997). Lack of a marked positive chronotropic response is in sharp contrast with cardiotonic drugs having a potent phosphodiesterase III-inhibitory action such as milrinone (Brunkhorst et al., 1989). Moreover, UK-1745 was demonstrated to exert antiarrhythmic action (Uchida et al., 1998). In chloroform-induced arrhythmias in mice, UK-1745 inhibited ventricular fibrillation more potently than mexiletine. Therefore, we thought that it would be of importance to examine the electropharmacological effect of UK-1745.

In guinea-pig papillary muscles stimulated at 0.5 Hz, UK-1745 very slightly prolonged the APD at its low concentrations and significantly decreased $\dot{V}_{max}$ at its high concentration (100 μM). These findings suggest that UK-1745 inhibits Na^+ and K^+ channels. In support of this concept is the observations in the patch–clamp experiments that UK-1745 at a concentration of 100 μM inhibited the outward tail current recorded on clamp back from depolarizing pulses and the Na^+ current elicited by depolarizing pulses. Therefore, UK-1745 might inhibit the delayed rectifier K^+ current (I_K) and the fast Na^+ current (I_{Na}). However, the prolonging effect of UK-1745 on the action potential was less prominent at its highest concentration. The inhibitory effects of UK-1745 on the Na^+ window current and the L-type Ca^{2+} current might counteract the action potential prolongation resulting from the K^+ channel inhibition. In contrast, milrinone slightly shortened the APD. The decrease in the APD might be attributable to an increase in the delayed rectifier K^+

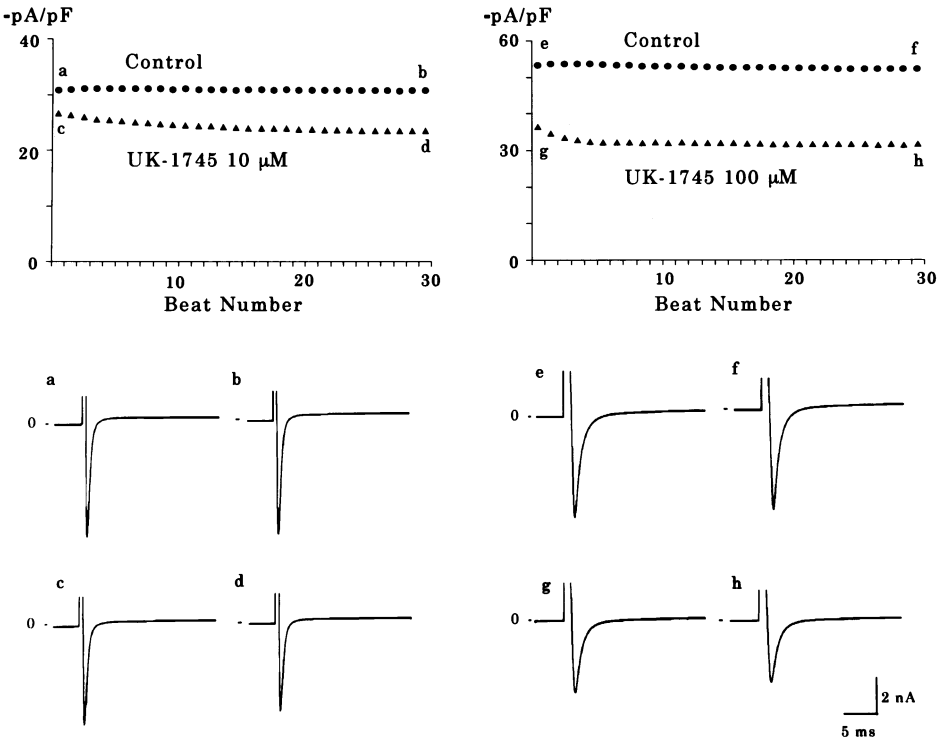

Fig. 7. Inhibitory effects of UK-1745 on the Na^+ current in single ventricular cells. A train of 30 pulses of 50-ms duration was applied to the cell after a pulse-free period in the absence (Control) and the presence of 10 (left) or 100 μM UK-1745 (right). Actual current traces induced by the first (a, c, e and g) and the 30th (b, d, f and h) depolarizing pulses are shown in the lower panels. Note that UK-1745 produced a tonic block of the Na^+ current.

current. It is well known that an increase in intracellular cAMP leads to increase in the K^+ current in isolated ventricular myocytes (Kameyama et al., 1985; Yazawa and Kameyama, 1990). These findings may imply that milrinone produces a greater increase in intracellular cAMP than UK-1745.

