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Inhibitory effect of erythromycin on potassium currents in rat ventricular myocytes in comparison with disopyramide

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Abstract

Disopyramide, a class Ia antiarrhythmic agent, has been reported to induce torsades de pointes (TdP) associated with excessive QT prolongation in electrocardiogram (ECG), especially when concomitantly administered with erythromycin, a macrolide antibiotic agent. In this study, we have evaluated the effects of erythromycin on action potential duration (APD) and potassium currents in rat ventricular myocytes in comparison with disopyramide. We have evaluated the relationship between in-vitro potassium current inhibition and in-vivo QT prolongation observed in a previous study. Action potentials and membrane potassium currents, including delayed rectifier current (I_K) and transient outward current (I_{to}), were recorded using a whole-cell patch clamp method in enzymatically-dissociated ventricular cells. Erythromycin and disopyramide prolonged APD in a concentration-dependent manner. Disopyramide (10–100 μM) and erythromycin (100 μM) led to increases in the APD at 90% repolarization level. Disopyramide reduced I_K (IC50 = 37.2 ± 0.17 μM) and I_{to} (IC50 = 20.9 ± 0.13 μM) while erythromycin reduced I_K (IC50 = 60.1 ± 0.29 μM) but not I_{to}. The observed prolongation of APD might be ascribed to the inhibition of potassium currents. Erythromycin produced the prolongation of APD and the inhibition of potassium currents with a lag time after addition of the drugs, which suggested that erythromycin might not reach potassium channels from outside the ventricular cells. The potency of disopyramide was almost equivalent under in-vitro and in-vivo conditions. However, potency of erythromycin in-vitro was far weaker than that in-vivo reported in a previous study, presumably due to a difference in the uptake of erythromycin into ventricular myocytes between in-vivo and in-vitro conditions. Therefore, when drug-induced risks of QT prolongation are to be evaluated, the difference of potencies between in-vitro and in-vivo should be taken into consideration.

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Introduction

It has been reported that concomitant administration of erythromycin, a macrolide antibiotic agent, and disopyramide, a class Ia antiarrhythmic agent, induced torsades de pointes (TdP) associated with electrocardiographic QT prolongation (Ragosta et al 1989; Kawamoto et al 1993). Disopyramide is well known to induce QT prolongation or TdP and erythromycin has been reported to induce TdP associated with electrocardiographic QT prolongation also (Brandriss et al 1994; Gitler et al 1994; Orban et al 1995). We have evaluated the risk of QT prolongation induced by erythromycin or disopyramide in rats in-vivo, and found that either drug induced QT prolongation in a concentration-dependent manner at the plasma concentrations corresponding to within the therapeutic range in man (Hanada et al 1999). The QT interval is considered to reflect the action potential duration (APD) in ventricular myocytes and is prolonged by the blockade of repolarizing K^+ channels, such as the delayed rectifier current (I_K) and the transient outward current (I_{to}). Most drugs associated with QT prolongation, such as quinidine, terfenadine or haloperidol, have been reported to block the delayed rectifier potassium current (I_K), particularly the rapid component of I_K (I_{Kr}) or cloned channels

encoded by human cloned potassium channels (HERG), which are believed to carry I_{Kr} (Witchel & Hancox 2000).

Erythromycin was reported to inhibit I_{Kr} in guinea-pigs and canine ventricular myocytes and HERG expressed in human embryonic kidney cells (Rubart et al 1993; Daleau et al 1995; Volberg et al 2002). However, so far the effects of erythromycin on I_{to} have not been quantitatively evaluated. In contrast, disopyramide has been reported to suppress I_K in guinea-pig, canine and rat ventricular myocytes, I_{Kr} in rabbit ventricular myocytes and I_{to} in goat Purkinje fibres and in rabbit and rat ventricular myocytes (Coraboeuf et al 1988; Hiraoka et al 1989; Virag et al 1998; Sanchez-Chapula 1999). A detailed analysis has yet to be conducted on the concentration–effect relationships of the drugs for the reduction of the K^+ currents, with the exception of the study by Virag et al (1998) on disopyramide in rabbit ventricular myocytes.

