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Effects of MCI-154, a new cardiotonic Ca^{2+} sensitizer, on ventricular arrhythmias and membrane ionic currents

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Abstract

We examined whether a new positive inotropic agent with Ca^{2+}-sensitizing activity, MCI-154, 6-[4-(4-pyridylamino)phenyl]-4,5-dihydro-3-(2*H*)-pyridazinone hydrochloride trihydrate, had deleterious effects on ventricular arrhythmias, since several phosphodiesterase III inhibitors have been shown to aggravate arrhythmias in our earlier studies. Continuous infusion of MCI-154 (1 μg/kg/min for 15 min) did not suppress or aggravate the arrhythmias generated in the two-stage coronary ligation-, digitalis- and adrenaline-induced canine arrhythmia models studied. Also in the case of a bolus injection of 30 μg/kg, MCI-154 did not aggravate the adrenaline-induced arrhythmias. To explain these results in vivo, a whole-cell voltage-clamp experiment on guinea-pig ventricular cells was performed. MCI-154 (10–100 μM) did not increase the inward Ca^{2+} current under the condition where these currents were increased by isoprenaline. These results indicate that MCI-154 does not aggravate ventricular arrhythmias and does not act on membrane currents associated with arrhythmogenesis. Thus, MCI-154 may become a useful positive inotropic agent with little arrhythmogenic effect.

Keywords: MCI-154 (6-[4-(4-pyridylamino)phenyl]-4,5-dihydro-3-(2*H*)-pyridazinone); Ventricular arrhythmia; Heart failure; Ca^{2+} current

1. Introduction

Positive inotropic compounds for the treatment of heart failure should act without inducing deleterious arrhythmias such as ventricular tachycardia or ventricular fibrillation. Cardiac glycosides and β-adrenoceptor agonists have been known to increase intracellular Ca^{2+} concentrations, hence they sometimes increase the myocardial oxygen demand and induce ventricular tachycardia and/or ventricular fibrillation in failing hearts. In recent years, positive inotropic compounds with a new mechanism of cardiotonic action have been developed. One type of these new drugs increases the Ca^{2+} sensitivity of the cardiac contractile system (Jonas et al., 1992; Kitada et al., 1987a, b, 1989a; Herzig et al., 1981; Ruegg et al., 1984). These Ca^{2+} sensitizers are expected to treat heart failure without increasing the risk of deleterious arrhythmias or sparing the energy for handling intracellular Ca^{2+}.

MCI-154, 6-[4-(4-pyridylamino)phenyl]-4,5-dihydro-3-(2*H*)-pyridazinone hydrochloride trihydrate, is a newly synthesized orally active positive inotropic agent (Narimatsu et al., 1987). In vivo experiments using anesthetized and conscious dogs revealed that MCI-154 in the dose range of 0.3–100 μg/kg exerted dose-dependent increases in dP/dt_{max} which accompanied simultaneous vasodilatory and weak positive chonotropic effects (Narimatsu et al., 1987; Satoh et al., 1988). MCI-154 has been reported to increase the myofibrillar Ca^{2+} sensitivity and the maximal Ca^{2+}-activated force in cardiac skinned fibers of the dog, guinea pig (Kitada et al., 1987a, b, 1989a, b) and human (Perreault et al., 1989), and also to improve the contractile function of depressed canine hearts (Abe et al., 1991, 1992, 1993). MCI-154 within the positive inotropic doses did not change the myocardial cyclic AMP levels (Kitada et al., 1987c) and the membrane Ca^{2+} current of ventricular cells (Katayama et al., 1987). If there are no changes in the cardiac depolarizing currents at positive inotropic doses of MCI-154, this drug should not alter the electrical behavior of the heart and hence should not aggravate ventricular arrhythmias. Since deleterious arrhythmias are often present in patients with congestive heart failure, it is of great importance to evaluate the proarrhythmic properties of MCI-154.

The present study was designed to investigate the effects of MCI-154 on various types of spontaneously occur-

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ring canine ventricular arrhythmias produced by coronary ligation, digitalis intoxication and halothane-adrenaline interactions. In previous studies using the same experimental models, we have shown that new inotropic agents with a phosphodiesterase III inhibitory action such as amrinone, milrinone, vesnarinone and OPC-18790 have arrhythmogenic effects (Hashimoto and Mitsuhashi, 1986; Hashimoto et al., 1990; Wu et al., 1993). We also examined the effects of MCI-154 on membrane ionic currents, especially increased Ca^{2+} currents induced by the β-adrenoceptor agonist, isoprenaline, in single ventricular cells of the guinea-pig using the whole-cell voltage-clamp method.

2. Materials and methods

2.1. Canine ventricular arrhythmia models

2.1.1. Production of two-stage coronary ligation-induced arrhythmia

Six female beagles weighing 8.5–11 kg were anesthetized initially with intravenous (i.v.) thiopental sodium 30 mg/kg and intubated. As reported earlier (Hashimoto et al., 1982), the chest was opened and a two-stage coronary ligation of the left anterior descending artery was performed under halothane anesthesia. Experiments were performed without anesthesia 24 and 48 h after coronary ligation. The lead II ECG, atrial electrogram from implanted electrodes sutured on the left atrial appendage, and instantaneous blood pressure and mean blood pressure were recorded continuously by a telemetry system (Nihon Kohden WEB-5000, Tokyo, Japan). MCI-154 1 μg/kg/min was continuously infused for 15 min in the right external jugular vein. Arterial blood samples were drawn from one lumen of the arterial double lumen cannula at 0, 5, 10, 15, 20, and 60 min after starting drug administration.

