Naunyn-Schmiedeberg's Arch Pharmacol (2000) 361 : 646–653
Digital Object Identifier (DOI) 10.1007/s002100000230

ORIGINAL ARTICLE

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Effects of JTV-519, a novel anti-ischaemic drug, on the delayed rectifier K^+ current in guinea-pig ventricular myocytes

Received: 2 August 1999 / Accepted: 10 January 2000 / Published online: 14 April 2000

Abstract We studied the effects of a newly synthesized anti-ischaemic agent, 4-[3-(4-benzylpiperidin-1-yl) propionyl]-7-methoxy-2, 3, 4, 5-tetrahydro-1, 4-benzothiazepine monohydrochloride (JTV-519) on the delayed rectifier potassium current (I_K), using guinea-pig ventricular myocytes and whole-cell voltage-clamp techniques, under blockade of the L-type calcium current ($I_{Ca,L}$) by D600 (1 μM) or nitrendipine (5 μM). The I_K in guinea-pig ventricular cells consists of two different components; the rapidly activating, E4031-sensitive component (I_{Kr}) and the slowly activating E4031-resistant component (I_{Ks}). Under steady-state conditions, JTV-519 (1 and 5 μM) did not change the amplitude of I_{Ks} remaining after blockade of I_{Kr} with 5 μM E4031. The effect of JTV-519 on I_{Kr} was assessed using short (50 ms) pulses which evoked a tail current that was sensitive to E4031 but not to chromanol 293B, a specific blocker of I_{Ks}. JTV-519 suppressed the I_{Kr} with a half-maximal inhibitory concentration of 1.2 μM. Selective inhibition of I_{Kr} by this agent was confirmed by using the "envelope of tails" test. These results suggest that the blockade of I_{Kr} may underlie the prolongation of action potential duration in ventricular muscle and QT-intervals alleged to occur in animal as well as human hearts.

Key words Action potential duration · Chromanol 293B · Delayed rectifier potassium current · E4031 · Guinea-pig ventricular myocytes · JTV-519 · Whole-cell voltage clamp

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Introduction

JTV-519, 4-[3-(4-benzylpiperidin-1-yl)propionyl]-7-methoxy-2, 3, 4, 5-tetrahydro-1, 4-benzothiazepine monohydrochloride is a newly synthesized agent possessing protective effects against myocardial ischaemia. The agent effectively prevented Ca^{2+} overload in a canine model of myocardial infarction and induced a quick recovery from myocardial stunning (Kaneko 1994). The effects of JTV-519 on cardiac membrane ionic currents have been studied using guinea-pig ventricular myocytes by Kimura et al. (Kimura et al. 1999). JTV-519 suppressed the Na^+ current (n=), the L-type Ca^{2+} current ($I_{Ca,L}$), and the inward rectifier K^+ current (I_{K1}), while it had no effect on the Na^+/Ca^{2+} exchange current. In healthy Japanese volunteers, JTV-519 prolonged QT intervals (personal communication, Research Institute of Japan Tobacco Co.). However, electrophysiological data on the action potential duration (APD) of ventricular muscles are controversial. JTV-519 prolonged the APD in canine ventricular papillary muscles (personal communication, Research Institute of Japan Tobacco Co.) and in coronary-perfused right ventricular myocardium of guinea-pigs (Ito et al. 1998), while it markedly shortened the APD of single ventricular myocytes obtained from guinea-pigs (Kimura et al. 1999).

The aim of the present study was to elucidate the mechanism(s) underlying the APD prolongation in ventricles that occurred in animal as well as in human hearts. In a preliminary experiment, we found that JTV-519 decreased the $I_{Ca,L}$ as reported by Kimura et al. (1999). Thus in the present study, we focused our attention on the effect of this agent on the delayed rectifier K^+ current (I_K) that plays a critical role in repolarization of ventricular action potentials. The I_K of the guinea-pig ventricle has been shown to consist of two distinct current systems: a rapidly activating delayed rectifier K^+ current (I_{Kr}) that is selectively blocked by E4031, and a slowly activating delayed rectifier K^+ current (I_{Ks}) that is insensitive to E4031 (Sanguinetti and Jurkiewicz 1990). We found that JTV-519 se-

lectively blocked I_{Kr}. A preliminary report on the subject has appeared elsewhere (Kiriyama et al. 1999).

Methods

Cell isolation. Single ventricular myocytes were isolated enzymatically from guinea-pig hearts as described by Taniguchi et al. (Taniguchi et al. 1981). In brief, guinea-pigs weighing 300–350 g were stunned by a blow to the neck, and the heart was quickly dissected and perfused through the aorta with Tyrode's solution gassed with 100% O_2 that contained (in mM): NaCl, 137; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; NaH_2PO_4, 0.16; $NaHCO_3$, 3.0; glucose, 5.5; and HEPES, 5.0 (pH 7.4 adjusted with NaOH). After 2–3 min of perfusion with Tyrode's solution, the perfusate was switched to Ca^{2+}-free Tyrode's solution containing collagenase (Type IV, Worthington Biomedical, Freehold, N.J., USA) for 10–15 min. After washout of the latter solution, and to harvest the cells, a piece of ventricular tissue was picked up and stirred in high K^+/low Cl^- solution of the following composition (in mM): KCl, 5; glutamic acid, 70; taurine, 10; oxalic acid, 10; KH_2PO_4, 5; HEPES, 5; glucose, 11; and EGTA, 0.5 (pH 7.4 adjusted with KOH, making a final $[K^+]$ of ~136 mM), in which the cells were stored at room temperature before use.