Consistent with previous reports (Malecot et al., 1986; Sutko et al., 1986; Fujino et al., 1988; Fischmeister and Hartzell, 1990; Kirstein et al., 1995; Varró and Papp, 1995), milrinone per se increased the L-type Ca^{2+} current. In addition, the drug also potentiated the isoproterenol- and histamine-induced increases in the Ca^{2+} current, indicating the phosphodiesterase-III inhibitory action of milrinone. On the other hand, UK-1745 failed to increase the Ca^{2+} current in concentrations examined and significantly decreased the current at its highest concentration (100 μM). These findings suggest that UK-1745 possesses a direct Ca^{2+} channel blocking action and the increasing effect of the drug on the Ca^{2+} current might be too small to overcome the run down or the direct inhibitory effect. UK-1745 at a concentration of 10 μM effectively antagonized the isoproterenol-induced increase in the Ca^{2+} current although the drug potentiated the histamine-induced increase in the Ca^{2+} current. UK-1745 has been shown to possess a β-adrenoceptor blocking action (Uchida et al., 1998; Sawada and Endoh, 1999). In isolated canine ventricular muscles UK-1745 antagonized the positive inotropic response to isoproterenol with a pA2 value of 5.70 (Sawada and Endoh, 1999). In addition, a receptor binding study showed that UK-1745 competitively inhibited the binding of [^{125}I]iodopindolol to rat cortical membrane (Kawada et al., 1996). The affinity of UK-1745 for β_1-adrenoceptors was about 10 times higher than that for β_2-adrenoceptors (Kawada et al., unpublished observation). Therefore, the inhibitory action of 10 μM UK-1745 on the isoproterenol-induced increase in the Ca^{2+} current might be mainly due to the β_1-adrenoreceptor blocking action and the contribution of the direct inhibitory action on the L-type Ca^{2+} channel might be small. Consistent with previous reports (Hescheler et al., 1987; Yazawa and Abiko, 1993), histamine slightly increased the Ca^{2+} current, probably via the activation of histamine-H_2 receptor-cAMP pathway, in guinea-pig ventricular myocytes. UK-1745 potentiated the histamine-induced increase in the Ca^{2+} current. This effect could be ascribed to the phosphodiesterase III-inhibitory action. UK-1745 was shown to selectively inhibit phosphodiesterase III from dog cardiac muscle with IC_{50} values of 11.5 μM (Uchida et al., 1998; Kawada et al., unpublished observation). It may be curious that UK-1745 increased the Ca^{2+} current in the presence of histamine but decreased the current in the presence of isoproterenol. The β-adrenoceptor blocking action of the drug might be more overt than the cAMP-generating action resulting from the phosphodiesterase III inhibition. UK-1745 increased the Ca^{2+} current in the presence of histamine but not in the basal condition. Since the in-

hibitory action of UK-1745 on the phosphodiesterase III might not be so strong as milrinone, the drug might increase the intracellular cAMP level markedly in the presence of the tonic stimulation of the cAMP-generating system but very slightly in the basal condition.

Although UK-1745 produced a mild positive inotropic response in guinea-pig papillary muscles, the drug failed to increase the Ca^{2+} current in guinea-pig ventricular cells. One may ask how UK-1745 produced a positive inotropic response in spite of the Ca^{2+} channel blocking action. One possible explanation may be that the stimulatory effect of UK-1745 on the Ca^{2+} current might be too small to overcome the run-down of the current, which is inevitable in such experimental conditions using patch–clamp techniques. The second explanation may be that the Ca^{2+} channel blocking action of UK-1745 might be voltage-dependent and the inhibitory effect on the current might be less prominent in normally-polarized ventricular muscles, in which a mild positive inotropic was observed. Accumulation of intracellular cAMP is known to lead to a positive inotropic response by producing an increase in transmembrane Ca^{2+} influx through phosphorylated Ca^{2+} channels and an increase in the accumulation rate of Ca^{2+} into the sarcoplasmic reticulum associated with phospholamban phosphorylation (Tada and Katz, 1982). Therefore, UK-1745 might produce a positive inotropy by increasing Ca^{2+} uptake and Ca^{2+} release through the sarcoplasmic reticulum even if the transmembrane Ca^{2+} influx was reduced. However, the half relaxation time of contraction, which reflects the accumulation rate of Ca^{2+} into the sarcoplasmic reticulum, was not shortened by UK-1745 although milrinone produced a marked positive inotropic response concomitantly with a significant decrease in the half-relaxation time. A slight action potential prolongation secondary to the K^+ channel inhibition by UK-1745 might partly mask the accelerated relaxation. Whatever the mechanism(s) involved, it can be concluded that UK-1745 can produce a mild positive inotropic response without excessive increase of transmembrane Ca^{2+} influx.