2.1.2. Production of digitalis-induced arrhythmia

Fourteen female beagles weighing 8–11.5 kg were anesthetized with i.v. pentobarbital sodium 30 mg/kg. As reported earlier (Hashimoto et al., 1985), 40 μg/kg ouabain was injected i.v. followed by an additional 10 μg/kg every 20 min until stable ventricular tachycardia was produced (total dose = 66 ± 5 μg/kg, $n = 14$). In one group of six beagles, MCI-154 1 μg/kg/min was continuously infused for 15 min in the right femoral vein. Arterial blood samples were drawn from one lumen of the arterial double lumen cannula at 0, 5, 10, 15, 20, and 60 min after starting drug administration. In another group of eight beagles, bolus injections of 15 μg/kg (two beagles) and 30 μg/kg (six beagles) of MCI-154 were given within seconds. Arterial blood samples were drawn from one lumen of the arterial double lumen cannula 1 min before and 1, 3, 5, and 60 min after drug administration. The lead II ECG, atrial electrogram from catheter tip electrodes in the right atrium, and instantaneous blood pressure and mean blood pressure were continuously recorded.

2.1.3. Production of adrenaline-induced arrhythmia

Twelve mongrel dogs of either sex, weighing 12–18 kg, were anesthetized initially with thiopental sodium 30 mg/kg. As reported earlier (Shibuya et al., 1983), after intubation, 1% halothane vaporized with 100% O_2 was administered with a volume-limited ventilator (20 ml/kg, 15 strokes/min). Adrenaline was infused through the right femoral vein from one lumen of a double lumen cannula at an initial dose of 1.5 μg/kg/min with a syringe pump. If multifocal ventricular tachycardia or premature ventricular contraction including bigeminal pulses were not induced or these arrhythmias converted spontaneously to sinus rhythm during the course of adrenaline infusion, the dose of adrenaline was increased by an increment of 0.5 μg/kg/min. Usually 1.5–3.0 μg/kg/min adrenaline was sufficient to produce multifocal ventricular tachycardia or ventricular bigeminy. In one group of six mongrel dogs, from 3 min after the start of adrenaline infusion, 1 μg/kg/min of MCI-154 was continuously infused for 15 min in the right femoral vein from another lumen of the double lumen cannula. In another group of six mongrel dogs, a higher dose of MCI-154 (30 μg/kg) was injected as a bolus in the right femoral vein within seconds. Arterial blood samples were drawn from one lumen of the arterial double lumen cannula; in the intravenous infusion group, samples were drawn 1 min before and 5, 10, and 15 min after, while in the bolus injection group samples were drawn 1 min before and 1, 3, 5, and 15 min, after starting drug administration. The lead II ECG, atrial electrogram from catheter tip electrodes in the right atrium, and instantaneous blood pressure and mean blood pressure were continuously recorded.

2.1.4. Statistical analysis

Data are expressed as means ± S.D. One-way analysis of variance (ANOVA) was used to compare the results obtained in each time-course group. If the ANOVA value was significant, comparisons between the pre- and post-drug values were made by one-way ANOVA followed by Scheffé's F-test to localize the significant difference; $P < 0.05$ was considered significant. For examining the correlation between the plasma concentrations of drug and another parameters, the method of Pearson's correlation coefficient followed by Fisher's r to z test was used. All analyses were done with Stat View 4.0 statistical software (Abacus Concepts, CA, USA) and Excel 4.0 (Microsoft Corporation, WA, USA) on a Macintosh personal computer.

2.1.5. Evaluation of antiarrhythmic effects

The severity of ventricular arrhythmia was expressed by the arrhythmic ratio: the number of ventricular ectopic

beats divided by the total heart rate, which is the number of ventricular ectopic beats plus the number of conducted beats. The ventricular ectopic beats were judged by the different shape of the ventricular complex from the normal QRS complex. The arrhythmic ratio before drug administration was almost 1, except for the adrenaline-induced arrhythmia model, in which the arrhythmic ratio was 0.5–1 for the purpose of examining whether MCI-154 increases the arrhythmic ratio and induces deleterious arrhythmias, i.e., induction of ventricular fibrillation. In all the arrhythmia models, there were no spontaneous improvements in these ratios after a saline infusion. If the values after drug administration were decreased or increased significantly from the 0 time value, the drug was judged to have antiarrhythmic or proarrhythmic effects, respectively.

2.1.6. Determination of MCI-154 plasma concentration

A sensitive and specific determination of MCI-154 in plasma was performed at Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan by the following radioimmunoassay method. The anti-MCI-154 antiserum was obtained using Japanese White rabbits immunized with complexes which are composed of a MCI-154 derivative and bovine serum albumin conjugated by glutaraldehyde in complete Freund's adjuvant. This antiserum was diluted to 5000-fold for the radioimmunoassay. ^{125}I-MCI-154 was synthesized by a modification of the chloramine-T method (Hunter and Greenwood, 1962; Greenwood et al., 1963). The ^{125}I-labelled MCI-154 was purified by exclusion chromatography on a Sephadex C-25 column (Pharmacia, Uppsala, Sweden). RIA for MCI-154 was carried out at 4°C in 63 mM phosphate buffer (pH 7.4, containing 13 mM EDTA-2Na, 230 μl). MCI-154 standard or unknown samples (20 μl) were preincubated with ^{125}I-MCI-154 (100 μl, approximately 8000 cpm), anti-MCI-154 antiserum (100 μl), and canine control serum (50 μl) for 24 h. After this preincubation, rabbit control serum (IBL, Fujioka, Japan, 100 μl) and goat anti-rabbit antiserum diluted to 20-fold (Nippon Tanner, Kobe, Japan, 100 μl) were added and subsequently incubated for 24 h at 4°C. The incubated solution was centrifuged at $1700 \times g$ for 30 min at 4°C. The supernatant was decanted and the radioactivity in the remaining pellet was determined. The sensitivity of measurement reached approximately 0.5 pg/assay tube.