Electrical recordings. Whole-cell membrane currents (Marty and Neher 1983) were recorded using a patch-clamp amplifier (Axopatch-1D, Axon Instruments, Foster City, Calif., USA). The current signals were filtered at 3 kHz and stored on magnetic tapes using a PCM data recording system (VR-10B, Instrutech, Elmont, N.Y., USA) for later off-line computer analyses. Patch pipettes were fabricated from capillary tubes (Narishige, Tokyo, Japan) using an electrode puller (model P-97, Sutter Instrument, Novato, Calif., USA) and a microforge (model MF-83, Narishige). The electrodes had a resistance of 2–4 MΩ. Cells were transferred to a water-jacketed recording chamber (0.8 ml volume) mounted on the stage of an inverted microscope (TMD, Nikon, Tokyo, Japan). The temperature of the bath (perfusing) solution (see below for the composition) was kept at 35–36 °C and the flow rate was maintained at 2–3 ml/min. The pipette solution contained (in mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 2; HEPES, 10; EGTA, 11; Na_2-ATP, 5; creatine phosphate (disodium salt), 5 (pH 7.2 adjusted with KOH). The composition of the bath solution was (in mM): NaCl, 137; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; NaH_2PO_4, 0.16; $NaHCO_3$, 3.0; glucose, 5.5; and HEPES, 5.0 (pH 7.4 adjusted with NaOH). The solution also contained 1 μM D600 or 5 μM nitrendipine to block $I_{Ca,L}$ when I_K was measured. The amplitude of I_K was measured either as a time-dependent current elicited during depolarizing pulses ($I_{K,depo}$) or as a tail current evoked on returning to a holding potential of –40 mV ($I_{K,tail}$).

Chemicals. JTV-519 (a gift from Japan Tobacco Co., Osaka, Japan) was prepared as a 1–10 mM stock solution in dimethylsulphoxide (DMSO). E4031 (a gift from Eisai, Tokyo, Japan) was prepared as a 1 mM stock solution in distilled water. Chromanol 293B (a gift from Hoechst Marion Roussel, Frankfurt, Germany) was prepared as a 10 mM stock solution in DMSO. Nitrendipine (a gift from Yoshitomi Pharmaceutical, Osaka, Japan) was prepared as a 10 mM stock solution in DMSO. D600 (a gift from Taisho Pharmaceutical, Osaka, Japan) was prepared as a 10 mM stock solution in ethanol. The final concentration of DMSO or ethanol did not exceed 0.5% of the total volume, and the highest concentration of DMSO (0.5%) had no effect on the membrane current and the contour of action potentials.

Data analysis. Voltage and current signals were digitized with sampling intervals of 1–4 ms, using a personal computer (NEC PC-H98, Tokyo, Japan), equipped with an analogue-to-digital converter (ADX-98 Canopus, Kobe, Japan). A personal computer (Vio PCG-723, Sony, Tokyo, Japan) with a software program (Sigma Plot, Jandel Scientific, Corte Madera, Calif., USA) was used for statistical analyses and for drawing the figures. Unless otherwise mentioned, the data are expressed as mean±SEM. Statistical analysis was performed using Student's *t*-test and $P<0.05$ was considered to indicate significant differences.

Results

Effect of JTV-519 on I_K

We firstly examined the effect of JTV-519 on the I_K composed of both rapidly activating (I_{Kr}) and slowly activating (I_{Ks}) components (Fig. 1). Depolarizing voltage steps (500 ms) were applied from a holding potential of –40 mV in 10 mV increments at a rate of 0.2 Hz. $I_{Ca,L}$ was blocked by nitrendipine (5 μM). The tail current of I_K evoked after

