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European Journal of Pharmacology 434 (2002) 125–131

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Differences in protective profiles of diltiazem isomers in ischemic and reperfused guinea pig hearts

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Received in revised form 30 October 2001; accepted 6 November 2001

Abstract

The effects of L-*cis* and D-*cis* diltiazem on the extracellular potassium concentration ($[K^+]_e$), pH and cardiac function were compared in ischemic guinea pig hearts. Before inducing ischemia, L-*cis* diltiazem (10 and 30 μM) reduced the left ventricular developed pressure (LVDP) with a marginal inhibition of heart rate (HR), whereas lower doses of the D-*cis* isomer decreased both LVDP and HR. L-*cis* Diltiazem only slightly inhibited the increase in $[K^+]_e$ and the decrease in pH but significantly inhibited ischemic contractures in contrast to the marked inhibition of these parameters produced by even low doses of the D-*cis* isomer. Notably, at equipotent doses for the ischemic parameters, L-*cis* diltiazem restored the left ventricular end-diastolic pressure (LVEDP) and HR after reperfusion to a greater extent than the D-*cis* isomer. These results suggest that the L-*cis* isomer may specifically improve postischemic function, in addition to the modest action on $[K^+]_e$ and pH, in guinea pig hearts.

Keywords: L-*cis* Diltiazem; Myocardial ischemia; K^+ accumulation; Ca^{2+} channel blocker; (Guinea pig)

1. Introduction

One of the most important pharmacological and clinical features of voltage-dependent Ca^{2+} channel blockers such as diltiazem (D-*cis* diltiazem) is their ability to protect the myocardium against the injury caused by ischemia/reperfusion. L-*cis* Diltiazem, an optical isomer of diltiazem, has been reported to elicit cardioprotective effects (Nasa et al., 1990; Xiao et al., 1997; Nishida et al., 1999) although its Ca^{2+} channel-blocking activity is 20–100 times lower than that of the D-*cis* isomer (Ikeda et al., 1991).

During ischemic periods, several changes in the normal pattern of electrophysiological events occur in cardiac cells. It is known that extracellular K^+ and H^+ gradually accumulate during myocardial ischemia (Harris et al., 1954; Gebert et al., 1971) and that the increase in extracellular K^+ concentrations ($[K^+]_e$) is characteristically triphasic (Hill and Gettes, 1980; Weiss and Shine, 1981, 1982; Kléber, 1984). An uneven distribution of myocardial $[K^+]_e$ has been reported in regional cardiac ischemia, and the electrophysiological abnormalities responsible for ventricular arrhythmias have been attributed to this (Coronel et al., 1995). Although these changes in ionic parameters may be of great pathophysiological importance, it has not yet been fully determined whether L-*cis* diltiazem, like other Ca^{2+} channel blockers, influences $[K^+]_e$ or pH during ischemia. The specific aims of the present study were therefore to investigate whether L-*cis* diltiazem inhibits various ionic and functional parameters during and after ischemia, and whether it shares the same mechanism of action as the D-*cis* isomer. To this end, using the D-*cis* isomer as a positive control for the blocking activity on voltage-dependent Ca^{2+} channels, we evaluated the protective effects of L-*cis* diltiazem against changes in $[K^+]_e$, myocardial pH, end-diastolic pressure and postischemic cardiac function in globally ischemic guinea pig hearts. We also compared the tension developed in isolated atria in the presence of the L-*cis* and D-*cis* isomers when $[K^+]_e$ was increased to values equivalent to those found during ischemia.

2. Materials and methods

2.1. Preparations

All experiments were conducted in accordance with The Guiding Principles of The Japanese Pharmacological Soci-

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0014-2999/02/$ - see front matter
PII: S0014-2999(01)01514-X

ety. Male guinea pigs (Hartley strain; 6–8 weeks old, weighing 350–400 g) were heparinized (1000 units/kg, i.p.) and killed by decapitation. The hearts were quickly removed and immediately immersed in ice-cold Krebs–Henseleit solution (KHS) with the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 10.