It has long been assumed and taught that β-adrenoceptor antagonists are contraindicated in heart failure because of their short-term adverse effects (Epstein and Braunwald, 1966). However, controlled clinical trials of several different β-adrenoceptor antagonists have shown that these drugs can reduce symptoms, improve left ventricular function, increase functional capacity and reduce the risk of death (Waagstein et al., 1993; CIBIS Investigators and Committees 1994; Packer et al., 1996). Although mechanism(s) underlying the beneficial effects of β-adrenoceptor antagonists have not been well-defined, reduction of the detrimental, noradrenaline-induced tachycardia and/or upregulation of cardiac β-adrenoceptors as a result of exposure to the receptor antagonist have been proposed (van Zwieten, 1996). Since UK-1745 shows β-adrenoceptor blocking action, it may also provide the beneficial outcome during long term treatment of congestive heart failure. However, in some patients it is difficult to administer a pure β-adrenoceptor antagonist because of exacerbation of cardiac contractile dysfunction (Charlap et al., 1989). In such clinical setting, a β-adrenoceptor antagonist with a mild cardiotonic action such as UK-1745 may be used for the improvement of quality of life as well as long term prognosis. In this context, a recent report (Shakar et al., 1998) has shown that combination therapy with enoximone, a phosphodiesterase inhibitor, and metoprolol, an β_1-adrenoceptor antagonist, markedly improved the estimated probability of survival in patients with severe, class IV heart failure. The mortality associated with the regimen was better than that observed in CONSENSUS for patients treated with enalapril (The CONSENSUS Trial Study Group, 1987).

UK-1745 was demonstrated to exert antiarrhythmic action against chloroform-induced arrhythmias in mice. Since propranolol was shown to prevent the development of ventricular fibrillation in this model (Lawson, 1968), the β-adrenoceptor blocking action of UK-1745 might play an important role. In addition, UK-1745 did not aggravate the ventricular arrhythmias induced by two-stage coronary ligation (Kawada et al., 1996), which was shown to be aggravated by cAMP-generating cardiotonic drugs such as amrinone and vesnarinone (Piwonka et al., 1983; Hashimoto and Mitsuhashi, 1986). From in vivo animal experiments the therapeutic concentration of UK-1745 would be expected to be from 0.3 to 10 μM. Therefore, the β-adrenoceptor blocking action may be more important for the efficacy against various arrhythmias than the direct inhibitory action on cardiac Na^+ and Ca^{2+} channels.

In conclusion, UK-1745 is a unique cardiotonic drug having β-adrenoceptor blocking and phosphodiesterase III-inhibitory actions in addition to the direct inhibitory actions on the Na^+, Ca^{2+} and K^+ channels with its high concentrations.

Acknowledgements

The authors thank M. Tamagawa, I. Sakurada and Y. Furusawa for their technical assistance and I. Sakashita for her secretarial assistance.

References

Brunkhorst, D., Leyden, H., Meyer, W., Nigbur, R., Schmidt-Schumacher, C., Scholz, H., 1989. Relation of positive inotropic and chronotropic effects of pimobendan, UD-CG212CI, milrinone and other phosphodiesterase inhibitors to phosphodiesterase III inhibition in guinea pig heart. Naunyn-Schmiedeberg's Arch. Pharmacol. 339, 575–583.

Charlap, S., Lichstein, E., Frishman, W.H., 1989. β-Adrenergic blocking drugs in the treatment of congestive heart failure. Med. Clin. North Am. 73, 373–385.

CIBIS Investigators and Committees, 1994. A randomized trial of beta-blockade in heart failure: the Cardiac Insufficiency Bisoprolol Study (CIBIS). Circulation 90, 1665–1673.

Epstein, S.E., Braunwald, E., 1966. The effects of beta adrenergic blockade on patterns of urinary sodium excretion: studies in normal subjects and in patients with heart disease. Ann. Intern. Med. 65, 20–27.

Eto, K., Hashimoto, K., Nakaya, H., 1998. Preferential inhibition of IKr by MCI-154, a putative cardiotonic Ca^{2+} sensitizer, in guinea pig atrial cells. Cardiovasc. Res. 38, 685–694.

Fabiato, A., Fabiato, F., 1979. Calculator programs for computing the composition of solution containing multiple metals and legend used for experiments in skinned muscle cells. J. Physiol. (Paris) 75, 463–505.

Fischmeister, R., Hartzell, H.C., 1990. Regulation of calcium current by low-Km cyclic AMP phosphodiesterases in cardiac cells. Mol. Pharmacol. 38, 426–433.