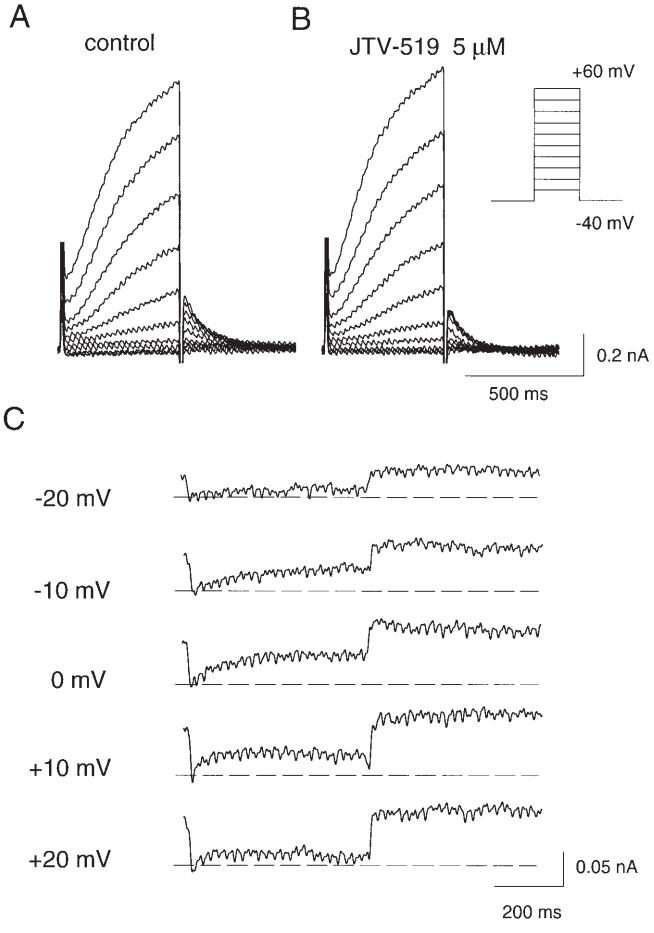

Fig. 1A–C Effects of JTV-519 (5 μM) on the delayed rectifier K^+ current (I_K) in guinea-pig ventricular myocytes. Under blockade of the L-type calcium current ($I_{Ca,L}$) with 5 μM nitrendipine, depolarizing voltage steps (from –30 to +60 mV, with the duration of 500 ms) in 10 mV increments were applied from a holding potential of –40 mV (see *inset* for the clamp protocol). Typical examples of the effect of JTV-519 on the time-dependent current ($I_{K,depo}$) and tail current ($I_{K,tail}$) are shown. JTV-519 suppressed the I_K (**A** and **B**). JTV-519-sensitive currents were obtained by subtracting the current after exposure to JTV-519 (5 μM) from the current in the absence of the drug (**C**). Note that JTV-519-sensitive current, activated during depolarizing pulses, reached a peak at 0 mV and decreased at more positive potentials

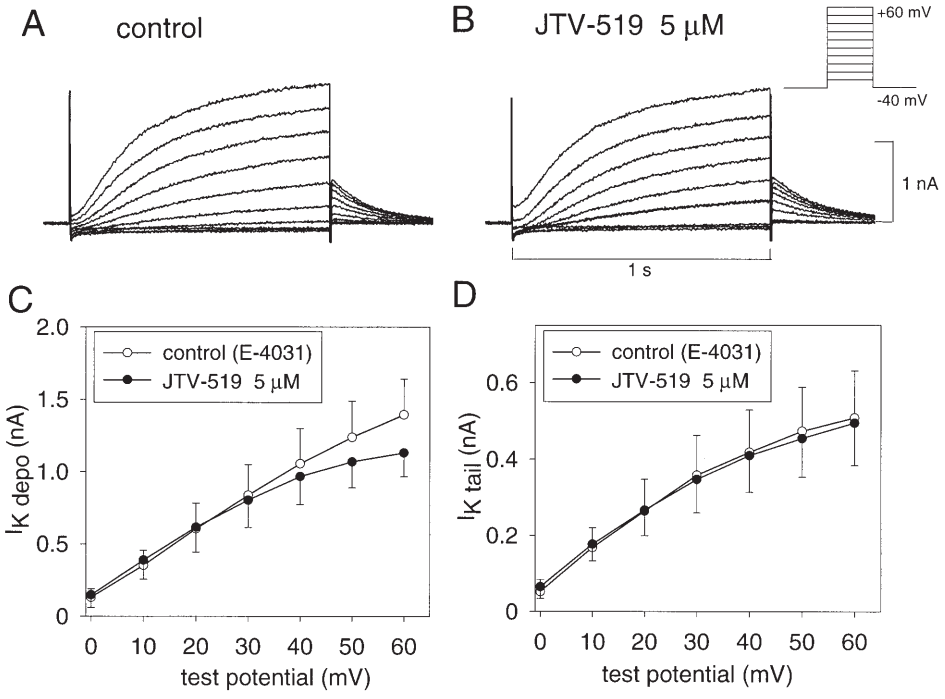

Fig. 2 **A, B** A typical example of the effect of JTV-519 (5 μM) on the slowly activating delayed rectifier K^+ current (I_{Ks}) in guinea-pig ventricular myocytes. Under blockade of $I_{Ca,L}$ with 1 μM D-600 and of the rapidly activating delayed rectifier K^+ current (I_{Kr}) with 5 μM E4031, depolarizing voltage steps (from –30 to +60 mV with the duration of 1 s; see *inset*) in 10-mV increments were applied from a holding potential of –40 mV. The leak current was subtracted. **C, D** Summary of the steady-state effects (5 min after application) of JTV-519 (5 μM) on I_{Ks} defined either as time-dependent outward current during depolarizing steps ($I_{K,depo}$, **C**) or tail current ($I_{K,tail}$, **D**) (n=4)

depolarizing to +60 mV decreased by 42.2±6.4% (n=4) after application of 5 μM JTV-519 for 5 min (Fig. 1A and B). Figure 1C shows the JTV-519-sensitive current obtained by subtracting the current in the presence of 5 μM JTV-519 from the current in the absence of the drug. The JTV-519-sensitive current activated during depolarizing pulses reached a peak at 0 mV and decreased at more positive potentials. As these characteristics are similar to those of E4031-sensitive component of I_K, i.e. I_{Kr} (Sanguinetti and Jurkiewicz 1990), we surmised that JTV-519 might suppress I_{Kr} with practically no effect on I_{Ks}.

