Effects of a Novel Cardioprotective Drug, JTV-519, on Membrane Currents of Guinea Pig Ventricular Myocytes

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ABSTRACT—We investigated effects of a novel cardioprotective drug, JTV-519 (4-[3-(4-benzylpiperidin-1-yl)propionyl]-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine monohydrochloride) on membrane currents of guinea pig ventricular myocytes by whole-cell voltage and current clamp methods. The fast Na^+ current (i_{Na}) was activated by ramp pulses from various holding potentials of -90 , -80 or -60 mV to 10 mV with various intervals. At 0.2 Hz, JTV-519 inhibited i_{Na} in a concentration-dependent manner with an IC_{50} of approximately 1.2 and $2 \mu\text{M}$ at the holding potential of -60 and -90 mV, respectively, implicating a voltage-dependent block. Increasing the pulse frequency from 1 to 2 or 3.3 Hz in the presence of $1 \mu\text{M}$ JTV-519 shortened the time-course and increased the level of i_{Na} block, indicating a frequency-dependent block. The time-course of i_{Na} blocking by JTV-519 was slower than that of lidocaine and similar to that of quinidine. Ca^{2+} current (i_{Ca}) and the inwardly rectifying K^+ current (i_{K1}) were also inhibited by JTV-519. JTV-519 decreased the duration and the height of the plateau of the action potential. We conclude that JTV-519 has frequency- and voltage-dependent blocking effects on i_{Na} as well as inhibition of i_{Ca} and i_{K1} .

Keywords: JTV-519, Na^+ channel, Na^+ channel inhibitor, Cardiac myocyte, Antiarrhythmic drug

JTV-519 (4-[3-(4-benzylpiperidin-1-yl)propionyl]-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine monohydrochloride) (K-201; Fig. 1), a newly synthesized drug, has been shown to have a cardioprotective effect against a sudden cardiac cell death due to over-contraction induced by high Ca^{2+} , and the effect was more potent than those of nicorandil, prazosin, propranolol, verapamil and diltiazem (1). The drug was found to bind allosterically to annexin V and inhibit annexin V-dependent Ca^{2+} entry (2, 3). The drug also induced vasorelaxation in the rat aorta and an increase in coronary blood flow in the dog heart (4). Recently, there have been increasing reports about the cardioprotective effects of this drug against ischemia/reperfusion-induced Ca^{2+} overload (5, 6) and stunning (7). The effect of the drug on the ionic channels,

however, had not yet been elucidated. We started investigating the drug effect on the Na^+ - Ca^{2+} exchange current in cardiac myocytes and found no effect. However, we found that the drug inhibited the Na^+ current (i_{Na}).

A large number of antiarrhythmic drugs have been known to inhibit Na^+ channels (8, 9). The mechanism of the block of these drugs has been interpreted by a modulated receptor theory (10–12). Recent development of the molecular technology has allowed identification of the binding sites of the antiarrhythmic drugs (13–15). We investigated the effects of JTV-519 on i_{Na} and compared them with those of other antiarrhythmic Na^+ -channel inhibitors such as lidocaine and quinidine. We also investigated the effects on other membrane currents such as Ca^{2+} current (i_{Ca}), the inwardly rectifying K^+ current (i_{K1}) and on the action potential.

MATERIALS AND METHODS

Cell preparation

Single cardiac ventricular cells were prepared from the guinea pig heart by a method essentially similar to the previously reported ones (16). Briefly, male guinea pigs (250–400 g) were deeply anesthetized with intra-

Fig. 1. Chemical structure of JTV-519 (K-201).

peritoneal injection of sodium pentobarbital. Under artificial ventilation, the heart was extracted and perfused with Ca^{2+} -free Tyrode solution containing collagenase, 5 mg/50 ml (Wako Chemical, Tokyo) and alkaline protease 1 mg/50 ml (Nagase, Tokyo) for about 10 min on the Langendorff apparatus. The dissociated cells were kept in a low Cl^- , high K^+ solution (KB solution) at 4°C .

Whole-cell-clamp

The whole-cell-clamp method was essentially the same as the originally reported (17). The patch clamp amplifiers were either EPC-7 (List, Darmstadt, Germany) or TM-1000 (Act ME, Tokyo). The current clamp was performed using the EPC-7. Borosilicate glass capillaries (Tokyo Rikagaku Kikai, Tokyo) with 1.5-mm external diameter were used to pull the patch pipettes, which had a resistance of 2–3 M Ω . The data were recorded on-line with a computer (PC9801RX; NEC, Tokyo) for later analysis using original software called RAM5 (National Institute for Physiological Sciences, Okazaki).