2.2. Whole-cell voltage-clamp experiments

2.2.1. Isolation of single cells

Adult male guinea-pigs (Hartley) weighing 350–450 g were anesthetized with i.p. pentobarbital sodium 30 mg/kg. The heart was excised following a thoracotomy and was mounted on a modified Langendorff perfusion system for retrograde perfusion at a hydrostatic pressure of 70 cm H_2O and a flow rate of approximately 10 ml/min. All the solutions were equilibrated with 100% O_2. The cell dissociation was carried out at 37°C by the following procedure. At first, the heart was perfused by a Langendorff system for 3 min with normal Tyrode's solution followed by a perfusion with zero-Ca^{2+} Tyrode's solution for 8 min. At the next step, after perfusion for 8–12 min with a low Ca^{2+} Tyrode's solution containing 46 U/ml of collagenase (Yakult, Tokyo, Japan), the surface of the ventricle was swollen and opaque at the end of this procedure. Finally, Kraft-brühe (KB) solution was perfused for 10 min. After this procedure, part of the left ventricle was dissected and placed in a beaker containing KB solution. The tissue was agitated gently with a Pasteur pipette with a relatively large bore (5 mm), yielding a satisfactory number of viable cells. These cells were then stored in O_2-saturated KB solution at a low temperature (4°C) until they were used.

2.2.2. Electrophysiological recordings and analysis

The gigaseal patch clamp technique was used in the whole-cell clamp configuration (Hamil et al., 1981). Glass microelectrodes, with a tip resistance of 3–4 MΩ when filled with the pipette solutions, were fabricated using 1.5-mm diameter glass pipettes (TW 150-6, World Precision Instruments, CT, USA) and a programmable microelectrode puller (Model P-87, Sutter Instrument Co., CA, USA). A patch/whole-cell clamp amplifier (EPC-7, List Electronic, Darmstadt, Germany) was used. All experiments were done at room temperature (25–28°C). Membrane potential and membrane current signals were monitored on a storage oscilloscope (Nihon Kohden, Model VC-10, Tokyo, Japan) and stored in a microcomputer (Compaq, PC-Prolinea) using an analog-to-digital conversion board (Digidata 1200 Interface, Axon Instrument, CA, USA) controlled by pClamp 5.5.1 software (Axon Instrument). Data were digitized at a sampling speed of 4 kHz after filtering at 3 kHz. All solutions flowed to a recording chamber of 1.0 ml at a speed of about 1.5 ml/min.

2.2.3. Solutions and drugs

All solutions were made using glass-distilled water and reagent grade chemicals. The composition of the normal Tyrode's solution (in mM) was: NaCl, 135.0; KCl, 5.4; glucose, 5.5; Hepes, 5.0; $CaCl_2$, 1.8; $MgCl_2$, 0.5; NaH_2PO_4, 0.33. The pH was adjusted to 7.4 with NaOH. Zero-Ca^{2+} or low Ca^{2+} Tyrode's solution was prepared by omitting the $CaCl_2$ or reducing it to 150 μM. The KB medium consisted of (in mM): glutamic acid monopotassium salt, 50.0; KCl, 25.0; taurine, 10.0; KH_2PO_4, 10.0; EGTA, 0.5; glucose, 10.0; Hepes, 10.0; $MgCl_2$, 3.0. The pH was adjusted to 7.4 with KOH. The solution in the recording pipette used for the whole-cell voltage-clamp measurements contained (in mM): potassium aspartate, 120.0; KCl, 30.0; EGTA, 10.0; Hepes, 5.0; ATP-2Na, 4.0; $MgCl_2$, 1.0. The pH was adjusted to 7.2 with KOH. All drugs were dissolved in normal Tyrode's solution. In some experiments the Ca^{2+} current (I_{Ca})was isolated from other membrane currents by using Cs^+-rich external and pipette

solutions. The Cs^+-rich external solution was prepared by replacing KCl in the normal Tyrode's solution with equimolar CsCl. The Cs^+-rich pipette solution consisted of (in mM): *l*-aspartate, 110.0; CsOH, 110.0; CsCl, 20.0; EGTA, 10.0; Hepes, 5.0; ATP-2K, 5.0; $MgCl_2$, 1.0. The pH was adjusted to 7.2 with CsOH and HCl.

Drugs used were MCI-154 (from Mitsubishi Chemical Corporation, Japan) and pimobendan (from Nippon Boehringer Ingelheim Corporation, Japan). The stock solution of MCI-154 10 mM was dissolved in distilled water and diluted to 10–100 μM with the normal Tyrode's or Cs^+-rich external Tyrode's solution. The stock solution of pimobendan 100 mM was dissolved in 100% dimethylsulfoxide (DMSO) and diluted to 1–100 μM with the normal Tyrode's or Cs^+-rich external Tyrode's solution.

3. Results

3.1. Two-stage coronary ligation-induced arrhythmia

After 1–2 days of coronary ligation, all dogs showed continuously occurring multifocal ventricular tachycardia. The arrhythmic ratios at 24 and 48 h after ligation were 0.96 ± 0.09 ($n = 6$) and 0.89 ± 0.18 ($n = 6$), respectively, showing that 48 h arrhythmias were less severe (Fig. 1A). Intravenous infusion of MCI-154 (15 μg/kg/15 min) was used in these studies, which produced prominent positive inotropic effects (about 50% increase of maximal dP/dt) in previous studies (Narimatsu et al., 1987; Abe et al., 1992). On both 24- and 48-h arrhythmias, this dose of MCI-154 showed no antiarrhythmic effects (i.e., no statistically significant decrease in the arrhythmic ratio), or any proarrhythmic effects (i.e. no increase in the arrhythmic ratio, or no induction of ventricular fibrillation or repetitive beats faster than control). The total heart rate and atrial rate were virtually unchanged except the transient increases 14–15 min after the start of the infusion of MCI-154 on 48-h arrhythmias (Fig. 1A). Blood pressure tended to decrease soon after starting the infusion of the drug. At 24 and 48 h after coronary ligation, maximal plasma concentrations of the drug were 21 ± 5 ($n = 6$) and 20 ± 7 ng/ml ($n = 6$) at 15 min after starting the infusion of the drug, respectively. No significant correlations were observed between the plasma concentrations of MCI-154 and the changes in the parameters in this study.

