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Electrophysiological effect of *l-cis*-diltiazem, the stereoisomer of *d-cis*-diltiazem, on isolated guinea-pig left ventricular myocytes

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Abstract

l-cis-Diltiazem, the stereoisomer of the L-type Ca^{2+} channel blocker *d-cis*-diltiazem, protects cardiac myocytes from ischemia and reperfusion injury in the perfused heart and from veratridine-induced Ca^{2+} overload. We determined the effect of *l-cis*-diltiazem on the voltage-dependent Na^+ current (I_{Na}) and lysophosphatidylcholine-induced currents in isolated guinea-pig left ventricular myocytes by a whole-cell patch-clamp technique. *l-cis*-Diltiazem inhibited I_{Na} in a dose-dependent manner without altering the current–voltage relationship for I_{Na} (K_d values : 729 and 9 μM at holding potentials of −140 and −80 mV, respectively). A use-dependent block of I_{Na}, the leftward shift of the steady-state inactivation curve and the delay of recovery from inactivation suggest that *l-cis*-diltiazem has a higher affinity for the inactivated state of Na^+ channels. In addition to I_{Na}, the lysophosphatidylcholine-induced currents were inhibited by *l-cis*-diltiazem in a similar concentration range. It is suggested that inhibition of both Na^+ channels and lysophosphatidylcholine-activated non-selective cation channels contributes to the cardioprotective effect of *l-cis*-diltiazem.

Keywords: *l-cis*-Diltiazem; *d-cis*-Diltiazem; Patch-clamp; Whole-cell; Ca^{2+} overload; Na^+ current; Lysophosphatidylcholine

1. Introduction

Myocyte death in ischemia/reperfusion may be due to cytosolic Ca^{2+} overload (Jeremy et al., 1992) mediated by reversal activation of the Na^+/Ca^{2+} exchanger (Silverman and Stern, 1994). Intracellular accumulation of Na^+ occurs after ischemia as a result of activation of the Na^+/H^+ exchanger (Karmazyn and Moffat, 1993; Nishida et al., 1993), depolarization, accumulation of lysophosphatidylcholine (Undrovinas et al., 1992), and Na^+ channel activation (Takeo et al., 1989; Butwell et al., 1993; Silverman and Stern, 1994). Previous studies have shown that *d-cis*-diltiazem, a benzothiazepine L-type Ca^{2+} channel blocker, but not dihydropyridine Ca^{2+} channel blockers, prevents ischemia and reperfusion injury of the heart (Watts et al., 1980; Nagai et al., 1983; Lopaschuk et al., 1992).

Recently, it was reported that *l-cis*-diltiazem, the stereo isomer of *d-cis*-diltiazem, also exerts cardioprotective effects in ischemia and reperfusion injury in the rat working heart (Nasa et al., 1990; Van Amsterdam et al., 1990). However, *l-cis*-diltiazem is less potent than the *d*-isomer in causing Ca^{2+} antagonism (Nagao et al., 1982). Therefore, the cardioprotective effect is not related to blockade of L-type Ca^{2+} channels. Furthermore, we have recently demonstrated that *l-cis*-diltiazem suppresses veratridine-induced Ca^{2+} and Na^+ accumulation in isolated rat myocytes (Itogawa et al., 1996) and speculated that a blocking action on Na^+ channels by *l-cis*-diltiazem may contribute to its cardioprotective effect.

In the present study, in order to clarify the effect of *l-cis*-diltiazem on Na^+ channel activity, we determined the Na^+ currents (I_{Na}) of guinea-pig left ventricular myocytes using a whole-cell patch-clamp technique. Furthermore, its effects on lysophosphatidylcholine-induced currents were also determined since lysophosphatidylcholine accumulates and induces Ca^{2+} overload in the heart during ischemia (Magishi et al., 1996).

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2. Materials and methods

2.1. Cell isolation

Ventricular myocytes were enzymatically isolated from hearts of male Hartley guinea-pigs (weight 300–400 g) according to the method described by Cavalié et al. (1983). The animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and the ascending aorta was cannulated in situ under artificial respiration. The heart was excised and perfused at 37°C via a Langendorff's apparatus, first with a nominally Ca^{2+}-free solution for 10 min, followed by collagenase solution for 5–8 min. Subsequently, the enzyme solution was washed out with a Kraftbrühe (KB) solution. The ventricular myocytes were dissociated by gentle stirring of the tissue fragments at 37°C. The dissociated cells were stored in KB solution at 4°C and were used for experiments within 12 h.