The K^+ currents and action potential configuration observed in rat ventricular myocytes appear to be different from those in human ventricular myocytes. In human ventricular myocytes, two different components, the rapid (I_{Kr}) and slow (I_{Ks}) components contribute to I_K, however such components are not observed in rat ventricular myocytes. In addition, in rat ventricular myocytes I_{to} can be observed more prominently than that observed in human ventricular myocytes. In this study, to elucidate the mechanisms of erythromycin- and disopyramide-induced QT prolongation observed in a previous rat in-vivo study (Hanada et al 1999), we undertook a quantitative investigation of the effects of disopyramide and erythromycin on APD and potassium currents (I_K or I_{to}) using rat ventricular myocytes. We evaluated the relationship between in-vitro potassium current inhibition and in-vivo QT prolongation observed in our previous study.

Materials and Methods

Chemicals

Erythromycin base (erythromycin) was obtained from Dainippon Pharmaceutical Co., Ltd (Osaka, Japan). Disopyramide and collagenase were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other reagents used were of analytical grade, as purchased from Wako Pure Chemical Industries.

Animals

Male Sprague-Dawley rats were purchased from Takasugi Jikken Doubutsu (Saitama, Japan). All experiments were performed according to the regulations of the Animal Research Committee of Chiba University Graduate School of Medicine and the Guide for the Care and Use of Laboratory Animals (NIH publication).

Cell isolation

Single ventricular cells were isolated from rat hearts by an enzymatic dissociation method. Briefly, male rats (250–400 g) were anaesthetized with an intraperitoneal injection of pentobarbital sodium. The heart was removed, immediately mounted on a Langendorff apparatus, and retrogradely perfused with normal N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES)-Tyrode solution for 5 min followed by nominally Ca^{2+}-free Tyrode solution for 5 min, and finally Ca^{2+}-free Tyrode solution containing 0.3 mg mL^{-1} collagenase for 25–32 min. All solutions were maintained at 36.0 ± 1.0 °C. Following enzymatic digestion, the hearts were perfused with a high-K^+-low-Cl^- solution (modified Kraftpieces Bruhe (KB) solution). Ventricular tissue was then cut into small pieces in the modified KB solution, and the pieces gently agitated to isolate cells. The cell suspension was filtered through a 100-μm-pore stainless-steel mesh and stored in a refrigerator (4 °C) for use on the same day.

The composition of the normal HEPES-Tyrode solution was (in mM): 143 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 5.5 glucose, and 5 HEPES-NaOH buffer (pH 7.4). The nominally Ca^{2+}-free Tyrode solution was prepared by omitting $CaCl_2$ from the normal Tyrode solution. The modified KB solution contained (in mM): 70 KOH, 50 L-glutamic acid, 40 KCl, 20 taurine, 20 KH_2PO_4, 3 $MgCl_2$, 10 glucose, 1 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), and 10 HEPES-KOH buffer (pH 7.4).

Electrophysiological recordings

Whole-cell membrane currents and action potential in rat ventricular myocytes were recorded by a patch-clamp method. Single ventricular cells were placed in a recording chamber (1 mL volume) and superfused with the HEPES-Tyrode solution at a rate of 3 mL min^{-1}. The temperature of the bath solution was maintained at 36.0 ± 1.0 °C. Patch pipettes were made from borosilicate glass capillaries (1.5 mm o.d.) using a vertical microelectrode puller (PB-7, Narishige, Tokyo, Japan). The tip resistance was 2–3 MΩ when filled with a solution containing (in mM): 110 L-aspartate, 20 KCl, 1 $MgCl_2$, 5 ATP-K_2, 5 phosphocreatine-K_2, 10 EGTA, 5 HEPES-KOH, and 1.42 $CaCl_2$, pH 7.4 adjusted by addition of KOH. The electrode was connected to a patch-clamp amplifier (Nihon Kohden CEZ-2300, Tokyo, Japan). Command pulse signals were generated by means of a 12-bit digital-to-analog converter, controlled using the pCLAMP software package (Axon Instruments, Foster City, CA). Current signals were digitized with a sampling interval of 1 kHz and stored on the hard disk of a DOS/V personal computer. Correction was made for a liquid junction potential of −8 mV between the pipette solution and bath solution. Following establishment of a gigaohm seal between the tip of the electrode and the cell membrane, the cell membrane was ruptured by negative pressure to generate the whole-cell configuration.