3.2. Digitalis-induced arrhythmia

When the same infusion rate (15 μg/kg/15 min) of MCI-154 used in the two-stage coronary ligation-induced arrhythmia experiments was used, MCI-154 had no antiarrhythmic effect, nor did it aggravate the arrhythmia, as shown in Fig. 1B (the control arrhythmic ratio; 0.96 ± 0.11,

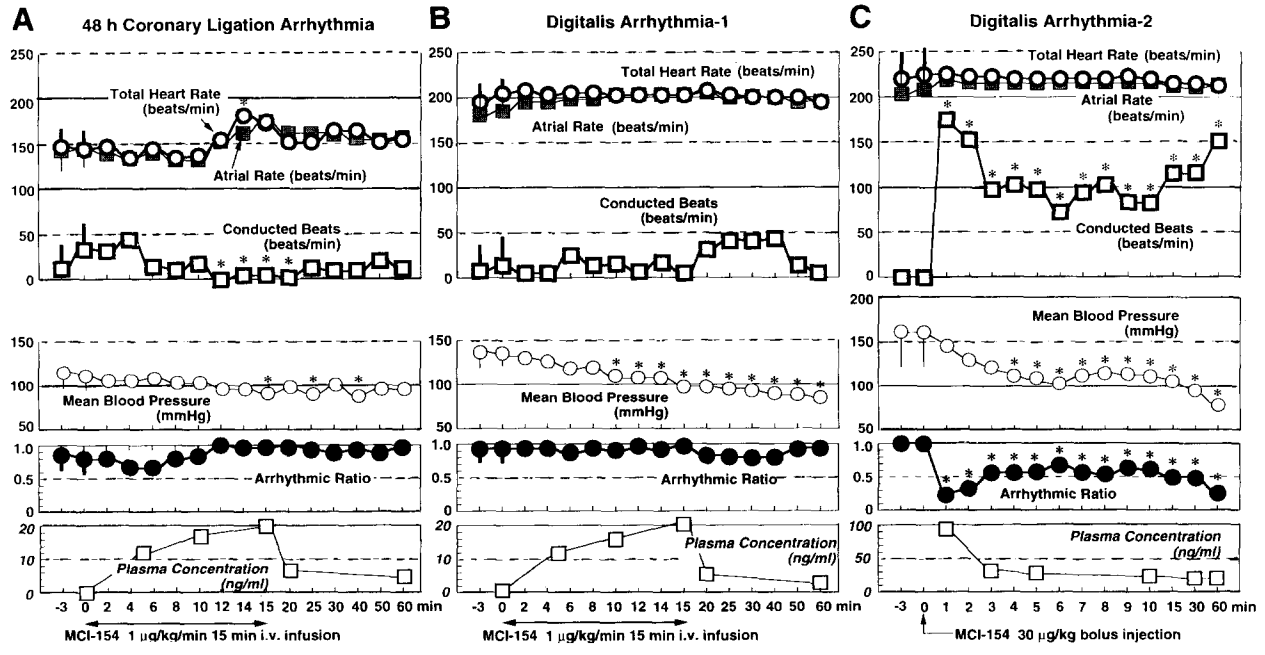

Fig. 1. (A) Summary of the effects of intravenous continuous infusion of MCI-154 (1 μg/kg/15 min, total 15 μg/kg) on the 48-h coronary ligation arrhythmia ($n = 6$) and the plasma concentrations of the drug ($n = 6$). (B) Digitalis arrhythmia-1; summary of the effects of intravenous continuous infusion of MCI-154 (1 μg/kg/15 min, total 15 μg/kg) on the digitalis arrhythmia ($n = 6$) and the plasma concentrations of the drug ($n = 6$). (C) Digitalis arrhythmia-2; summary of the effects of intravenous bolus injection of 30 μg/kg of MCI-154 on the digitalis arrhythmia ($n = 6$) and the plasma concentrations of the drug ($n = 5$). On these three graphs, * marks represent significant changes from 0 time values ($P < 0.05$); S.D.s are shown only at -3 and 0 time. The total heart rate represents the number of ventricular ectopic beats plus the number of conducted beats, and the ventricular ectopic beats were judged by the different shape of the ventricular complex from the normal QRS complex. The arrhythmic ratio represents the number of ventricular ectopic beats divided by the total heart rate.

$n = 6$). MCI-154 did not change the total heart rate and atrial rate. Blood pressure decreased gradually soon after starting the infusion of the drug. The maximal plasma concentration of the drug reached 20 ± 5 ng/ml ($n = 6$) at 15 min after starting the infusion of the drug. No significant correlation was observed between the plasma concentrations of MCI-154 and the changes in the parameters examined in this study.

An i.v. bolus injection of MCI-154 (30 μg/kg) suppressed the arrhythmia immediately after injection, but this effect lasted only for 1–2 min, as shown in Fig. 1C (the arrhythmic ratio decreased from 1.0 at the control to 0.25 ± 0.42 at 1 min, $n = 6$). However, a lower bolus injection of 15 μg/kg MCI-154 did not suppress or aggravate this digitalis-induced arrhythmia ($n = 2$, data not shown). MCI-154 reduced the blood pressure soon after the bolus injection. The peak plasma concentration of MCI-154 was 94 ± 31 ng/ml ($n = 5$) at 1 min after a 30 μg/kg bolus injection. The increase in the conducted beats and decrease in the arrhythmic ratio correlated with the change in the plasma concentration of MCI-154 ($r = -0.471$, $P = 0.004$), but other parameters did not correlate with the change in the plasma concentration.