The KB solution contained (mM): potassium glutamate 70, KCl 25, oxalic acid 10, KH_2PO_4 10, taurine 10, glucose 11, HEPES 10 and EGTA 0.5 (pH 7.4 by KOH). The normal Tyrode solution used in this procedure had the following composition (mM): NaCl 135, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 11 and HEPES 5 (pH 7.4). Ca^{2+} was omitted in Ca^{2+}-free Tyrode solution. The collagenase solution was prepared by adding 70–80 units/ml collagenase (Collagenase Type I, Yakult, Tokyo, Japan) and 10 μM $CaCl_2$ to Ca^{2+}-free Tyrode solution.

2.2. Electrophysiological measurements

Transmembrane currents were recorded with the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981) by use of a patch-clamp amplifier (EPC-8, HEKA Elektronik, Lambrecht, Germany). The resistance of the electrodes was 2–4 MΩ when filled with internal solution. The junction potential between the pipette solution and the extracellular medium was adjusted to zero before the microelectrode contacted the surface of myocytes. In whole-cell recordings, data were collected by and stored on a Macintosh computer running HEKA pulse software. Currents were filtered at 3 kHz. The sampling frequency of the A/D converter was 10 kHz. Current signals were stored on a computer hard disc. The cells were placed in a chamber (volume 0.15 ml) attached to the stage of an inverted microscope (TMD, Nikon, Tokyo, Japan). The chamber was continuously perfused at a constant rate of 1 ml/min with modified Tyrode solution at 19°C. For I_{Na} recordings, the external solution had the following composition (mM): NaCl 10.0, tetramethylammonium chloride 130.0, $CaCl_2$ 1.8, $CoCl_2$ 1.0, CsCl 5.0, $MgCl_2$ 1.2, glucose 11.0 and HEPES 20.0 (pH 7.3, adjusted by tetramethylammonium hydroxide). The pipette solution was composed of (mM): NaF 5.0, CsF 125.0, K_2ATP 5.0, K_2 creatine phosphate 5.0, EGTA 5.0, and HEPES 5.0 (pH 7.2, adjusted with CsOH). For lysophosphatidylcholine-induced current recordings, a Tyrode solution containing 2 mM $BaCl_2$ and 3 μM nisoldipine to block K^+ channel currents and the L-type Ca^{2+} channel current was used as the external solution. The pipette solution was composed of (mM): CsCl 110.0, CsOH 20.0, $MgCl_2$ 3.0, MgATP 5, K_2 creatine phosphate 5.0, 1,2-bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) 5.0, HEPES 10.0, $CaCl_2$ 0.35, aspartate 20.0 (pH 7.2, adjusted by CsOH). For study of the effects of *N*-methyl-D-glutamine (NMG), the external solution and the pipette solution were composed of (mM): NMG-aspartate 140.0, BAPTA 1.0, HEPES 10, $BaCl_2$ 2.0, nisoldipine 0.003(external, pH 7.4); NMG-aspartate 140.0, $MgCl_2$ 3, MgATP 5.0, K_2 creatine phosphate 5.0, BAPTA 5.0, HEPES 10.0 (pipette, pH 7.2), respectively. After a giga seal (>10 GΩ) was formed by negative pressure, additional pressure was added to rupture the membrane under the tip of the electrode. Capacitive currents and series resistance were compensated by use of analog circuitry and leak subtraction was also used in some experiments. The mean seal resistance was 34 ± 6 GΩ ($n = 12$) with series resistance compensation set at 50–70%, the mean series resistance was 4.0 ± 1 MΩ ($n = 12$) and the mean cell capacitance was 130 ± 20 pF ($n = 12$). P/N subtraction was not routinely done and there was no evident effect on macroscopic currents when it was used.

2.3. Chemicals

l-cis-Diltiazem was synthesized at Tanabe Seiyaku (Osaka, Japan). L-α-lysophosphatidylcholine (palmitoyl) was obtained from Sigma (St. Louis, MO, USA). Other chemicals of the highest grade were purchased commercially.