We used mainly ramp pulses to induce i_{Na} in the present experiments by using a function generator (NF, Tokyo). The ramp pulse is convenient, because an I-V curve can be obtained by one pulse. However, the voltage axis of the I-V curve obtained by the ramp contains a time factor. Therefore, if the current is time-dependent, the I-V curve does not reflect the steady state I-V curve. If a drug modifies the time-course of the current, the I-V curve may shift in the voltage axis. The drug effects on the voltage-dependency and the time dependency are inseparable in the I-V curve of the ramp clamp data. Therefore we analyzed only the peak amplitude of i_{Na} and not its I-V curve.

Solutions and drugs

Tyrode solution contained the following: 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2 , 1 mM MgCl_2 , 0.33 mM NaH_2PO_4 , 5.5 mM glucose, 5 mM HEPES (pH 7.4). Na^+ current was measured either at 140 or 28 mM Na^+ . The low Na^+ external solution was made by mixing Na^+ - and *N*-methyl-*D*-glucamine (NMG) external solutions. The external solutions contained the following: 140 mM NaCl or NMG, 1.8 mM CaCl_2 , 1 mM MgCl_2 , 5 mM HEPES (pH 7.4) and 10 μM nifedipine (Sigma, St. Louis, MO, USA). The pH of NMG solution was adjusted with HCl. The pipette solution contained the following: 120 mM CsOH, 20 mM CsCl, 54 mM aspartic acid, 3 mM MgCl_2 , 5 mM MgATP, 20 mM BAPTA, 20 mM HEPES (pH 7.2). For the K^+ internal solution, Cs^+ was replaced by K^+ . JTV-519 was provided by Japan Tobacco, Inc. (Tokyo). All the chemicals used were of the highest grade available.

Statistics

The data were expressed as the mean $\pm$ S.D. The statistical significance was analyzed by the Z test for the tonic block and by analyses of variance (ANOVA) for the frequency-dependent block. Differences with $P < 0.05$ were considered significant.

RESULTS

Tonic block

To see the tonic effect of the drug, i_{Na} was activated every 5 sec by depolarizing ramp pulses from -120 to -5 mV with the speed of 0.7 V/sec from two different holding potentials: -60 and -90 mV (Fig. 2A). The 140 mM Na^+ external solution was used. After recording the control i_{Na} , JTV-519 at 0.3, 1, 3 and 10 μM was applied cumulatively in the external solution. JTV-519 decreased the peak amplitude of i_{Na} in a concentration-dependent manner. i_{Na} was readily restored upon washing out the drug as shown in Fig. 2B. I-V curves in response to the ramp pulses were superimposed in Fig. 2C. A positive shift of the I-V curve was seen as the drug concentration increased. This shift, however, was also observed in the absence of the drug, when the current amplitude was decreased by high frequency activation. Therefore the shift was not necessarily due to the drug. The concentration-response curve measured at -60 mV was shifted to the right compared to that at -90 mV, indicating that the drug effect was voltage-dependent (Fig. 2D). The concentration of JTV-519 that gave a 50% block of i_{Na} (IC_{50}) was approximately 1.2 μM at the holding potential of -60 mV and 2 μM at -90 mV.

The effect of JTV-519 was also examined by depolarizing square pulses of 30-msec duration to -30 mV from the holding potential of -90 mV (Fig. 3). The drug did not significantly change either the activation or the inactivation time-course of i_{Na} but only diminished the peak at 1 μM . These results indicate that JTV-519 has tonic and voltage-dependent inhibitory effects on the cardiac i_{Na} .

Phasic block

Frequency dependency of the drug inhibition was examined with three different pulse intervals: 300 msec (3.3 Hz), 500 msec (2 Hz) and 1000 msec (1 Hz). The holding potential was -90 mV. Figure 4 shows i_{Na} activated by 15 ramp pulses with the 3 different intervals in the presence of 1 μM JTV-519. The control i_{Na} before applying the drug hardly changed during the 15 pulses (not shown). In the presence of JTV-519, i_{Na} in response to the 15th pulse decreased to $64 \pm 20\%$ ($n=6$) of the first current in the series, $65 \pm 17\%$ ($n=5$), and $77 \pm 11\%$ ($n=5$) at 3.3, 2 and 1 Hz, respectively. The percentage of i_{Na} decrease was significantly different between 3.3 or 2 Hz and 1 Hz. The

half time of the block was 1.0 ± 0.3 ($n=6$), 1.4 ± 0.2 ($n=5$) and 2.6 ± 0.5 sec ($n=5$) at 3.3, 2 and 1 Hz, respectively. The time-course of the block was significantly faster as the frequency increased, indicating that the drug inhibited the channel in a frequency-dependent manner.