3.3. Adrenaline-induced arrhythmia

As reported previously (Shibuya et al., 1983), adrenaline infusion for 3 min at a rate of 1.5–3.0 μg/kg/min induced multifocal or monofocal ventricular tachycardia with almost all the beats consisting of ventricular ectopic beats. Infusion of MCI-154 at the same speed (15 μg/kg/15 min) as used in the previous two experiments did not change the arrhythmic ratio or the total heart rate, atrial rate and conducted beats, as shown in Fig. 2A. Blood pressure decreased gradually after starting the infusion of the drug. The decrease in blood pressure correlated with the plasma concentration of MCI-154 ($r = -0.464$, $P = 0.02$). The peak plasma concentration was 9 ± 1 ng/ml, $n = 6$, at 15 min after infusion. Other parameters in this experiment did not correlate with the change in the plasma concentrations.

Since we have demonstrated the proarrhythmic effects (e.g. production of ventricular fibrillation) of other inotropic agents, such as amrinone, milrinone, sulmazole, vesnarinone and OPC-18790, using the halothane-adrenaline-induced ventricular tachyarrhythmia model and bolus injection of the drugs (Hashimoto and Mitsuhashi, 1986; Hashimoto et al., 1990; Wu et al., 1993), a bolus injection of MCI-154 (30 μg/kg) was also examined. Since this dose produced a 70% increase of dP/dt_{max} in anesthetized dogs (Narimatsu et al., 1987), we thought that 30 μg/kg was a supramaximal dose for the treatment of heart failure. This dose of MCI-154 induced transition from ventricular tachycardia to ventricular fibrillation in one dog, but five other dogs survived without any changes in the arrhythmic ratio as shown in Fig. 2B (the arrhythmic

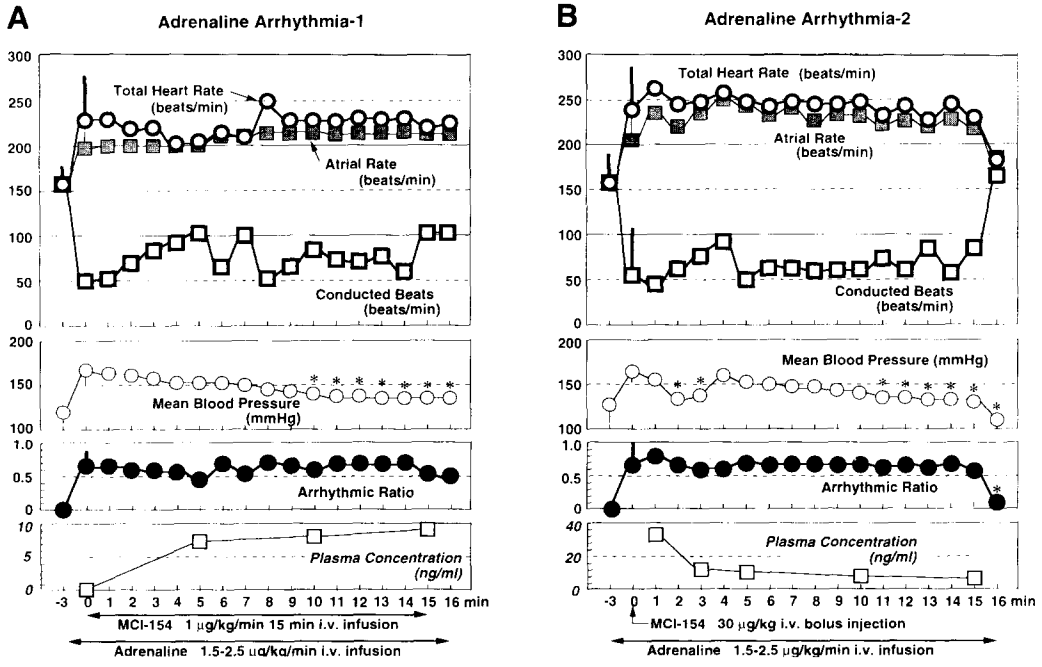

Fig. 2. (A) Adrenaline arrhythmia-1; summary of the effects of intravenous continuous infusion of MCI-154 (1 μg/kg/15 min, total 15 μg/kg) on the adrenaline arrhythmia ($n = 6$) and the plasma concentrations of the drug ($n = 6$). (B) Adrenaline arrhythmia-2; summary of the effects of intravenous bolus injection of 30 μg/kg of MCI-154 on the adrenaline arrhythmia ($n = 5$) and the plasma concentrations of the drug ($n = 5$). This dose of MCI-154 produced the transition from ventricular tachycardia to ventricular fibrillation in one dog, but the other five dogs survived without changes in the arrhythmic ratio. On both graphs, * marks represent significant changes from 0 time values ($P < 0.05$); S.D.s are shown only at −3 and 0 time.

ratio of the control = 0.70 ± 0.27, the peak ratio = 0.82 ± 0.25 after 1 min, not significant). The peak plasma concentration was 32 ± 9 ng/ml ($n = 5$) 1 min after the bolus injection. No significant correlation was observed between the plasma concentrations of MCI-154 and the parameters examined in this study.

3.4. Effects of MCI-154 on the membrane ionic current

For the study of the inward Ca^{2+} currents (I_{Ca}) and the delayed rectifier K^+ current (I_K), myocytes were kept at a holding potential of −40 mV (for inactivating the Na^+ currents) and then depolarized for 550 ms to various command voltages at a frequency of 0.25 Hz. To obtain a current-voltage relationship (*I-V* curve), the command voltage was increased from −40 mV to +60 mV in 10-mV steps. A concentration of 10 μM MCI-154 (3.6 μg/ml) alone did not affect the *I-V* curve of I_{Ca} and I_K, as shown in Fig. 3. The holding current and the apparent reversal potential for I_{Ca} were unchanged (Fig. 3B). Similar results were obtained in five other cells ($n = 6$).