Effect of JTV-519 on I_{Ks}

To obtain more quantitative data, we examined the effect of JTV-519 on the I_{Ks} which remained after blockade of I_{Kr} with 5 μM E4031 (Fig. 2). Depolarizing voltage steps (1 s) were applied from a holding potential of –40 mV in 10 mV increments at a rate of 0.2 Hz. $I_{Ca,L}$ was blocked by D600 (1 μM). As E4031 (5 μM) was included in the perfusate, a time-dependent outward current that developed during depolarizing pulses ($I_{K,depo}$) and a tail current that was evoked on return to the holding potential ($I_{K,tail}$) should represent I_{Ks} alone (Sanguinetti and Jurkiewicz 1990, 1991). The steady state effect of the agent was assessed at 5 min after application of JTV-519, because in our preliminary experiments (n=4) the amplitude of the current measured 5 min after application of the agent was comparable to that measured 8 min after the drug application. Figure 2B shows that 5 μM JTV-519 affected neither $I_{K,depo}$ nor $I_{K,tail}$. In four cells examined, the $I_{K,depo}$ measured at +60 mV (pulse duration, 1 s) was 1.06±0.09 nA and 1.39±0.25 nA under control condition and which were decreased to 0.88±0.09 nA and 1.13±0.16 nA after application of 1 μM and 5 μM JTV-519, respectively. However, these changes were not significant. Meanwhile, the peak $I_{K,tail}$ evoked after depolarization to +60 mV was 0.43±0.07 and 0.51±0.12 nA under control condition and which were slightly decreased to 0.39±0.07 and 0.49±0.11 nA in the presence of 1 μM and 5 μM JTV-519, respectively. These changes were also not significant. In Fig. 2C and D, the amplitudes of the $I_{K,depo}$ and $I_{K,tail}$ before and 5 min after application of 5 μM JTV-519 were plotted against the membrane voltages. At potentials higher than +40 mV, the amplitude of $I_{K,depo}$, and not of $I_{K,tail}$, tended to decrease in the presence of JTV-519, albeit the difference did not reach statistical significance (P=0.12 for 5 μM JTV-519). Even when the concentration of JTV-519 was increased up to 20 μM, $I_{K,depo}$ and $I_{K,tail}$ did not seem to be decreased (n=1, data not shown). These results indicate that JTV-519 does not suppress I_{Ks} in the steady-state condition.

"Envelope of tails" test

We then examined the effect of JTV-519 on I_{Kr} using an envelope of tails test, which enabled us to separate the current components included in I_K (Noble and Tsien 1969; Sanguinetti and Jurkiewicz 1990, 1991). Depolarizing voltage steps to +50 mV were applied from a holding potential of –40 mV with increasing pulse duration. Figure 3A shows typical examples of the effect of E4031 on the current recorded using the envelope of tails test. In the absence of E4031 (Fig. 3A, top), short pulses (<0.2 s)

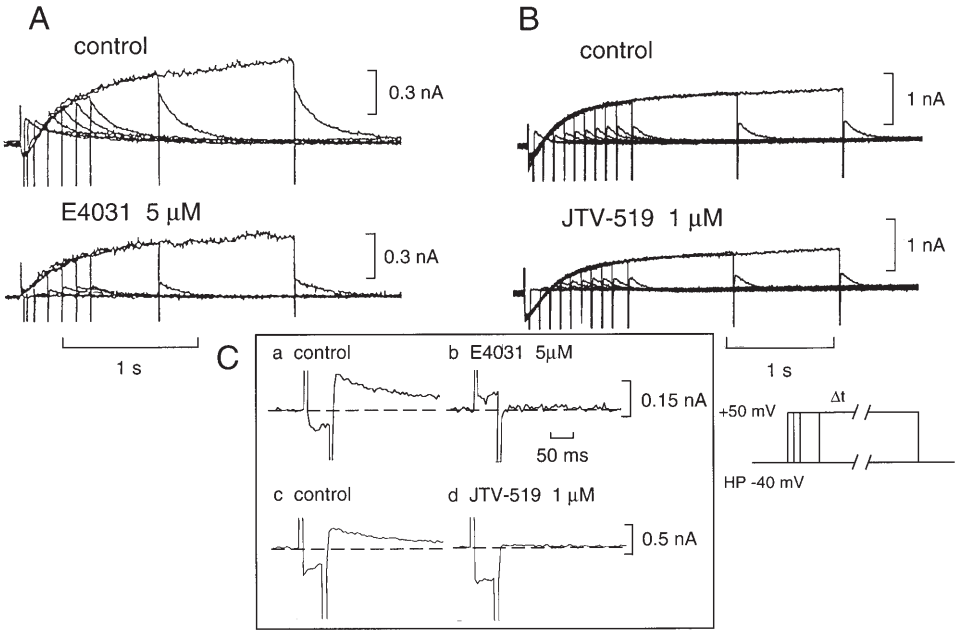

Fig. 3 Comparison of "envelope-of-tails" tests in between E4031 (**A**) and JTV-519 (**B**). Depolarizing pulses to +50 mV were applied from a holding potential of –40 mV with increasing duration of the pulses (from 50 to 3000 ms). Current traces obtained before (*control*) and in the presence of 5 μM E4031 or 1 μM JTV-519. **C** Isolated and expanded traces from portions of **A** and **B**

practically failed to activate $I_{K,depo}$, whereas a large $I_{K,tail}$ was evoked on return to the holding potential (-40 mV). This occurred because the I_{Kr} has a characteristic of inward-going rectification. Namely, I_{Kr} was quickly and totally inactivated at +50 mV and did not contribute to $I_{K,depo}$, but it contributed to the build-up of $I_{K,tail}$ as I_{Kr} channels were able to recover from the inactivation immediately after clamping back to –40 mV (Sanguinetti and Jurkiewicz 1990, 1991). On the other hand, I_{Ks} is activated very slowly with a considerable delay, so that the contribution of I_{Ks} to either $I_{K,depo}$ or $I_{K,tail}$ is negligible when the depolarizing pulse duration is fairly short (<0.2 s). Application of E4031 (5 μM) markedly decreased the $I_{K,tail}$ activated by short pulses (Fig. 3A, bottom), suggesting that the contribution of I_{Kr} to the tail current was quite large when the pulse duration was short. Application of JTV-519 (1 μM) also decreased $I_{K,tail}$ activated by short pulses as shown in Fig. 3B.