Current clamp experiments

Current clamp experiments were performed in the whole-cell recording mode at 36.0 ± 1.0 °C. After establishment

of the whole-cell clamp mode, cells were stimulated with rectangular 2-ms currents through the pipette at a rate of 0.2 Hz. Following the stabilization of action potential configuration, effect of each drug (erythromycin or disopyramide) on the action potential was evaluated. The concentrations of erythromycin used were 10, 30 and 100 μM, and those of disopyramide were 3, 10, 30 and 100 μM.

Voltage clamp experiments

According to the method of Slawsky & Castle (1994), either the delayed rectifier K^+ current (I_K) or the transient outward current (I_{to}) was measured during depolarizing pulses of 100 ms to +40 mV from a holding potential of −90 mV. The Na^+ current was inactivated by a 15-ms depolarization from −90 mV to −20 mV before the depolarization used to evoke the outward K^+ current.

The amplitudes of I_K and I_{to} were determined by selective inhibitors of each current i.e. tetraethylammonium (TEA) and 4-aminopyridine (4AP), respectively. For the measurement of I_K, a HEPES-Tyrode solution containing 3.0 mM $CoCl_2$ and 3.0 mM 4AP was perfused to inhibit I_{Ca} and I_{to}. Then the Co^{2+}-4AP solution containing the test drug was perfused, and the effect of each drug on I_K was evaluated. Finally, I_K was completely suppressed by application of the Co^{2+}-4AP solution containing 80 mM TEA. For the measurement of I_{to}, a Co^{2+}-TEA solution (pH 7.4) containing 3 mM $CoCl_2$ and 80 mM TEA was initially perfused to inhibit I_{Ca} and I_K. Thereafter, the Co^{2+}-TEA solution containing the test drug was perfused, and the effect of each drug on I_{to} was evaluated. Finally, I_{to} was completely suppressed by application of the Co^{2+}-4AP-TEA solution. No more than three concentrations of test drug were applied to an individual cell to preserve the stability of the cell. Currents were recorded after attainment of a steady state for the effect of the test drugs (i.e. > 6 min for erythromycin and approximately 3 min for disopyramide). After all the concentrations of test drug were applied, Co^{2+}-4AP or Co^{2+}-TEA solution was re-perfused to wash-out the test drug.

Data analysis

The amplitude of I_K or I_{to} was evaluated by a method described by Slawsky & Castle (1994). I_K was derived as the difference between the current remaining at the end of the 100-ms depolarizing pulse and zero current level. I_{to} was derived as the integral of the outward current, measured from the initiation of a 100-ms depolarizing pulse to +40 mV, with respect to the "steady-state" current remaining at the end of the pulse. The inhibitory effect of the test drug was normalized by the maximum blockade (A_{drug}(%)). The relationship between A_{drug} (%) and IC50 (μM) (the drug concentration that effects 50% reduction of each potassium current) is described as follows:

$$A_{drug}(\%) = 100\ (1 - (C/(IC50 + C))) \quad (1)$$

where C is the drug concentration (μM). IC50 was calculated by fitting the data to equation 1, using a non-linear least-squares program MULTI (Yamaoka et al 1981), with a modified Marquardt method.

Statistical analysis

All experimental values are presented as mean ± s.d. The one-way analysis of variance was employed for statistical analysis of the data in the current clamp experiment (n = 4 for disopyramide, n = 7 for erythromycin). Thereafter, we performed the Dunnett's test as a post hoc test. P values of less than 0.05 were considered significant.

Results

Effects of disopyramide and erythromycin on APD

Action potentials were recorded from rat ventricular myocytes by a whole-cell patch clamp method. The resting membrane potential was found to be −78.8 ± 3.0 mV, and the action potential duration at 90% repolarization level (APD_{90}) was 30.7 ± 15.9 ms (n = 11).

Figure 1 shows the representative effects of disopyramide and erythromycin on the action potential. Disopyramide prolonged APD_{90} in a concentration-dependent manner (Figure 1A). The increases in APD_{90} after 10, 30 or 100 μM disopyramide were 18.7 ± 9.0, 34.2 ± 8.8 and 53.1 ± 16.7 ms, respectively (n = 4) (Figure 1C). Erythromycin prolonged APD_{90} in a concentration-dependent manner although higher concentrations of erythromycin were required to prolong APD_{90} compared with disopyramide (Figure 1B and D). The increase in APD_{90} with 100 μM erythromycin was 26.0 ± 16.5 ms (n = 7).