We also compared the effects of MCI-154 with those of another Ca^{2+} sensitizer, pimobendan, on the isolated normal I_{Ca} and the isoprenaline-increased I_{Ca} in the Cs/Cs condition. Fig. 4A,C show typical tracings of the isolated I_{Ca} when 100 μM MCI-154 (a) and 100 μM pimobendan (b) were applied. Fig. 4A shows the effects on the control I_{Ca}, whereas Fig. 4C shows the effects on the increased I_{Ca} elicited by 10 nM isoprenaline. The membrane potential was clamped at −40 mV to inactivate the Na^+ current and then depolarized to 0 mV for 200 ms at a frequency of 0.1 Hz. Fig. 4B shows the effects of various concentrations of MCI-154 and pimobendan on the control peak I_{Ca} obtained in a whole-cell voltage-clamp experiment. After rupture of the cell membrane, peak I_{Ca} tended to decrease, showing a slight 'run down'. However, 10–100 μM MCI-154 did not increase the I_{Ca} at 10 min after administration of the drug and did not overcome this 'run down' (10 μM: 94 ± 5% vs. control (100%), not significant ($n = 5$); 100 μM: 90 ± 8%, not significant ($n = 6$), Fig. 4B) and also 100 μM MCI-154 did not increase the augmented I_{Ca} elicited by 10 nM isoprenaline (92 ± 10% vs. isoprenaline-induced I_{Ca} (100%), not significant ($n = 7$), Fig. 4C (a)). Pimobendan 1–100 μM increased the control I_{Ca} at 5–8 min after administration of the drug (1 μM: 111 ± 5% vs. control (100%), $P < 0.05$ ($n = 4$); 10 μM: 126 ± 7%, $P < 0.01$ ($n = 4$); 100 μM: 143 ± 9%, $P < 0.01$ ($n = 6$), Fig. 4B). Moreover, 100 μM pimobendan increased the augmented I_{Ca} (118 ± 7% vs. isoprenaline-induced I_{Ca} (100%), $P < 0.05$ ($n = 4$), Fig. 4C (b)). DMSO 0.1% was used to dissolve 100 μM pimobendan. DMSO 0.1% alone did not affect the I_{Ca} (101 ± 6% vs. control (100%), not significant ($n = 4$)).

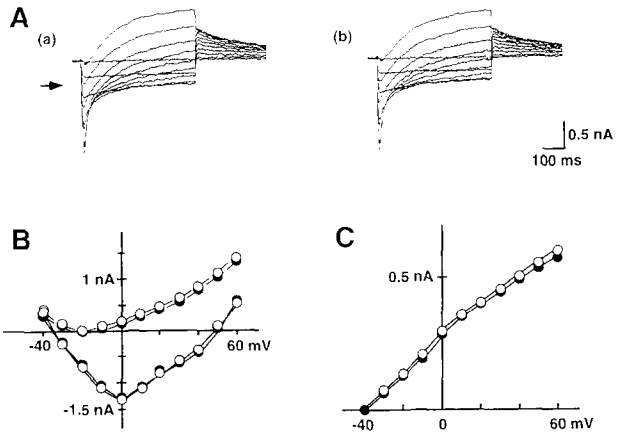

Fig. 3. Effects of MCI-154 on the current-voltage relation of membrane currents in normal Tyrode's solution. (A) Typical current tracings under 'predrug' control conditions (a), after 10-min application of 10 μM MCI-154 (b). Arrow head indicates zero current level. (B) Current-voltage relationships for peak of I_{Ca} and current after 550-ms test pulse in the absence (○) and presence of MCI-154 (●). (C) Current-voltage relationships for amplitude of the outward tail current in the absence (○) and presence of MCI-154 (●).

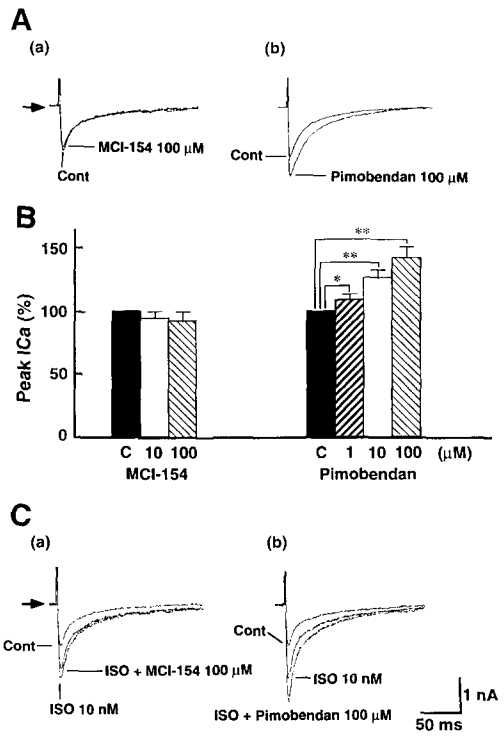

Fig. 4. Comparative effects of MCI-154 and pimobendan on the isolated I_{Ca} and isoprenaline-increased I_{Ca} in the Cs/Cs condition. (A) (a) Superimposed typical recordings of I_{Ca} under 'predrug' control conditions, after 10 min application of 100 μM MCI-154. (b) Superimposed typical recordings of I_{Ca} under control conditions, after 8-min application of 100 μM pimobendan. (B) Concentration-response peak I_{Ca} for MCI-154 and pimobendan. Control = 100% * $P < 0.05$, ** $P < 0.01$. (C) (a) Superimposed typical recordings of I_{Ca} under 'predrug' control conditions, after application of 10 nM isoprenaline (ISO) and then after 10 min application of 100 μM MCI-154. (b) Superimposed typical recordings of I_{Ca} under 'predrug' control conditions, after application of 10 nM isoprenaline and then after 6-min application of 100 μM pimobendan. Arrow head indicates zero current level.