2.4. Statistical analysis

The results represent means ± S.E.M. Each experiment was done on myocytes isolated from different animals. Statistical comparisons were made by using the paired Student's *t*-test. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Effect of l-cis-diltiazem on I_{Na}

We examined the effect of *l-cis*-diltiazem on I_{Na}, which was evoked from different holding potentials (V_h), i.e., V_h −140, −110, −90 and −80 mV. Fig. 1A shows typical traces of I_{Na} evoked by 20-ms step depolarization from V_h −90 mV to various potential levels ranging from −80 to +50 mV in 10-mV steps. To avoid intricate traces, currents depolarized to −70, −50, −30, −10, +10, +30 and +50 mV were traced in Fig. 1A.

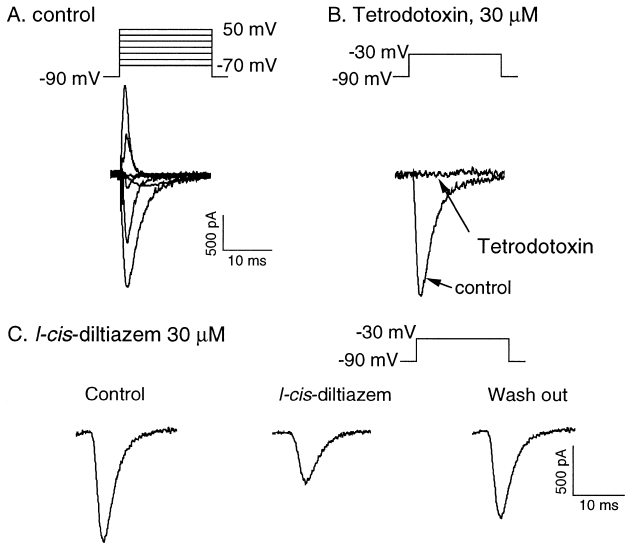

Fig. 1. Typical traces of I_{Na}. Current traces of I_{Na} elicited by a stepwise depolarization to +50 mV from V_h of −90 mV (20 ms duration) in 10-mV steps at 0.05 Hz. To avoid intricate traces, currents depolarized to −70, −50, −30, −10, +10, +30 and +50 mV were traced (A). Inhibitory effect of 30 μM tetrodotoxin on the current that was evoked at a V_h from −90 to −30 mV (20 ms duration) at 0.2 Hz (B). Current traces of I_{Na} elicited by a test potential to −30 mV from V_h of −90 mV before (left), during (center) superfusion with 30 μM *l-cis*-diltiazem and following washout (right) (C).

To verify that the phasic current was carried entirely by ions passing through Na^+ channels, the effect of tetrodotoxin on the membrane current was examined. Fig. 1B shows the current before and during superfusion with 30 μM tetrodotoxin, indicating that the phasic current was carried by ions passing through Na^+ channels.

Fig. 1C indicates a typical trace of inhibition by 30 μM *l-cis*-diltiazem of I_{Na}, which was evoked from V_h −90 to −30 mV depolarizing pulses at 0.2 Hz.

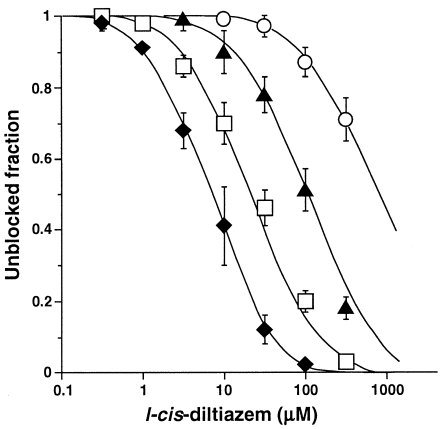

Fig. 3. Dose–response curve for the *l-cis*-diltiazem-induced block of I_{Na}. The amplitude of peak I_{Na} elicited by 20-ms depolarizing pulses to −30 mV from V_h −140 (open circle), −110 (closed triangle), −90 (open square) and −80 mV (closed diamond) is plotted against *l-cis*-diltiazem concentration. Relative values of I_{Na} normalized by taking the control as unity are plotted against the drug concentrations. Each point and vertical bar represents the mean ± S.E.M. of 4–6 experiments. Currents were recorded at a frequency of 0.2 Hz.