Comparison with lidocaine and quinidine

Time-course of the frequency-dependent blocking effect of JTV-519 was compared with those of lidocaine and quinidine, well-known frequency-dependent antiarrhythmic drugs. As shown in Fig. 5, JTV-519 at $1 \mu\text{M}$, lidocaine at $10 \mu\text{M}$ and quinidine at $20 \mu\text{M}$ were applied at 3.3-Hz ramp pulse protocol similar to that shown in Fig. 4. The drug concentrations were chosen to block the current roughly by half. At the 15th pulse, $10 \mu\text{M}$

lidocaine decreased i_{Na} to $71 \pm 9\%$ of the initial i_{Na} with the half time of $<0.38 \pm 0.08$ sec ($n=4$). Quinidine at $20 \mu\text{M}$ decreased it to $34 \pm 5\%$ with the half time of 0.9 sec ($n=3$), while JTV-519 at $1 \mu\text{M}$ decreased it to $64 \pm 20\%$ ($n=6$) with the half time of 1.0 ± 0.3 sec ($n=6$), as described above. Thus JTV-519 inhibited i_{Na} with a time-course similar to that of quinidine but was slower than lidocaine.

Effect on i_{Ca}

Effect of JTV-519 on i_{Ca} was investigated by using a step pulse protocol. Square pulses of 300-msec duration were applied every 10 sec from the holding potential of -70 mV. The external solution contained NMG without Na^+ to eliminate i_{Na} . Figure 6 shows representative traces

Fig. 2. Tonic effect of JTV-519. A: i_{Na} was induced by a ramp pulse shown at the upper part. The lower part indicates the membrane current in response to the above voltage pulse. B: Upper part indicates the chart record of voltage and the lower part, the current. JTV-519 was applied cumulatively as indicated. The envelope of the current shows the blocking effect on i_{Na} by the drug. C: Current-voltage relations obtained in panel B were superimposed. Labels correspond to panel B. D: Concentration-response relations of JTV-519. Data obtained with the holding potentials at -60 mV (□) and -90 mV (■) are plotted. * indicates $P < 0.05$. Number of cells are indicated in the parenthesis.

Fig. 3. I_{Na} obtained by square pulses illustrated in the above inset. Control and i_{Na} in the presence of $1 \mu\text{M}$ JTV-519 were superimposed.

of i_{Ca} and its I-V curves before, during and after washing off JTV-519. The drug inhibited i_{Ca} reversibly. The concentration-response curve was plotted in Fig. 7. The IC_{50} value of JTV-519 on i_{Ca} was about $3 \mu\text{M}$.

Effect on the inward rectifier K^+ current (i_{K1})

Effect of JTV-519 on i_{K1} was investigated using the ramp pulse protocol. Voltage pulses were given from the holding potential of -40 mV to 40 mV , then to -90 mV and back to the holding potential with the speed of 0.52 V/sec . JTV-519 inhibited i_{K1} irreversibly. Figure 8 shows the representative I-V curves plotted from the descending portion of the ramp. The concentration-response curve was obtained by measuring i_{K1} at -90 mV (Fig. 7). IC_{50} of JTV-519 on i_{K1} was about $5 \mu\text{M}$.

Effect on the action potential

Action potentials were recorded with 10-sec intervals using K^+ -pipette solution and Tyrode external solution, and the effect of $1 \mu\text{M}$ JTV-519 was tested. In all the 3 cells tested, the action potential duration was shortened and the height of the plateau was decreased. Figure 9 shows representative data. In this cell, the resting potential was -74 mV and the action potential duration measured at 90% repolarization was 500 msec in the control. JTV-519 at $1 \mu\text{M}$ decreased the height of the plateau and the duration of the action potential to 300 msec but did not significantly change the resting potential, which was at -73 mV in the presence of the drug. The effect recovered completely on washing off the drug. On the

Fig. 4. Frequency-dependency of JTV-519 effect. i_{Na} in response to 15 ramp pulses with different intervals in the presence of JTV-519 ($1 \mu\text{M}$). The pulse interval is shown above each set of current traces. The holding potential was -90 mV .

Fig. 5. Comparison of JTV-519 with lidocaine and quinidine. The pulse interval was 300 msec. Currents were activated by the same ramp pulse protocol used in Fig. 4.