Fujino, K., Sperelakis, N., Solaro, R.J., 1988. Sensitization of dog and guinea pig heart myofilaments to Ca^{2+} activation and the inotropic effect of pimobendan: comparison with milrinone. Circ. Res. 63, 911–922.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J., 1981. Improved patch–clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. 391, 85–100.

Hara, Y., Nakaya, H., 1995. SD-3212, a new class I and IV antiarrhythmic drug: a potent inhibitor of the muscarinic acetylcholine-receptor-operated potassium current in guinea-pig atrial cells. Br. J. Pharmacol. 116, 2750–2756.

Hashimoto, K., Mitsuhashi, H., 1986. Effects of OPC-8212, a new positive inotropic agent, on canine ventricular arrhythmias. Br. J. Pharmacol. 88, 915–921.

Hescheler, J., Tang, M., Jastorff, B., Trautwein, M., 1987. On the mechanism of histamine induced enhancement of the cardiac Ca^{2+} current. Pflügers Arch. 410, 23–29.

Hosokawa, T., Mori, T., Fujiki, H., Kinoshita, S., Takemoto, K., Imaizumi, T., Noda, T., Ohura, M., Tominaga, M., Yabuuchi, Y., 1992. Cardiovascular actions of OPC-18790: a novel positive inotropic agent with little chronotropic action. Heart Vessels 7, 66–75.

Isenberg, G., Klockner, U., 1982. Calcium tolerant ventricular myocytes prepared by preincubation in a ''KB medium''. Pflügers Arch. 395, 6–18.

Kameyama, M., Hofmann, F., Trautwein, W., 1985. On the mechanism of β-adrenergic regulation of the Ca channel in the guinea-pig heart. Pflügers Arch. 405, 285–293.

Kawada, M., Sawanobori, K., Sonoki, H., Inoue, K., Mizuno, K., Itou, T., Kyoutani, Y., Uchida, Y., 1996. Cardiovascular effects of furo[2,3-e]indole derivative UK-1745, a novel cardiotonic agent. Jpn. J. Pharmacol. 71 (Suppl. I), 229 pp.

Kirstein, M., Rivet-Bastide, M., Hatem, S., Benardeau, A., Mercadier, J.J., Fischmeister, R., 1995. Nitric oxide regulates the calcium current in isolated human atrial myocytes. J. Clin. Invest. 95, 794–802.

Lawson, J.W., 1968. Antiarrhythmic activity of some isoquinoline derivatives determined by a rapid screening procedure in the mouse. J. Pharmacol. Exp. Ther. 160, 22–31.

Malecot, C.O., Bers, D.M., Katzung, B.G., 1986. Biphasic contractions induced by milrinone at low temperature in ferret ventricular muscle: role of the sarcoplasmic reticulum and transmembrane calcium influx. Circ. Res. 59, 151–162.

Nakaya, H., Tohse, N., Takeda, Y., Kanno, M., 1993. Effects of MS-551, a new class III antiarrhythmic drug, on action potential and membrane currents in rabbit ventricular myocytes. Br. J. Pharmacol. 109, 157–163.

Packer, M., Medira, N., Yushak, M., 1984. Hemodynamic and clinical limitations of long-term inotropic therapy with amrinone in patients with severe chronic heart failure. Circulation 70, 1038–1047.

Packer, M., Carver, J.R., Rodeheffer, R.J., Ivanhoe, R.J., Zeldis, S.M., Hendrix, G.H., Bommer, W.J., Elkayam, U., Kukin, M.L., Mallis, G.I., Sollano, J.A., Shannon, J., Tandon, P.K., DeMets, D.L., 1991. Effect of oral milrinone on mortality in severe chronic heart failure. N. Engl. J. Med. 325, 1468–1475.

Packer, M., Bristow, M.R., Cohn, J.N., Colucci, W.S., Fowler, M.B., Gilbert, E.M., Shusterman, N.H., 1996. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N. Engl. J. Med. 334, 1349–1355.

Piwonka, R.W., Healey, J.F., Caniff, P.C., Farah, A.E., 1983. Electrophysiological actions of amrinone in dogs with cardiac lesions. J. Cardiovasc. Pharmacol. 5, 1052–1057.

Ravens, U., Flüß, M.O., Li, Q., Himmel, H.M., Wettwer, E., Klocknow, M., Lues, I., 1997. Stereoselectivity of actions of the calcium sensitizer [+]-EMD60263 and its enantiomer [−]-EMD60264. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 733–742.