The currents evoked by a short (50 ms) pulse are shown in Fig. 3C on a magnified scale to demonstrate that both E4031 and JTV-519 depress the $I_{K,tail}$ evoked by the short pulse, suggesting that these agents share the same characteristic, that is the blockade of I_{Kr}.

In Fig. 4, the ratio of the peak $I_{K,tail}$ to $I_{K,depo}$, i.e. $I_{K,tail}/I_{K,depo}$ is plotted against duration of the depolarizing pulses. In the absence of the drug (open circles), the ratio obtained at short pulses (<0.2 s) had larger values than those obtained with longer pulses, thereby indicating coexistence of I_{Kr} and I_{Ks} in the I_K. JTV-519 significantly decreased the ratio obtained with short pulses (closed circles), but did not change the ratio with longer pulses. After washout of the drug (open triangles), the ratio obtained with short pulses (<0.2 s) was restored to the control value. These results again lend support to the notion that JTV-519 selectively blocks I_{Kr}.

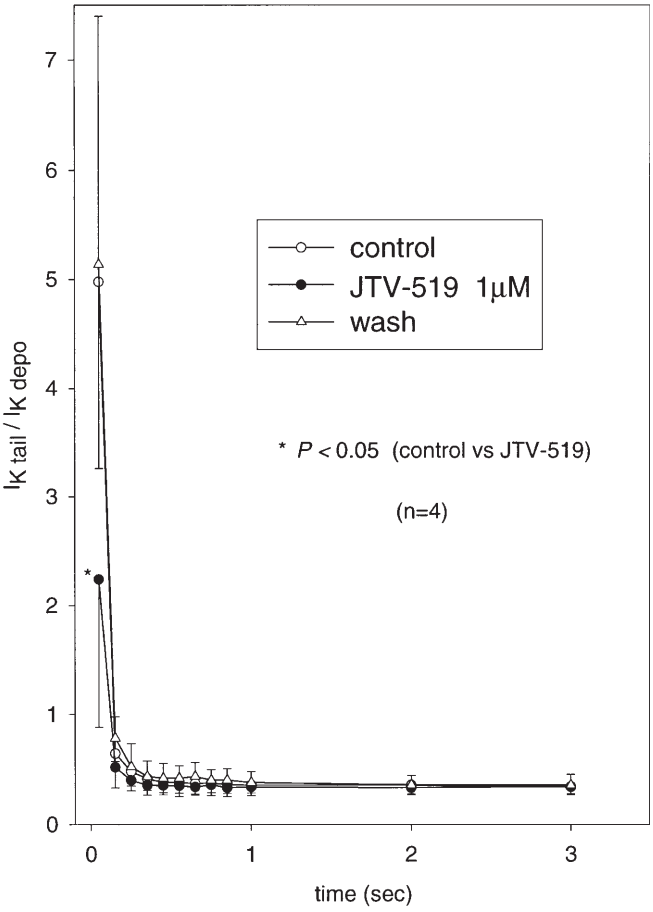

Fig. 4 The ratio of $I_{K,tail}$ to the time-dependent currents activated during the pulse ($I_{K,depo}$) (*ordinate*) as a function of the duration of the depolarizing pulse (*abscissa*). Mean±SEM, *n*=4. *Open circles* control; *filled circles* JTV-519; *open triangles* after wash-out of the drug