Effects of disopyramide on I_K and I_{to}

Disopyramide inhibited I_K and I_{to} in a concentration dependent manner (Figure 2). Suppression of I_K and I_{to} was observed immediately after introduction of disopyramide and disappeared upon wash-out of the drug. The concentration–effect relationship for disopyramide was analysed using a sigmoid E_{max} equation (eqn 1) (Figure 2C and D). The calculated IC50 values of disopyramide for I_K and I_{to} were 37.2 ± 0.17 μM (= 12.6 ± 0.06 μg mL^{-1}) and 20.9 ± 0.13 μM (= 7.1 ± 0.04 μg mL^{-1}), respectively.

Effects of erythromycin on I_K and I_{to}

Figure 3 shows the effects of erythromycin on I_K and I_{to}. Erythromycin suppressed I_K in a concentration-dependent manner (Figure 3A), although compared with disopyramide higher concentrations of erythromycin were required for the suppression (Figure 2). Moreover, erythromycin did not significantly affect I_{to}. In contrast to disopyramide, a relatively long time was needed to attain the steady-state inhibitory effect on I_K. In addition, the inhibitory effect of erythromycin on I_K persisted even after drug wash-out. The concentration–effect

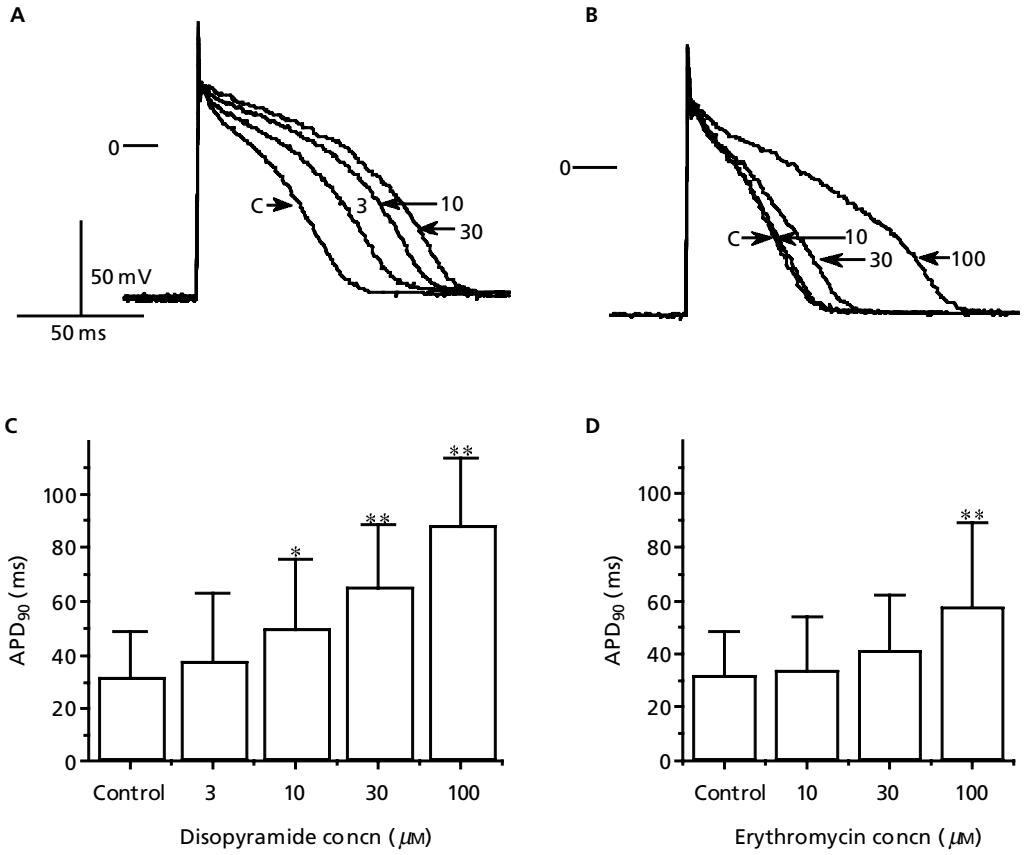

Figure 1 Effects of disopyramide or erythromycin on the action potential duration (APD) of isolated rat ventricular myocytes. Panel A shows the representative changes of APD recorded after 3-min exposure to 3, 10 and 30 μM disopyramide. Panel B shows representative changes of APD recorded after 6-min exposure to 10, 30 and 100 μM erythromycin. Panel C summarizes concentration-dependency effects of disopyramide on APD. Panel D summarizes concentration-dependency effects of erythromycin on APD. Data shown are absolute values. Mean $\pm$ s.d.; n = 4 for disopyramide, n = 7 for erythromycin; *P < 0.05, **P < 0.01 compared with control with Dunnett's test following analysis of variance.