Fig. 6. Effect of JTV-519 on i_{Ca} . The top panel illustrates the square pulses to induce i_{Ca} . Middle panels illustrate families of i_{Ca} . Lower panels indicate I-V curves that correspond to the above current traces. The peak (●) and quasi steady state (○) measured just before the end of the pulse.

average, the control resting potential was -74 ± 1 mV ($n=3$) and the action potential duration was shortened to $49 \pm 12\%$ ($n=3$) of the control.

Fig. 7. Concentration-response curves of i_{Ca} (□) and i_{K1} (◆).

Fig. 8. Effect of JTV-519 on i_{K1} . i_{K1} at different concentrations of the drug were superimposed.

Fig. 9. Effect of JTV-519 on the action potential. The control resting potential was -74 mV. Control and the action potential after washing out the drug were almost the same.

DISCUSSION

The present study showed that JTV-519 has a voltage- and frequency-dependent blocking effect on i_{Na} . The time-course of inhibition of JTV-519 was slower than that of lidocaine and similar to that of quinidine. Therefore JTV-519 is not a 'fast' drug but can be categorized as an 'intermediate' drug (18). It has been reported that lidocaine blocks the Na^+ channel mainly at the inactivated state (19, 20), while quinidine is an open channel blocker and has no affinity to the resting or inactivated state of the Na^+ channel (20–22). JTV-519 has a time-course of blocking similar to that of quinidine but it also showed a tonic block at the holding potential of -90 mV, indicating that JTV-519 could also block the resting state of the channel. Therefore, although the time-course was similar, JTV-519 might have a mechanism of action different from quinidine. Another difference from lidocaine or quinidine was that JTV-519 was approximately 10 times more potent than the former drugs. The half-maximum concentration of lidocaine was higher than $300 \mu M$ (19), while IC_{50} of the tonic block of JTV-519 was $1-2 \mu M$.

The square pulse protocol revealed that JTV-519 decreased the peak amplitude of i_{Na} without changing the activation and inactivation kinetics. This effect was different from that of penticainide, which decreased the peak but slowed the inactivation phase (23).

Unlike quinidine, which prolongs the action potential duration, JTV-519 shortened it by 50% at $1 \mu M$. At this concentration, JTV-519 inhibited i_{Na} to 65% and i_{Ca} to 80%. We tested whether the blocking effect against these currents could explain the shortening of action potential by the drug by using the commercially available cardiac

computer model software, Oxsoft Heart (Oxsoft Ltd., Oxford, UK). Running the guinea pig ventricular model, the action potential duration was shortened only to 93% upon blocking i_{Na} and i_{Ca} to the above degrees (not shown). Adding the parameter of blocking i_{K1} prolonged the action potential duration to 110% of the control. Therefore, it is difficult to explain 50% block of the action potential duration only by the drug effects on i_{Na} , i_{Ca} and i_{K1} . The drug may have some other additional effects, for example, activation of some components of the delayed rectifier K^+ channels. Kiyosue and Arita (24) reported that tetrodotoxin at $60 \mu M$ shortened the action potential duration by 10% in guinea pig myocytes, which they attributed to the presence of a slow component of i_{Na} . Other workers (25, 26) also reported the presence of slow inactivating sodium current. However, we did not see the inhibition of the slow component of i_{Na} by JTV-519 using square pulses.

The resting potential was not affected by JTV-519 in the action potential recording (Fig. 9), although JTV-519 at $1 \mu M$ decreased i_{K1} to 70% (Fig. 7). The reason for the unchanged resting potential might be because the resting potential was at -74 mV in this cell, which coincided with the reversal potential of i_{K1} shown in the I-V curves in Fig. 8. In the computer model described above, decreasing the conductance of i_{K1} did not affect the resting potential.

We conclude that JTV-519 inhibits i_{Na} in a voltage- and frequency-dependent manner. The drug also blocked i_{Ca} and i_{K1} , and the action potential shortening was observed. We did not examine whether or not the Ca^{2+} channel inhibition was voltage dependent. If it is, it could block supraventricular tachyarrhythmia. However, the Ca^{2+} channel inhibition may lead to inhibition of contraction. Indeed, JTV-519 inhibits contraction of the guinea pig papillary muscle at concentrations higher than $1 \mu M$ (H. Miyai et al., personal communication). Kaneko et al. (1–3, 27) reported that this drug binds to annexin V. Inagaki et al. (28) more recently reported that the drug could activate protein kinase C. It is unknown whether annexin V or protein kinase C is involved in the inhibitory effects of this drug on the ionic channels. The mechanism of the anti-stunning effect of the drug has been suggested to be different from that of the K^+ channel openers (29). Further study is required to elucidate the multiple effects of this drug.

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