The effect of *l-cis*-diltiazem on the *I–V* relationship for V_h −140, −90 or −80 mV at 0.2 Hz is shown in Fig. 2. During control, the peak current was induced by a −30-mV depolarizing pulse and outward currents were observed with +10-mV depolarizing pulses. *l-cis*-Diltiazem produced an inhibitory effect on I_{Na} either depolarized from V_h −90 and −80 mV without causing significant changes in the *I–V* relationship (Fig. 2B and C).

The relationship between the concentration of *l-cis*-diltiazem and the inhibitory effect on I_{Na} is summarized in Fig. 3. I_{Na} was elicited with a V_h of −140, −110, −90 or −80 to −30 mV at 0.2 Hz. After observation of control current, *l-cis*-diltiazem at each concentration was superfused for 3 min and then the effect of *l-cis*-diltiazem

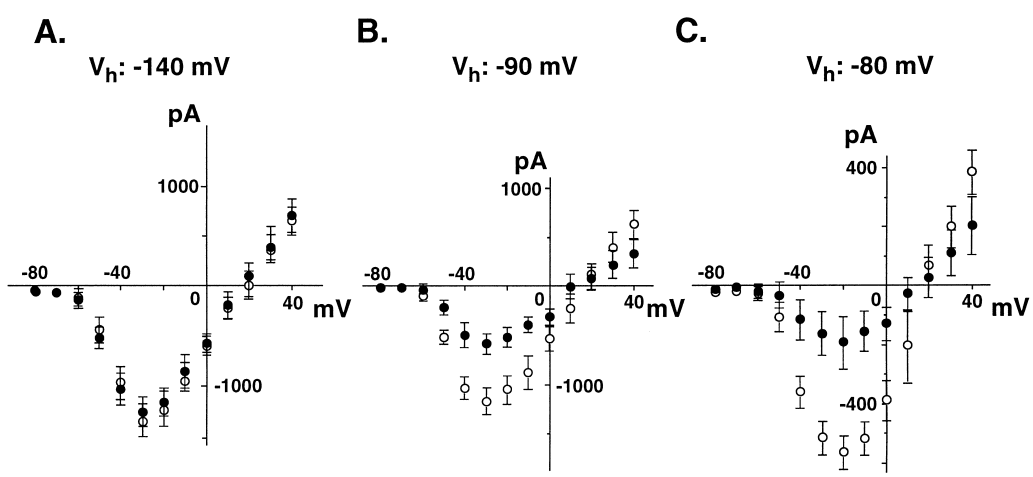

Fig. 2. Inhibitory effect of 30 μM *l-cis*-diltiazem on current (I)–voltage (V) relationships for I_{Na}. *I–V* relationships of I_{Na} under control conditions (open circle) and after 3 min superfusion with *l-cis*-diltiazem (closed circle). I_{Na} elicited on 20-ms step depolarizations from V_h −140 (A), −90 (B) and −80 mV (C) to various potential levels ranging from −80 to +40 mV, respectively. The pulse protocol was repeated every 20 s. Each point and vertical bar represents the mean ± S.E.M. of 4–6 experiments.

was measured when its action had stabilized. The effect of *l-cis*-diltiazem of each concentration was studied in individual cells. I_{Na} values relative to the control with a holding potential of −140, −110, −90 or −80 mV were plotted against the drug concentrations. *l-cis*-Diltiazem reduced the I_{Na} in a dose-dependent manner and the dose–response curve was shifted in the direction of lower concentrations by increasing the holding potential, suggesting that *l-cis*-diltiazem has a higher affinity for the inactivated state than for the resting state of the Na^+ channel. The apparent dissociation constants (K_d) were 729.3 ± 33.0, 185.4 ± 7.6, 24.8 ± 6.4 and 8.6 ± 3.0 μM at −140, −110, −90 and −80 mV, respectively ($n = 3$–6). Hill coefficients were 1.00, 1.15. 1.01 and 1.17 at holding potentials of −140, −110, −90 and −80 mV, respectively, as calculated using the following equation:

$$\text{Relative} \quad I_{Na} = 1/\left[1 + (D)^n/K_d\right]$$

where D is *l-cis*-Diltiazem concentration, K_d is an apparent dissociation constant and n is the Hill's coefficient. In each experiment, the effect of *l-cis*-diltiazem was reversible following 10 min of washing.