4. Discussion

The present study has shown that a new cardiotonic Ca^{2+} sensitizer MCI-154 at a dose of 1 μg/kg/min for 15 min (total 15 μg/kg) caused no deleterious effects on the canine ventricular arrhythmias produced by coronary ligation, digitalis intoxication and halothane-adrenaline interaction. Moreover, a bolus injection of 30 μg/kg MCI-154 did not aggravate adrenaline- and digitalis-induced arrhythmias except in the case of one ventricular fibrillation in the adrenaline-induced arrhythmia experiments. Supporting the results of these in vivo experiments, MCI-154 (10–100 μM) did not influence the I_{Ca} increased by isoprenaline in isolated guinea pig ventricular cells (Figs. 3 and 4). From these findings, we conclude that MCI-154 may be a useful positive inotropic agent in the treatment of heart failure, where catecholamine-related deleterious arrhythmias are often present.

The most interesting and important finding of the present study is the lack of proarrhythmic effects of MCI-154 on the halothane-adrenaline arrhythmia models. MCI-154 did not suppress or aggravate the arrhythmias when a continuous infusion (1 μg/kg/min for 15 min, total 15 μg/kg) was applied. Moreover, even in the case of a bolus injection of 30 μg/kg, MCI-154 induced ventricular fibrillation only in one dog, and did not aggravate arrhythmias in the other five of six dogs. At the doses used in the present experiments, MCI-154 has been reported to increase dP/dt_{max} of the left ventricular pressure by more than 50–70% (Narimatsu et al., 1987; Abe et al., 1992) and to alleviate the regional contractile abnormalities caused by ischemia and reperfusion in anesthetized open-chest dogs (Abe et al., 1991, 1992, 1993). In addition, our results on the plasma concentration of MCI-154, which reached to a level of 20–100 ng/ml and corresponds to 50–200 nM, indicate that the doses in the present study are enough to show positive inotropic effects. In vitro experiments showed that MCI-154 at a concentration of over 10 nM exerted a positive inotropic action in chemically skinned fibers from the canine right ventricular muscle (Kitada et al., 1989a). Also another in vitro study had shown that MCI-154 (0.1–100 nM) causes 10–90% increases of dP/dt_{max} (approximately 60% increase at the concentration of 10 nM) in the Langendorff perfused heart (Abe et al., 1996). Thus, the results of the present study indicate that MCI-154 does not aggravate ventricular arrhythmias at positive inotropic doses. In our previous studies, phosphodiesterase III inhibitors, such as amrinone, milrinone, vesnarinone and OPC-18790, all aggravated the adrenaline-induced ventricular tachyarrhythmias (i.e. production of ventricular fibrillation), and only about one tenth of the positive inotropic doses of these drugs could be administered without severely aggravating this arrhythmia (Hashimoto and Mitsuhashi, 1986; Hashimoto et al., 1990; Wu et al., 1993). It is quite reasonable that these positive inotropic agents increase I_{Ca} of sarcolemma via a cyclic AMP-dependent pathway (Iijima and Taira, 1987; Momose and Sasayama, 1990; Takase et al., 1994) and potentiate the already increased adrenaline-induced I_{Ca} and/or affect I_K, and hence aggravate the arrhythmia. In agreement with these aggravating effects, our previous studies have shown that the halothane-adrenaline arrhythmia can be suppressed by a cyclic AMP-dependent process, i.e. by class II β-adrenoceptor antagonists and class IV Ca^{2+} channel blockers (Shibuya et al., 1983; Hashimoto et al., 1991). Thus, the fact that MCI-154 did not aggravate adrenaline-induced arrhythmias, unlike other positive inotropic agents, strongly indicates that MCI-154 exerts its positive inotropic effect through a pure Ca^{2+}-sensitizing property, not by inhibiting phosphodiesterase III. Recently, Bethke et al. (1993) have shown that MCI-154 has an inhibitory effect on phosphodiesterase III obtained from guinea-pig left ventricular tissues. However, this previous study also clearly suggests that MCI-154 induces a positive inotropic effect though the cyclic AMP-independent mechanism, because the inhibitory effect of MCI-154 on phosphodiesterase III is about 1.5-fold less potent than that of milrinone, while the inotropic effect of MCI-154 is about 800-fold more potent than that of milrinone (Bethke et al., 1993). Moreover, Kitada et al. (1987c) also reported that myocardial cyclic AMP levels did not increase when MCI-154 augmented myocardial contractility. The Ca^{2+}-sensitizing action of MCI-154 has been extensively demonstrated in skinned cardiac fibers (Kitada et al., 1987a, b, 1989a, b; Perreault et al., 1989) and purified contractile proteins, i.e., troponin C, troponin I and troponin T (Liao and Gwathmey, 1994). Therefore, MCI-154 exerts a positive inotropic effect through Ca^{2+} sensitization, and hence does not aggravate ventricular arrhythmias produced by halothane-adrenaline interaction.