Reddy, S., Benatar, D., Gheorghiade, M., 1997. Update on digoxin and other oral positive inotropic agents for chronic heart failure. Curr. Opin. Cardiol. 12, 233–241.

Satoh, H., Hashimoto, K., 1984. Effect of 3,4-dihydro-6[4-(3,4-dimethoxy-benzoyl)-1-piperazyl]-2(1*H*)-quinolinone (OPC-8212) on the membrane currents of rabbit sino-atrial node. Arzneim.-Forsch. 34, 376–380.

Sawada, H., Endoh, M., 1999. Pharmacological characterization of effects of UK-1745, a novel cardiotonic agent with β-adrenoceptor-blocking action, in aequorin-loaded canine right ventricular muscle. J. Mol. Cell. Cardiol. 31, 1047–1052.

Shakar, S.F., Abraham, W.T., Gilbert, E.M., Rabertson, A.D., Lowes, B.D., Zisman, L.S., Ferguson, D.A., Bristow, M.R., 1998. Combined oral positive inotropic and beta-blocker therapy for treatment of class IV heart failure. J. Am. Coll. Cardiol. 31, 1336–1340.

Sutko, J.L., Kenyon, J.L., Reeves, J.P., 1986. Effects of amrinone and milrinone on calcium influx into the myocardium. Circulation 73, 11152–11158.

Tada, M., Katz, A.M., 1982. Phosphorylation of the sarcoplasmic reticulum and sarcolemma. Annu. Rev. Physiol. 44, 401–423.

Tanaka, H., Furukawa, T., Hayafuji, M., Habuchi, Y., 1991. Modulation of the delayed K^+ current by histamine in guinea pig ventricular myocytes. Naunyn-Schmiedeberg's Arch. Pharmacol. 344, 582–588.

The CONSENSUS Trial Study Group, 1987. Effects of enalapril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N. Engl. J. Med. 316, 1429–1435.

Tohse, N., Nakaya, H., Kanno, M., 1992. α_1-Adrenoceptor stimulation enhances the delayed K^+ current of guinea pig ventricular cells through the activation of protein kinase C. Circ. Res. 71, 1441–1446.

Tsien, R.Y., Rink, T.J., 1980. Neutral carrier ion-sensitive microelectrode for measurement of intracellular calcium. Biochem. Biophys. Acta 599, 623–638.

Uchida, Y., Kawada, M., Sawanobori, K., Sonoki, H., Inoue, K., Mizuno, K., Itou, T., Takunoki, Y., Tsukamoto, M., Ohashi, Y., Kyoutani, Y., Shimizu, N., Fujii, M., Nakamura, M., 1998. Cardiovascular effects of (2*RS*,3*SR*)-2-aminomethyl-2,3,7,8-tetrahydro-2,3,5,8,8-pentamethyl-6*H*-furo-[2,3-e] indol-7-one hydrochloride (UK-1745), a novel cardiotonic agent with vasodilatory and antiarrhythmic properties. Arzneim.-Forsch. 48, 219–231.

Uchida, Y., Sonoki, H., 1996. Effect of UK-1745, a novel furoindole derivative with both cardiotonic and antiarrhythmic actions, on chronic congestive heart failure in dogs. Jpn. J. Pharmacol. 71 (Suppl. I), 227 pp.

Uretsky, B.F., Jessup, M., Konstam, M.A., William, G., Leier, C.V., Benotti, J., Murali, S., Herrmann, H.C., Sandberg, J.A., 1990. Multicenter trial of oral enoximone in patients with moderate to moderately severe congestive heart failure. Lack of benefit compared with placebo. Circulation 82, 774–780.

van Zwieten, P.A., 1996. Current and newer approaches in the drug treatment of congestive heart failure. Cardiovasc. Drugs Ther. 10, 693–702.

Varró, A., Papp, J.G., 1995. Classification of positive inotropic actions based on electrophysiologic characteristics: where should calcium sensitizer be placed? J. Cardiovasc. Pharmacol. 26 (Suppl 1), S32–S44.

Waagstein, F., Bristow, M.R., Swedberg, K., Camerini, F., Fowler, M.B., Silver, M.A., 1993. Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy for the Metoprolol in Dilated Cardiomyopathy (MDC) Trial Study Group. Lancet 342, 1441–1446.

Yazawa, K., Abiko, Y., 1993. Modulation by histamine of the delayed outward potassium current in guinea-pig ventricular myocytes. Br. J. Pharmacol. 109, 142–147.

Yazawa, K., Kameyama, M., 1990. Mechanism of receptor-mediated modulation on the delayed outward potassium current in guinea-pig ventricular myocytes. J. Physiol. (London) 421, 135–150.