3.2. l-cis-Diltiazem-induced use-dependent block of I_{Na}

To examine the *l-cis*-diltiazem-induced use-dependent block of I_{Na}, repeated pulses at a V_h from −90 to −30 mV depolarization were applied at 0.5, 2 and 10 Hz. The current amplitude for each pulse was normalized relative to that of the first pulse in the train and was plotted as a function of the pulse number in Fig. 4. *l-cis*-Diltiazem progressively decreased I_{Na} in a frequency-dependent manner. Although there was no inhibition at the first pulse, the inhibition was more pronounced as the pulse number increased in this experiment.

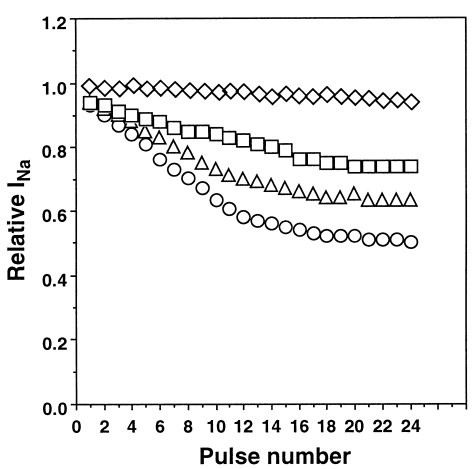

Fig. 4. Use-dependent block of I_{Na} by 10 μM *l-cis*-diltiazem. Depolarizing pulses (20 ms duration) to −30 mV from V_h −90 mV were applied at different pulse intervals (diamond, control at 10 Hz; square, 0.5 Hz; triangle, 2 Hz; circle, 10 Hz). The peak I_{Na} for each pulse was normalized to that for the first pulse. Each point represents the mean of 4–6 experiments.

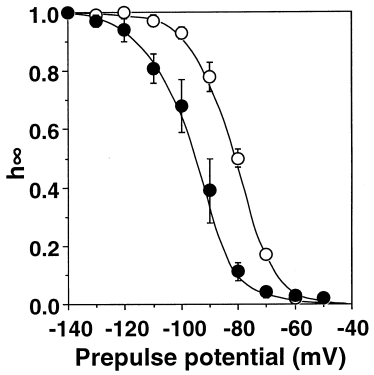

Fig. 5. Steady-state inactivation (h_∞) curves for I_{Na} in the presence (closed circle) or absence of 30 μM *l-cis*-diltiazem (open circle). Prepulses to selected conditioning potentials were applied for 3 s from V_h −140 to −50 mV in 10-mV steps. After repolarizing to V_h for 1 ms, a test pulse to −30 mV was applied. Each two-pulse sequence was applied at 20-s intervals. Resultant currents were recorded before and 3 min after superfusion with *l-cis*-diltiazem. The steady-state inactivation curves were obtained by normalizing the current values to the peak I_{Na} at −140 mV. Steady-state inactivation curves were fitted according to the Boltzmann equation; $h_\infty = 1/\{1 + \exp[(V - V_{1/2}]/s\}$, where V is the conditioning potential, $V_{1/2}$ is the potential giving half-maximal effect, and s is the slope factor. Each point and vertical bar represents the mean ± S.E.M. of 6 experiments.

3.3. Effect of l-cis-diltiazem on steady-state inactivation curves

To study the high affinity of *l-cis*-diltiazem for inactivated Na^+ channels, the effect of *l-cis*-diltiazem on the steady-state inactivation curves was examined using a standard double-pulse protocol. *l-cis*-Diltiazem shifted the curve to more negative levels (Fig. 5). From the fit of the data according to Boltzmann equation, $V_{1/2}$ was calculated as −80.8 ± 0.9 mV and −94.9 ± 3.1 mV without causing a change in the slope factor (7.0 ± 0.5 mV and 7.0 ± 0.9 mV) in the control condition and in the presence of *l-cis*-diltiazem, respectively. The finding that *l-cis*-diltiazem blocked I_{Na} in a voltage-dependent manner suggests that this drug has a higher affinity for the inactivated state of the channel than for the resting state.