relationship for erythromycin was similarly analysed using a sigmoid E_{max} equation (eqn 1) (Figure 3A). The calculated IC50 was determined to be 60.1 ± 0.29 μM ($= 44.1 \pm 0.21$ μg mL^{-1}).

Discussion

We have evaluated the inhibition of outward K^+ currents in rat ventricular myocytes. Two different components of outward K^+ currents in rat ventricular myocytes, i.e. TEA-sensitive I_K and 4AP-sensitive I_{to}, have been studied kinetically and pharmacologically. Himmel et al (1999) reported two other outward K^+ currents in rat ventricular myocytes, the novel delayed rectifier current I_{Kx} and the steady-state current I_{ss}. In that study it was reported that I_{Kx} was significantly blocked by 10 mM TEA and by 1 mM 4AP and, in contrast, I_{ss} was slightly reduced by TEA or 4AP. From these results, I_{Kx} could be mostly reduced under our conditions using 3 mM 4AP or 80 mM TEA, however I_{ss} could remain in part. In fact, even when the 4AP-TEA solution was applied, unknown residual currents were observed in this study. In addition, Himmel et al (1999) reported I_K was only slightly reduced by 1 mM 4AP, thus it was possible that I_K was reduced in part by 4AP under our present conditions. However, we have evaluated the inhibitory effect of the drugs as normalized values by maximum blockade, therefore, we consider that our results would not be changed largely.

Disopyramide produced a concentration-dependent prolongation of APD_{90} at lower concentrations (3–30 μM) in agreement with previous results (Kus & Sasyniuk 1975; Campbell 1983; Schanne et al 1986). Disopyramide-induced prolongation of APD has been attributed to the inhibition of repolarizing potassium currents. Disopyramide has been reported to reduce the delayed rectifier current (I_K), particularly the rapid component of I_K (I_{Kr}), and to competitively bind to the binding site of dofetilide (Hiraoka et al 1989; Duff et al 1995; Virag et al 1998). Moreover, disopyramide has been shown to inhibit I_{to} in sheep Purkinje fibres, rabbit and rat ventricular myocytes (Coraboeuf et al 1988; Hiraoka et al 1989;