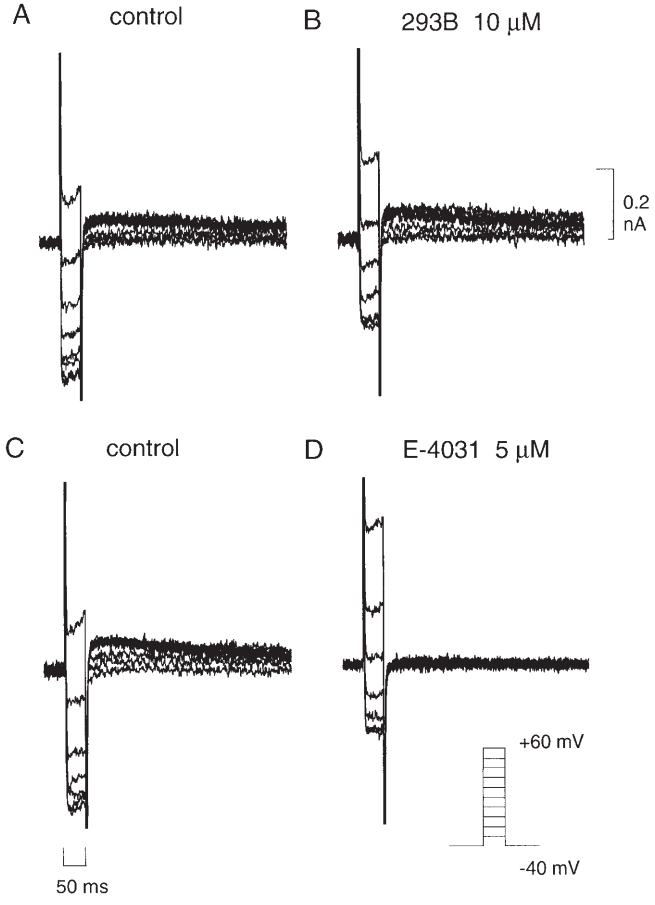

Fig. 5 Effects of 10 μM chromanol 293B (**A** and **B**) and 5 μM E4031 (**C** and **D**) on $I_{K,tail}$ evoked by short depolarizing pulses (50 ms) to between –30 and +60 mV from a holding potential of –40 mV in 10 mV increments. *Inset*: protocol of voltage clamps applied

Concentration-dependent block of I_{Kr} by JTV-519

We then studied the concentration/inhibition relationship of JTV-519 on I_{Kr}, using a short pulse protocol (duration 50 ms), which enabled us to isolate I_{Kr} from I_{Ks} without using pharmacological tools. The I_{Ks} is activated with a considerable delay after depolarization, while I_{Kr} is activated quickly after the start of the depolarizing pulses. Therefore, with the use of very short pulses (50 ms), only I_{Kr} could be activated.

Figure 5 demonstrates that the tail currents evoked by 50-ms pulses are not contaminated by I_{Ks}. Chromanol 293B (10 μM), a specific blocker of I_{Ks} (Bleich et al. 1997; Bosch et al. 1998; Busch et al. 1996; Schreieck et al. 1997) did not inhibit the $I_{K,tail}$ evoked by the pulses (Fig. 5A and B), while E4031 (5 μM) completely abolished it (Fig. 5C and D). Even when the concentration of chromanol 293B was increased up to 50 μM, the $I_{K,tail}$ evoked by 50-ms pulses was not changed (n=3, data not shown). Therefore, it is reasonable to consider that $I_{K,tail}$ evoked by very short pulses (50 ms) consists exclusively of I_{Kr}. Thus, we assessed the concentration-dependent effect of JTV-519 on I_{Kr} by using the short-pulse protocol. As shown in Fig. 6, JTV-519 (0.2, 1 and 5 μM) decreased the $I_{K,tail}$ in a concentration-dependent manner. We plotted the degree of inhibition against the drug concentrations used and fitted the data to the following theoretical equation, assuming a 1:1 interaction between drug molecules and receptors (channels):

$$\text{inhibition of } I_{Kr} = I_{max}[C/(K_d+C)] \quad (1)$$

where I_{max} is the maximum attainable inhibition (expressed as a percentage of control), K_d the apparent dissociation constant (in μmole/litre) and C the drug concen-

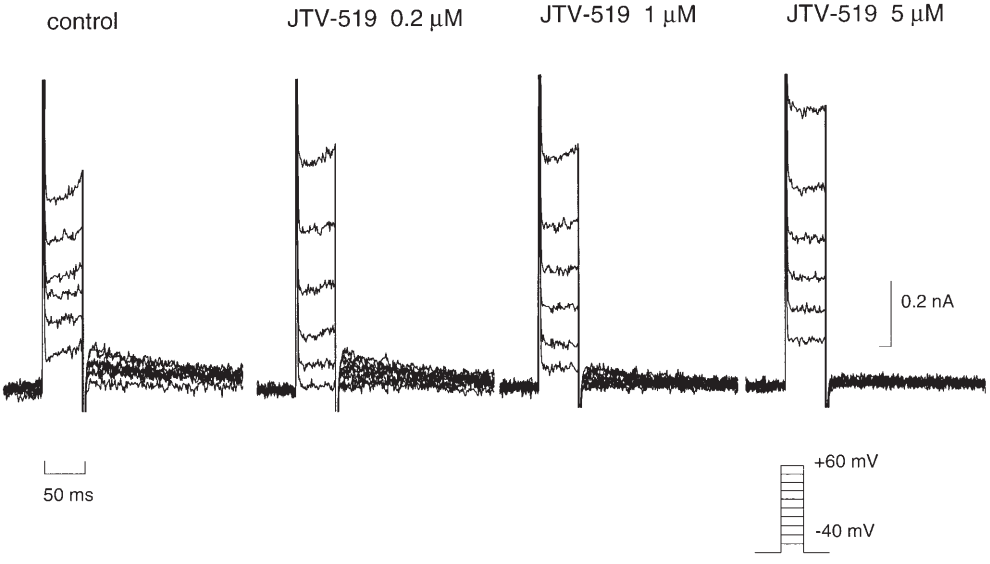

Fig. 6 Concentration-dependent effects of JTV-519 (0.2, 1 or 5 μM) on the tail currents evoked by short depolarizing pulses (50 ms) (i.e. I_{Kr}) from a holding potential of –40 mV in 10 mV increments. *Inset*: protocol of voltage clamps applied