3.4. Effect of l-cis-diltiazem on recovery from inactivation

Recovery of I_{Na} from inactivation was assessed using a standard two-pulse protocol shown in Fig. 5C. A conditioning pulse to −30 mV for 3 s was followed by various recovery periods and then by a test pulse to −30 mV. Fig. 6 (panel A) shows representative traces of I_{Na}, in the presence of 100 μM *l-cis*-diltiazem, during conditioning and test pulses with interpulse durations as indicated. In Fig. 6 (panel B), the unrecovered fraction of I_{Na} $(1 - I_t/I_p)$ was semilogarithmically plotted as a fraction of the recovery time.

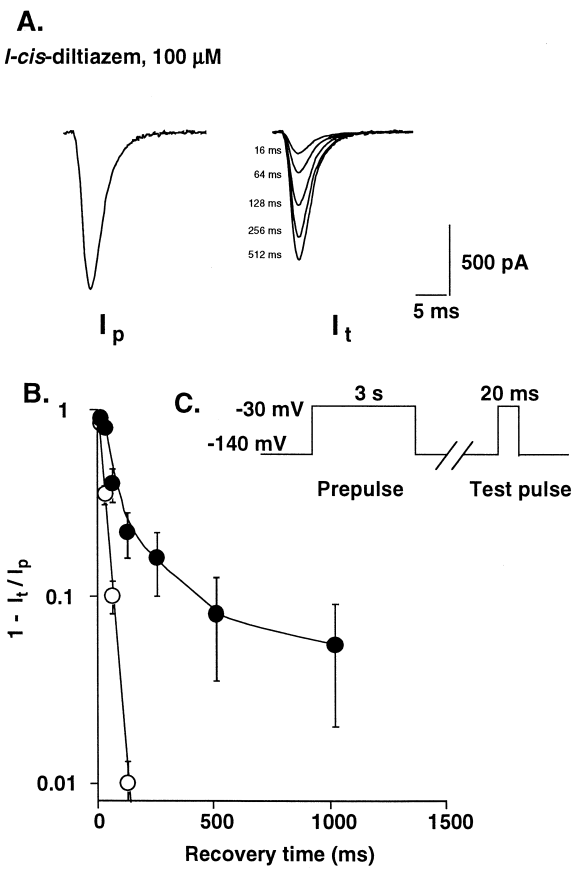

Fig. 6. Effect of 100 μM *l-cis*-diltiazem on the recovery of I_{Na} from inactivation. Recovery of I_{Na} from inactivation was assessed using a two-pulse protocol as shown in the inset of C. A prepulse (I_p) to −30 mV for 3 s was followed by various recovery periods and then by a test pulse (I_t) to −30 mV. In panel A, records of I_{Na} elicited by I_p and I_t in the presence of 100 μM *l-cis*-diltiazem were superimposed. Each two-pulse sequence was applied at 30 s intervals. In panel B, the unrecovered fraction of I_{Na} ($1-I_t/I_p$) was semilogarithmically plotted as a function of the recovery intervals. Open and closed circles indicate I_{Na} values during the control and in the presence of *l-cis*-diltiazem, respectively. Each symbol and vertical bar represents the mean ± S.E.M. ($n=3$).

Under control conditions, the unrecovered fraction was expressed by a single-exponential function with a time constant of 19 ± 4 ms, whereas in the presence of 100 μM *l-cis*-diltiazem the fraction was expressed by a double-exponential function with a time constant of 49 ± 7 ms and 890 ± 61 ms ($n=3$). These findings suggest that *l-cis*-diltiazem molecules dissociated with a relatively slow time constant of 890 ms from the inactivated Na^+ channels. Recovery was nearly complete after a 10-min recovery period in drug-free solution.

3.5. Effect of l-cis-Diltiazem on lysophosphatidylcholine-induced membrane current

Using ramp pulses (between −150 and +50 mV from V_h −50 mV, 0.8 V/s), we measured the effect of *l-cis*-diltiazem on lysophosphatidylcholine-induced non-selective cation currents. Lysophosphatidylcholine (10 μM) was