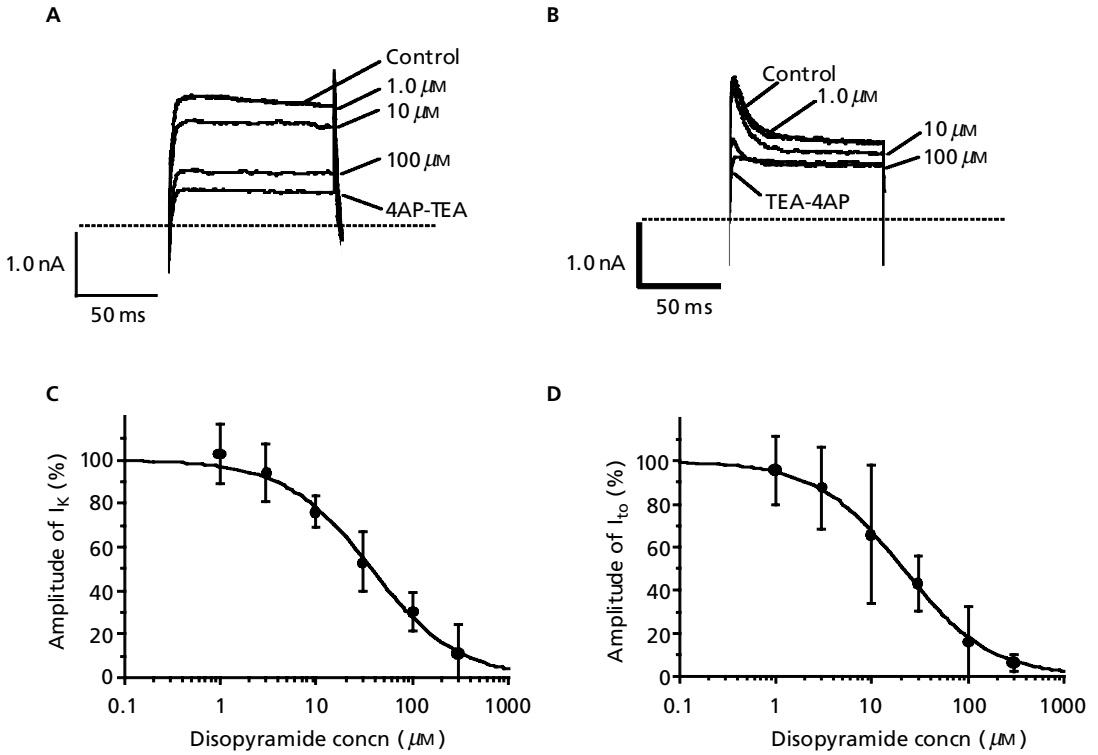

Figure 2 Inhibition of the delayed rectifier K^+ current (I_K) or the transient outward K^+ current (I_{to}) by disopyramide. Panel A shows the original current traces of I_K currents and panel B the I_{to} currents under control conditions, in the presence of 1, 10 and 100 μM disopyramide, and both 4AP and TEA. Panel C shows the concentration–effect curve for the inhibition of I_K and panel D for I_{to}, produced by disopyramide. Equation 1 was fitted to the data points. The IC50 values of disopyramide were 37.2 ± 0.17 and 20.9 ± 0.13 μM for inhibition of I_K and I_{to}, respectively (mean $\pm$ s.d., $n = 5$).

Virag et al 1998; Sanchez-Chapula 1999). Consistent with these reports, we found that disopyramide inhibited both I_K and I_{to} at concentrations corresponding to the therapeutic range of disopyramide (5.9–15 μM as a trough level). Virag et al (1998) reported the EC50 of disopyramide for inhibition of I_{Kr} or I_{to} in rabbit ventricular myocytes (EC50 = 1.8 μM for I_{Kr}, 14.1 μM for I_{to}). In comparison with the IC50 values in this study, it is similar for I_{to}, but not for I_K. They evaluated the I_{Kr} currents, adopted peak tail current amplitude at -40 mV as effect of disopyramide and calculated the EC50 value. In addition to the inter-species difference, these differences may be possible reasons why the EC50 calculated in their study was different from our result. In respect to inward rectifier current (I_{K1}), disopyramide was reported to cause a slight inhibition of I_{K1} at a high concentration (30–60 μM), which, however, is above the therapeutic range (Martin et al 1994; Virag et al 1998). The potency of disopyramide for the inhibition of I_K and I_{to} was almost equal to those for the prolongation of APD. Therefore, the prolongation of APD observed in our in-vitro study might be attributed to the inhibition of both I_K and I_{to} by disopyramide.

In contrast to the above results for disopyramide, erythromycin produced significant and concentration-dependent prolongation of APD_{90} at higher concentrations (100 μM), which was consistent with previous reports using in-vivo canine model and guinea-pig myocytes (Rubart et al 1993; Daleau et al 1995; Antzelevitch et al 1996). This erythromycin-induced prolongation of APD is considered to be a result of the suppression of I_{Kr} (Daleau et al 1995; Antzelevitch et al 1996). In this study, we confirmed that erythromycin inhibited I_K, but not I_{to}, in a concentration-dependent manner in rat ventricular myocytes. Rubart et al (1993) suggested that erythromycin did not inhibit I_{to} from their in-vivo study, but our results provided direct evidence that erythromycin did not affect I_{to}. Erythromycin was reported not to affect I_{K1} (Daleau et al 1995). In addition, the effective concentration of erythromycin for the reduction of I_K was almost equivalent to those for the prolongation of APD. Therefore, the inhibitory effect of erythromycin on I_K may be the primary cause of the prolongation of APD induced by erythromycin.