Our electrophysiological study showed that MCI-154 at a concentration of 10 μM did not affect either the magnitude and time course of the control I_{Ca} and I_K (Fig. 3), or the isoprenaline-augmented I_{Ca} (Fig. 4), indicating that this agent at an appropriate positive inotropic dose has no influence on the membrane currents which must cause halothane-adrenaline-induced ventricular arrhythmias. As the maximal plasma concentration of MCI-154 was approximately 40 ng/ml (100 nM) in our adrenaline-induced arrhythmia experiments, the concentration of up to 100 μM must be enough to evaluate whether this drug could affect the membrane currents. Katayama et al. (1987) have also shown that MCI-154 alone at a concentration of 1 μM failed to increase I_{Ca} in isolated guinea pig ventricular cells. Additionally, although Katayama et al. (1987) showed that MCI-154 at high concentrations over 100 μM slightly inhibited the inward rectifier K^+ current (I_{K1}), we did not confirm their results and instead showed that 10 μM MCI-154 did not affect the holding current in the normal Tyrode's condition (Fig. 3). In contrast, another Ca^{2+} sensitizer, pimobendan (Ruegg et al., 1984), which also has a potent phosphodiesterase III inhibitory activity

(Brunkhorst et al., 1989), dose-dependently increased the peak magnitude of the control I_{Ca} and isoprenaline-augmented I_{Ca} in the ventricular cells of guinea pig (Westfall et al., 1992) and dog (Kanai et al., 1994). We also confirmed this effect of pimobendan in the guinea pig (Fig. 4). These data suggest that pimobendan may aggravate the adrenaline-induced arrhythmia even though this drug has a Ca^{2+}-sensitizing property, like MCI-154 does. Actually, Lynch et al. (1988) had demonstrated that pimobendan aggravated the non-sustained ventricular tachycardia produced by the programmed electrical stimulation method to sustained ventricular tachycardia or ventricular fibrillation, as does milrinone (Lynch et al., 1989). We had also shown that another Ca^{2+} sensitizer, sulmazole, at an inotropic dose aggravated the adrenaline-induced arrhythmia (Hashimoto and Mitsuhashi, 1986). Altogether, it may be reasonable to conclude that MCI-154 is not deleterious to halothane-adrenaline-induced arrhythmia, unlike other inotropic drugs of phosphodiesterase III inhibitors or Ca^{2+} sensitizers.

With the coronary ligation-induced arrhythmias, MCI-154 did not change the arrhythmic ratio. In our previous study, vesnarinone showed arrhythmogenic effects on 48-h coronary ligation arrhythmias, i.e. arrhythmias became polymorphic rather than monomorphic, or repetitive beats occurred at a faster rate, though it had no deleterious effects on 24-h coronary ligation arrhythmias (Hashimoto and Mitsuhashi, 1986). Milrinone (Hashimoto et al., 1990) and OPC-18790 (Wu et al., 1993) also tended to aggravate 48-h coronary ligation arrhythmias. All these agents have high phosphodiesterase III inhibitory activity (Bethke et al., 1993; Brunkhorst et al., 1989; Endoh et al., 1994; Hosokawa et al., 1992; Kitada et al., 1991) and increased the total heart rate and atrial rate. However, a forskolin derivative NKH477, which also increases intracellular cyclic AMP, had no arrhythmogenic effects on 48-h coronary ligation-induced arrhythmias despite there being an acute increase in the total heart rate (Hirasawa et al., 1993). At present, the role of increased intracellular cyclic AMP on the genesis of this ischemic ventricular arrhythmia is not clear, but the acute increase in the total heart rate, which NKH477 produced, increases the oxygen consumption and possibly causes further deterioration of the failing hearts (Katz, 1992). Therefore MCI-154, which did not increase the total heart rate and atrial rate, can be used safely even with acute myocardial ischemia and infarction.

It is still the case that digitalis is a first choice positive inotropic compound for the treatment of heart failure. On digitalis-induced arrhythmias, 15 μg/kg MCI-154, both given as a continuous i.v. infusion or as a bolus injection, had no antiarrhythmic or aggravating effects on these arrhythmias. Moreover, a bolus injection of a supramaximal dose of MCI-154 (30 μg/kg), which increased the dP/dt_{max} more than 70% (Narimatsu et al., 1987), unexpectedly decreased the arrhythmic ratio. Though there have been no reports that explain this antiarrhythmic mechanism of MCI-154, such as the Na^+ channel-blocking effect, these findings suggest that MCI-154 can be used in combination with digitalis without increasing the risk of arrhythmia. Previously we have reported that amrinone (Hashimoto et al., 1990), sulmazole (Hashimoto and Mitsuhashi, 1986) and NKH477 (Hirasawa et al., 1993) have strong positive chonotropic effects and have antiarrhythmic effects on digitalis arrhythmia by an overdrive suppression mechanism, but MCI-154 had no positive chonotropic effect on the digitalized dogs in our study. There have been reports of a weak positive chonotropic effect in dog (Hosono and Taira, 1987; Sawaki et al., 1993) or of a negative chonotropic effect in guinea pig (Allert and Adams, 1990). Therefore this effect of MCI-154 may not be explained by the overdrive suppression of the ventricular automatic foci by the increased atrial rate (Hashimoto et al., 1990; Hirasawa et al., 1993). Thus, the antiarrhythmic mechanism of the supramaximal dose of MCI-154 on digitalis arrhythmia is difficult to explain on the basis of our previous results that coronary ligation-induced and digitalis-induced arrhythmias are suppressed by class I Na^+ channel blockers (Hashimoto et al., 1991).

Since malignant arrhythmias, which may result in sudden death, often occur in the failing heart, it is important that treatment for heart failure should not aggravate the preexisting arrhythmias. In the present study, we have shown that MCI-154 does not aggravate ventricular arrhythmias in three canine experimental models. Thus, the practical importance of our results is that MCI-154 can be used as a positive inotropic drug concomitant with catecholamine and/or digitalis, or in patients with myocardial infarction without increasing the risk of arrhythmias and the oxygen demand compared to other recently developed positive inotropic agents. As the mechanism of generation of the arrhythmias in the failing heart is still unknown, further studies may be needed with other models simulating the pathophysiological setting of heart failure besides our three experimental models.

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