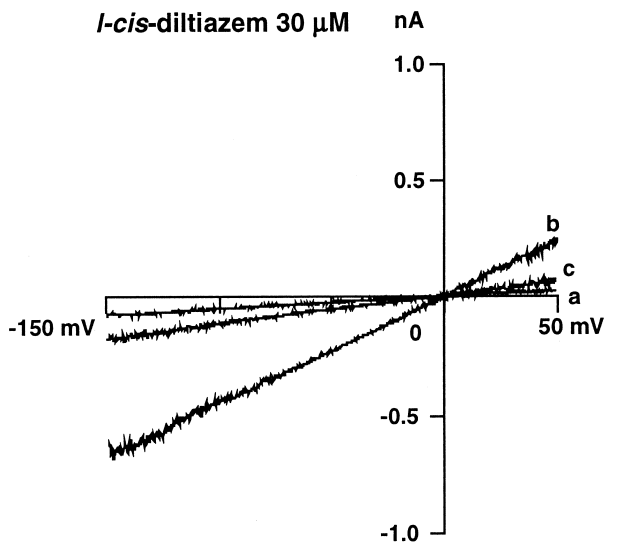

Fig. 7. Effect of 30 μM *l-cis*-diltiazem on 10 μM lysophosphatidylcholine-induced membrane current. (a) current–voltage ($I-V$) curve before superfusion with lysophosphatidylcholine, (b) $I-V$ curve 600 s after beginning superfusion with lysophosphatidylcholine, (c) $I-V$ curve 300 s after the addition of 30 μM *l-cis*-diltiazem in the presence of lysophosphatidylcholine (900 s after addition of lysophosphatidylcholine). Reversal potential of the lysophosphatidylcholine-induced current was 0 mV.

superfused in the Tyrode solution 3 min after the whole cell clamp was established. Lysophosphatidylcholine increased the membrane current slowly but markedly in ventricular cells. Fig. 7 shows the $I-V$ relations before, 600 s after the start of lysophosphatidylcholine addition

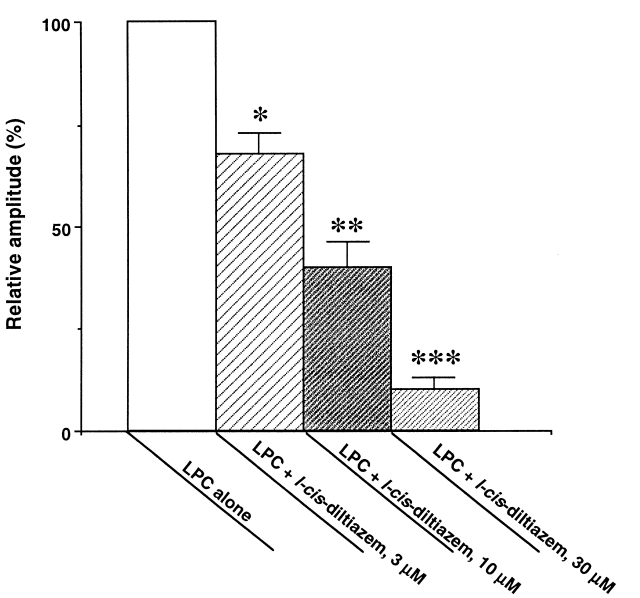

Fig. 8. Inhibitory effect of *l-cis*-diltiazem on lysophosphatidylcholine-induced non-selective cation current. The current amplitude after superfusion with *l-cis*-diltiazem at −100 mV was normalized by the current amplitude of lysophosphatidylcholine alone at −100 mV as 100%. Each column represents the mean ± S.E.M. of 6 experiments. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. control.

and 300 s after the addition of 30 μM *l-cis*-diltiazem in the presence of lysophosphatidylcholine (900 s after addition of lysophosphatidylcholine). Membrane conductance in the absence and presence of lysophosphatidylcholine was 0.8 and 5.2 nS, respectively. The reversal potential of the lysophosphatidylcholine-induced current was 0 mV, suggesting a non-selective cation current. *l-cis*-Diltiazem dose dependently and significantly inhibited the lysophosphatidylcholine-induced current (Figs. 7 and 8). The calculated IC_{50} value was 6.1 ± 1.2 μM.