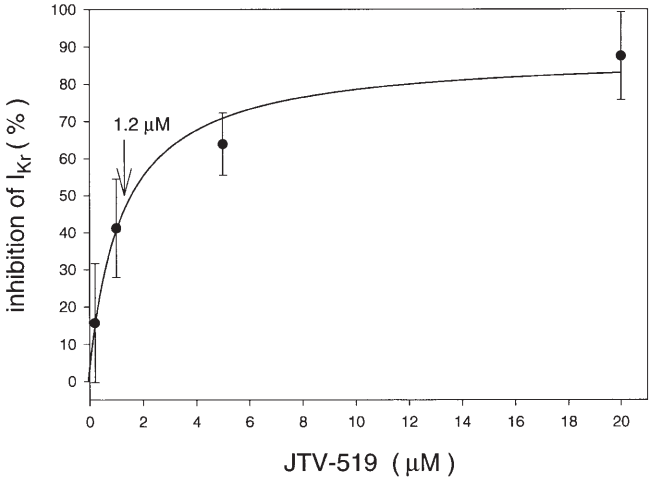

Fig. 7 Concentration/inhibition curve of JTV-519 on $I_{K,tail}$ evoked by short pulses (50 ms) of 60 mV (n=4). The data were fitted to the following equation: % inhibition=$I_{max}C/(C+K_d)$, where C is the concentration of JTV-519; I_{max}, the maximal attainable inhibition; K_d, the apparent dissociation constant. Data from three cells

tration in μmole/litre. The calculated I_{max} and K_d were 88.1% and 1.2 μM, respectively (Fig. 7).

Effects of JTV-519 on the action potential

The effect of JTV-519 on the action potential was tested at stimulation frequencies of 0.2 and 1 Hz. As shown in Fig. 8, JTV-519 (1 μM) shortened the APD at 90% repolarization level (APD_{90}) at both frequencies (Fig. 8), a finding similar to that reported by Kimura et al. (Kimura et al. 1999). We also found that the APD_{90} shortening caused by JTV-519 was frequency dependent. In all four cells tested, JTV-519 (1 μM) shortened the APD_{90} by 16.3±5.8% at 0.2 Hz (from 152.2±34.0 to 121.9±18.3 ms, n=4), while at 1 Hz, JTV-519 shortened the APD_{90} by 30.0±9.2% (149.8±34.7 to 95.6±6.9 ms). The percentage shortening was significantly less for 0.2 Hz than for 1 Hz (P<0.05).

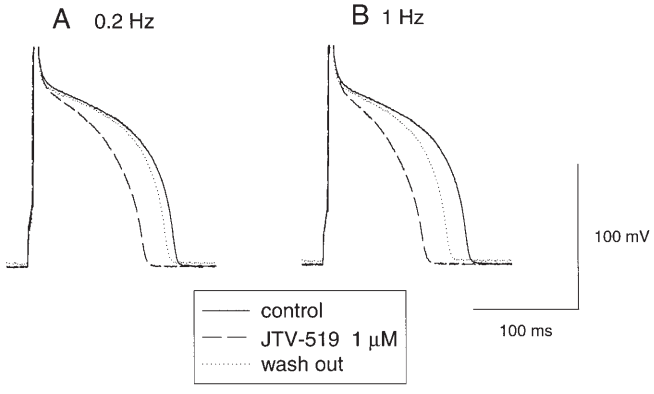

Fig. 8 An example of the effect of JTV-519 (1 μM) on the action potential duration recorded from a ventricular myocyte stimulated at frequencies of **A** 0.2 Hz and **B** 1 Hz

These findings suggest that JTV-519 shortened the APD_{90} perhaps via blockade of $I_{Ca,L}$ (Kimura et al. 1999), while this shortening effect of JTV-519 was much alleviated at a lower (0.2 Hz) stimulation frequency.

Discussion

Effect of JTV-519 on I_K

The delayed rectifier K^+ current (I_K) in the cardiac muscle includes at least three different current systems, namely the ultra-rapidly activating K^+ current, I_{Kur}, the rapidly activating K^+ current, I_{Kr} and the slowly activating K^+ current, I_{Ks}. Each of these is sensitive to a certain specific blocker. I_{Kur} is blocked by low concentrations of 4-aminopyridine (Fedida et al. 1993; Wang et al. 1993). I_{Kr} is blocked selectively by E4031, sotalol or dofetilide (Sanguinetti and Jurkiewicz 1990) and the I_{Ks} is blocked by chromanol 293B (Bleich et al. 1997; Bosch et al. 1998; Busch et al. 1996; Schreieck et al. 1997). The concomitant existence of I_{Kr} and I_{Ks} in guinea-pig ventricular cells has been suggested using a envelope of tails test (Sanguinetti and Jurkiewicz 1990), while I_{Kur} is not present in ventricular cells (Li et al. 1996). In the envelope of tails test, the ratio of the tail current to the time-dependent current activated during pulses ($I_{K,tail}/I_{K,depo}$) was not constant with regard to duration of the pulses (Fig. 4). This means that a very small fraction of $I_{K,depo}$ is activated even by a large depolarizing pulse (+50 mV) when the duration was fairly short (<0.2 s); this is because I_{Kr} activated at +50 mV is negligible due to its strong inward-going rectification property and the activation of I_{Ks} requires much longer time of depolarization. In contrast, $I_{K,tail}$ is large because I_{Kr} is rapidly reactivated or rapidly recovered from its inactivation when the membrane was repolarized (clamped back) to –40 mV. Therefore, the ratio ($I_{K,tail}/I_{K,depo}$) was quite large for the short depolarizing pulses. When the pulse duration was increased (>0.3 s), the ratio diminished because I_{Ks}, which is activated more slowly and has little inward-going rectification, becomes the dominant current (Fig. 4). JTV-519 decreased the ratio ($I_{K,tail}/I_{K,depo}$) at short pulses just as did E4031, a selective blocker of I_{Kr} (Sanguinetti and Jurkiewicz 1990; Wang et al. 1996). Such findings suggest that JTV-519 selectively blocked I_{Kr}.