It took several minutes for erythromycin to exert a steady state for the inhibitory effect on the potassium current (i.e. >6 min for erythromycin while approximately 3 min for disopyramide). This was consistent with the finding of our in-vivo study (Hanada et al 1999) where delayed QT prolongation was observed upon administration of erythromycin but not of disopyramide. In addition, erythromycin-induced K^+ current inhibition was apparently persistent by wash-out. It may not be due to any current rundown because disopyramide-induced K^+

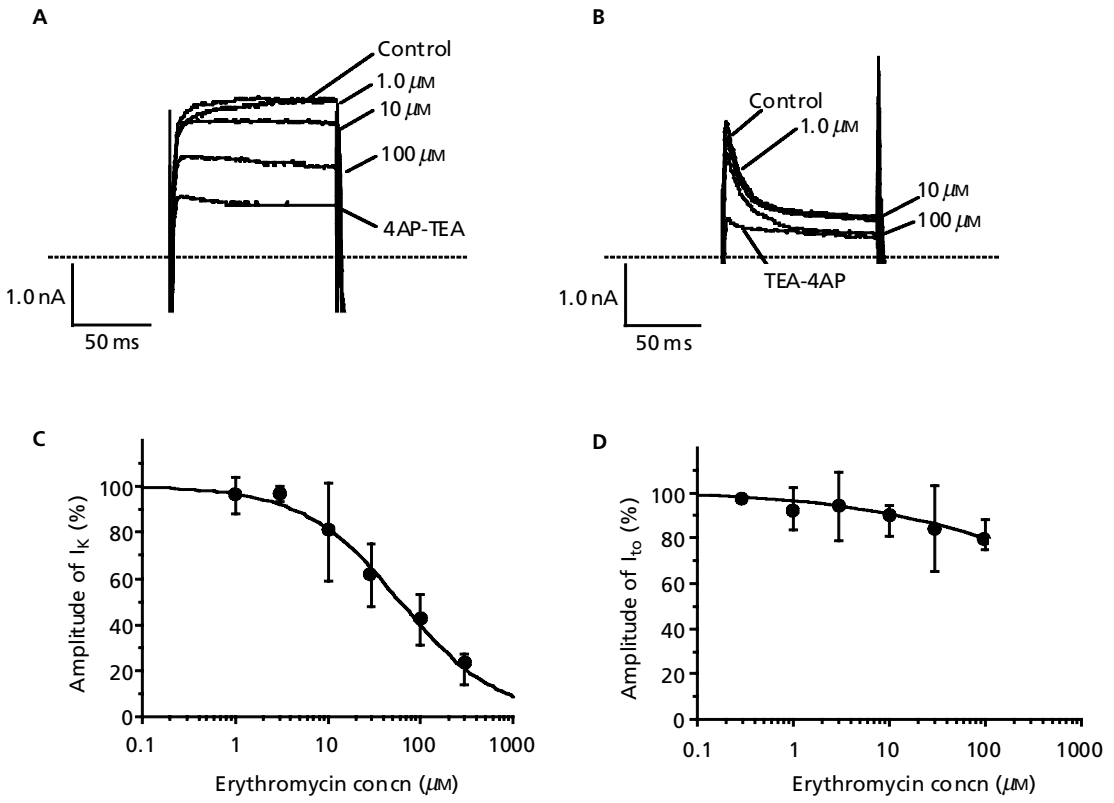

Figure 3 Inhibition of the delayed rectifier K^+ current (I_K) or the transient outward K^+ current (I_{to}) by erythromycin. Panel A shows the original current traces of I_K currents and panel B the I_{to} currents under control conditions, in the presence of 1, 10 and 100 μM erythromycin, and both 4AP and TEA. Panel C shows the concentration–effect curve for the inhibition of I_K and panel D for I_{to} produced by erythromycin. Equation 1 was fitted to the data points. The IC50 values of erythromycin were 60.1 ± 0.29 μM for inhibition of I_K (mean $\pm$ s.d., $n = 5$).

current inhibition was immediately reversed by wash-out. From these observations we considered that erythromycin might distribute into the ventricular cell membrane, and thereafter it might affect the potassium channels from the inside of the cell, not from outside the cell. It has been reported that erythromycin was effective in the inside-out membrane patch of Xenopus oocytes expressing HERG or Kv1.5 and of human embryonic kidney cells expressing Kv1.5 (Roy et al 1996; Rampe & Murawsky 1997). Those reports support a hypothesis that erythromycin gets access to and affects the channels from inside the cell. Another possible explanation for the slow onset of erythromycin may be that erythromycin might have some characteristic kinetics i.e. the slow binding to the channels. In addition, we did not perform the experiments to study voltage or frequency dependence of channel blockade by erythromycin. The difference of effective concentrations of erythromycin in-vivo and in-vitro may be in part explained by the state-dependent blockade by erythromycin, if any. Further study is necessary to clarify the underlying mechanisms.