4. Discussion

Previous studies showed that *l-cis*-diltiazem caused suppression of veratridine-induced Ca^{2+} elevation and cell death. The present study demonstrated the direct *l-cis*-diltiazem-induced suppression of I_{Na} in a dose-dependent manner. The K_d of this drug was 729 μM at a holding potential of −140 mV and 8.6 μM at −80 mV, suggesting that *l-cis*-diltiazem has a higher affinity for the inactivated state than for the resting state of the Na^+ channel. The voltage dependence of its effects was similar to that of the class I antiarrhythmic agent lidocaine. As holding potentials were depolarized, the inhibitory effect of lidocaine on I_{Na} increased (Sunami et al., 1991). The observation that the inactivation curve for the Na^+ channels was shifted in a negative direction also suggested that this drug had a higher affinity for inactivated Na^+ channels. The slow recovery from inactivation and use-dependent inhibition of I_{Na} suggests that *l-cis*-diltiazem binds to the inactivated state of Na^+ channels with a slow dissociation rate.

According to Bean et al. (1983) and Sanguinetti and Kass (1984), the dissociation constant for *l-cis*-diltiazem binding to Na^+ channels in the inactivated state (K_i) can be calculated from the equation:

$$\Delta V_{0.5} = V_s \ln\{(1 + [D]/K_i)/(1 + [D]/K_r)\}$$

where $\Delta V_{0.5}$ and V_s are the shift evoked by *l-cis*-diltiazem in the amplitude of the voltage-dependent inactivation curve and the slope factor of the inactivation curve, respectively, $[D]$ is the concentration of *l-cis*-diltiazem and K_r is the dissociation constant for *l-cis*-diltiazem binding to the Na^+ channels in the close-available state. Using the value of 14.1 mV for $\Delta V_{0.5}$ and 7.0 mV for V_s obtained with 30 μM *l-cis*-diltiazem and 729 μM for K_r, the K_i was calculated to be 4.41 μM, suggesting that *l-cis*-diltiazem had an about 165 times higher affinity for the inactivated than for the closed-available state of the Na^+ channel. Hence, the cardioprotective effect of *l-cis*-diltiazem may be explained by its action on the inactivated Na^+ channel. Because of the high affinity of *l-cis*-diltiazem for the depolarized state, *l-cis*-diltiazem would inhibit Na^+ channels and depress conduction efficiently in injured or partially depolarized myocardium.

Intracellular Ca^{2+} accumulation is considered to be the final stage leading to cell death during ischemia-reperfusion injury (Farber, 1982). Prevention of Ca^{2+} overload is expected to protect myocytes from cell death. In the present study, we also demonstrated the inhibition of lysophosphatidylcholine-induced currents at concentrations similar to those for inhibition of I_{Na}. Lysophosphatidylcholine accumulates in the myocardium during ischemia and reperfusion injury, and the influx of cations through non-selective cation channels occurs in cardiac myocytes (Magishi et al., 1996). In our experiment, the reversal potential of the lysophosphatidylcholine-induced current was 0 mV. Furthermore, we studied the effect of *N*-methyl-D-glutamine (NMG), which has a positive ionic charge with a greater molecular radius than Na^+ and K^+, using 140 mM NMG-aspartate both in the external and pipette solutions. Under these conditions, 10 μM lysophosphatidylcholine increased the current with a reversal potential of 0.12 ± 1.49 mV ($n = 3$)(not shown). These results support the view that the lysophosphatidylcholine-induced current is a non-selective cation current, which is consistent with previous reports (Magishi et al., 1996). Since lysophosphatidylcholine may mediate the ischemia and reperfusion injury of cardiac cells (Nasa et al., 1990; Van Amsterdam et al., 1990), it is possible that the cardioprotective effect of *l-cis*-diltiazem is partially dependent on the inhibition of this current. It is of interest to note that *l-cis*-diltiazem is a specific inhibitor of cyclic nucleotide-gated non-selective cation channels in the retina (Chen et al., 1993; Picones and Korenbrot, 1995). The presence of channel proteins that share structural homology with cyclic nucleotide-gated channels has been demonstrated in the heart (Gong and Kraus, 1998). While the molecular identity of the channel mediating the lysophosphatidylcholine-induced current has to be determined, *l-cis*-diltiazem would be a useful tool for elucidating the physiology of cardiac non-selective cation channels.

In summary, *l-cis*-diltiazem inhibited I_{Na} and lysophosphatidylcholine-induced influx of cation currents. These effects may underlie the cardioprotection provided by *l-cis*-diltiazem and also suggest the antiarrhythmic potential of this agent in ischemia and reperfusion stress.

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