The concentration/inhibition relationship of the I_{Kr} block by JTV-519 was examined using short pulses (50 ms) which evoked the tail current that consisted exclusively of I_{Kr}. The validity of this method was verified in the experiments to demonstrate that the $I_{K,tail}$ evoked by very short pulses was completely abolished by E4031 (Fig. 5D) but not by chromanol 293B, a selective blocker of I_{Ks} (Fig. 5B).

JTV-519 inhibited the tail current consisting of the I_{Kr} alone in a concentration-dependent manner (Figs. 6 and 7). According to the formulation of the drug-receptor complex (Eq. 1), the I_{max} and K_d were 88.1% and 1.2 μM, respectively (Fig. 7). In a previous study using the same specimen (Sanguinetti and Jurkiewicz 1990), the half-

maximum inhibition of I_{Kr} for E4031 was reported to be 0.397 μM, thereby suggesting that JTV-519 is less potent than E4031 for inhibition of I_{Kr}.

Effects of JTV-519 on action potentials

In experiments using ventricular tissues (multicellular preparations) of dogs (personal communication, Research Institute of Japan Tobacco Co.) and guinea-pigs (Ito et al. 1998), and also using ECG recordings in healthy volunteers (personal communication, Research Institute of Japan Tobacco Co.), it was reported that JTV-519 prolonged the ventricular repolarization. However, in isolated guinea-pig ventricular cells, JTV-519 markedly shortened the APD (Kimura et al. 1999). In our study using the same model, we observed similar shortenings of the APD in the presence of 1 μM JTV-519 (Fig. 8). One possibility is that the inhibition of $I_{Ca,L}$ caused by this drug (Kimura et al. 1999; unpublished observation by K. Kiriyama, T. Kiyosue, M. Arita) prevailed over the APD lengthening to be caused by the inhibition of I_{Kr} in single cells and shortened the APD. The shortening of APD by JTV-519 was less marked at a lower stimulation frequency (0.2 Hz). It is well known that authentic I_{Kr} blockers such as E4031 or dofetilide have reverse use-dependent effects on APD: the lower the stimulation frequency, the more marked the prolongation of APD. Thus, the apparent alleviation of APD shortening at a lower stimulation frequency (0.2 Hz) might have been due to the suppression of I_{Kr} by JTV-519 as was shown in the present study. However, at this moment, no information is available as for the use-dependent effect of JTV-519 on $I_{Ca,L}$. Therefore, we are not able to rule out the alternative possibility that use-dependent suppression of $I_{Ca,L}$ caused the rate-dependent shortening of APD, thereby leading to relatively minor APD shortening at lower stimulation frequencies. As I_{Kr} is known to exist in human ventricles and to play an important role in the initiation of action potential repolarization (Li et al. 1996), the inhibition of I_{Kr} by JTV-519 documented in the present study may contribute, at least in part, to the prolongation of QT intervals, alleged to occur in patients treated with this drug.

Cardioprotective effects of JTV-519

The present study demonstrated that JTV-519 inhibited I_{Kr}, an effect shared with class III antiarrhythmic drugs such as E4031, sotalol, or dofetilide. The half-maximal inhibitory concentration of I_{Kr} was 1.2 μM, indicating that the efficacy of JTV-519 is less potent than E4031 (0.397 μM). The inhibition of I_{Kr} by JTV-519, however, would not be directly related to the alleged cardioprotective effects of this agent. Recently, a few reports have appeared concerning the cardioprotective effects of this drug. Intravenous application of JTV-519 was effective in reducing the necrotic area in canine models of acute myocardial infarction (Miyai et al. 1998b). The drug was found to bind allosterically to annexin-V and to inhibit annexin-V-dependent Ca^{2+} entry (Kaneko 1997a, 1997b). The drug also induced vasorelaxation in the rat aorta and increased coronary blood flow in the dog heart (Miyai et al. 1998a). Recently, a beneficial effect of this drug to the ischaemia-induced myocardial damage was attributed, at least in part, to the activation of δ isoform of protein kinase C (Inagaki et al. 2000).

In clinical settings, however, one must remember that, at concentrations known to be effective in preventing ischaemic damage in animals (0.17–0.54 μM) (Miyai et al. 1998b), JTV-519 also inhibited a large fraction of I_{Kr} which may play a role to prolong the QT interval. Such a class-III effect of JTV-519 could be beneficial in preventing lethal tachyarrhythmias of the reentrant type occurring in case of acute myocardial infarction, by lengthening the effective refractory period. However, the effect of prolongation of the QT interval, if any, is sometimes pro-arrhythmic, particularly in patients with background disorders in ventricular repolarization (Jackman et al. 1988). Further clinical evaluations are to be done.

Acknowledgements We thank Dr. Hans-Lang for his kind supply of chromanol 293B and Ms. K. Moriyama for her secretarial services. All procedures and experiments described here met the Guideline Principles stipulated by the Physiological Society of Japan and the Animal Ethics Committee of the Oita Medical University for Animal Experiments.

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