The IC50 value of erythromycin for I_K was 60.1 μM, while in-vivo QT prolongation of 20–30 ms was observed at 0.94–3.4 μM in our previous study (Hanada et al 1999), indicating discrepancy between the potency for the inhibition of potassium currents in-vitro and that for QT prolongation in-vivo. This was not the case for disopyramide. In our previous report, QT prolongation of 20–30 ms was evoked by 9.1–13.7 μM disopyramide. In general, the plasma free drug manifests the effect. Therefore, taking the plasma free fraction (fp) of disopyramide (fp of disopyramide = 0.84) into consideration, the above in-vivo concentration could be regarded as 7.7–11.5 μM unbound disopyramide, which could produce significant prolongation of APD, as well as 7.8–24.3% suppression of I_K and 27.5–35.9% suppression of I_{to}, as suggested in this study. Therefore, the in-vitro electrophysiological potencies of disopyramide correlated well with that in-vivo. In respect to erythromycin, taking the fp value of erythromycin into consideration, the discrepancy between in-vitro and in-vivo would increase.

A difference in the effective concentration of erythromycin between in-vitro and in-vivo conditions was mentioned by Rubart et al (1993). Those authors found that the effective concentrations of erythromycin to induce early afterdepolarizations in canine Purkinje fibres were different from those at which multiform ventricular arrhythmias were evoked under in-vivo conditions. They suggested a possibility that erythromycin distributed into the ventricle in-vivo, and that the intracellular concentration of erythromycin became higher than that in plasma.

In other words, erythromycin might distribute into the myocytes to interact with the potassium channels from inside the cells. In fact, the inhibitory effect of erythromycin on potassium channels was observed only in an inside-out mode in Xenopus oocytes expressing HERG or Kv1.5 and in human embryonic kidney cells expressing Kv1.5 (Roy et al 1996; Rampe & Murawsky 1997). Therefore, erythromycin might possibly interact with the channels from the inside of the cell. In-vitro electrophysiological characteristics of a drug, therefore, should be carefully interpreted in estimating its in-vivo electrocardiographic effects. Further study is needed to fully elucidate this discrepancy in the effective concentration of erythromycin between in-vitro and in-vivo conditions.

Disopyramide and erythromycin might induce QT prolongation within or over the therapeutic concentrations (5.9–15 μM for disopyramide as a trough level, 0.7–4.1 μM for erythromycin). Taking the free plasma fraction in man into consideration, the plasma free disopyramide concentrations in the clinical setting were almost equal to those at which the inhibition of K^+ currents were observed in this study, however the plasma free erythromycin concentrations were much lower than those in this study. Since inter-species difference for the K^+ currents in ventricular myocytes is known, there may be a limitation to quantitatively estimate QT prolongation in man from this study using rat ventricular myocytes. However, by studying in-vitro and in-vivo in the same species, we found that the inhibitory potency of a drug for the K^+ currents in ventricular myocytes obtained in-vitro does not consistently reflect the potency for QT prolongation in-vivo. Therefore, the electrophysiological characteristics of a drug obtained in-vitro should be carefully interpreted in estimating the electrocardiographic effects in-vivo.

Conclusions

In conclusion, erythromycin and disopyramide prolonged APD in rat ventricular myocytes; disopyramide inhibited I_K and I_{to}, while erythromycin only inhibited I_K. Therefore, erythromycin-induced QT prolongation and APD prolongation might result from the inhibition of I_K. However, the in-vitro inhibitory potency of erythromycin for I_K turned out to be considerably weaker compared with that for QT prolongation in-vivo previously reported by us, though this was not the case for disopyramide. The electrophysiological characteristics of a drug obtained in-vitro should be carefully interpreted in estimating the electrocardiographic effects in-vivo.

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