2.2. Isolated perfused hearts

When contractions had ceased, the aortic arch was cannulated and the heart was perfused in an antegrade manner at a constant pressure of 55 mm Hg with KHS aerated with 95% O_2 and 5% CO_2 at 37 °C (pH 7.5). The O_2 tension of the perfusate was maintained above 600 mm Hg throughout all the experiments, except during the ischemic period. The heart was kept in a humidified atmosphere (~150 mm Hg O_2) in a temperature-controlled chamber (37 °C). The left ventricular developed pressure (LVDP) was measured using a thin latex balloon, which was inserted via the left atrium into the cavity of the left ventricle (LV). The balloon was connected to a pressure transducer (MPU-0.5, Toyo Baldwin, Tokyo, Japan) and a signal amplifier (AP-621G, Nihon Kohden, Tokyo, Japan). The left ventricular end-diastolic pressure (LVEDP) was adjusted to 5–10 mm Hg by inflating the balloon. Heart rate (HR) was measured using a tachometer (AT-601G, Nihon Kohden) into which the LVDP signal was fed. These parameters were recorded simultaneously using a multichannel recorder (WR3701, Graphtec, Japan).

2.3. Force measurements in isolated left atria

The left atrium was dissected from each heart and suspended in an organ bath containing 10 ml normal KHS. A resting tension of 2 g was loaded onto the atrial muscle, which was then stimulated via a pair of electrodes using a rectangular pulse (frequency: 4 Hz, duration: 3 ms) at twice the threshold voltage. The tension developed in the atrium was measured isometrically using a force–displacement transducer.

2.4. Measurement of extracellular K^+ and H^+ concentrations

The $[K^+]_e$ and extracellular H^+ concentrations (pH_e) were measured using a K^+-sensitive electrode (Hill et al., 1978) and a pH electrode (Johnson et al., 1990), respectively. A thin polyethylene tube (internal diameter [i.d.], 1 mm; external diameter, 1.5 mm) was placed in a flame and stretched to reduce the i.d. to 0.2 mm, then filled with 500 mM KCl solution saturated with AgCl and sealed with a polyvinyl chloride membrane. This membrane contained valinomycin for the K^+ electrode and tridodecylamine for the pH electrode. The K^+- and pH-sensitive electrodes were connected to a high-impedance amplifier (MEZ-7200, Nihon Kohden) via an Ag–AgCl wire and a reference Ag–AgCl electrode. The voltage difference was amplified using a bioelectric amplifier (AB-621B, Nihon Kohden) and displayed by a pen recorder (FBR-252A, TOA Electric, Tokyo, Japan). Before and after each experiment, the K^+ electrode was calibrated using isotonic KCl–NaCl solution ($[K^+]$ = 3, 6, 12 and 24 mM, total ionic concentration = 149 mM) and the pH electrode was calibrated using HEPES–NaOH buffers (pH 6.5, 7.0, 7.5 and 8.0). Electrodes, which showed a voltage change of 56–62 mV/decade of change in $[K^+]$ or pH, were used. Time constants of the voltage change for the K^+ and H^+ electrodes were estimated to be approximately 200 and 100 ms, respectively, by means of voltage measurement after the calibration solution with a certain $[K^+]$ or the pH was switched to that with the other $[K^+]$ or pH. The K^+ and pH electrode tips were inserted into the LV free wall to a depth of 1–1.5 mm.

2.5. Experimental protocol

For experiments involving perfused heart preparations, the hearts were equilibrated under spontaneous beating conditions for 30 min. They were then perfused with KHS containing the required drug concentration for 10 min, after which global ischemia was induced and maintained for 30 min. $[K^+]_e$, pH_e and ischemic contractures were measured during the ischemic period. The hearts were then reperfused with drug-free KHS, and postischemic functional parameters were measured after reperfusion for 30 min. In control experiments, some of the hearts were perfused with the drug-free KHS for 10 min. Global ischemia was then induced and maintained for 30 min and subsequently reperfused with the drug-free KHS.

In the study of negative inotropic activity at various $[K^+]_e$ values, each left atrium was paced at 4 Hz and equilibrated for 30 min. The buffer was then exchanged for either normal KHS ($[K^+]_e$ = 5.9 mM) or modified $[K^+]_e$ KHS prepared by substituting some of the KCl with a specific amount of the equimolar NaCl (final $[K^+]_e$ = 2.9) or by substituting some of the NaCl with the equimolar KCl (final $[K^+]_e$ = 11.9 mM). Concentration–tension curves were constructed by cumulative addition of the relevant drug to the organ bath, and each response was expressed as a percentage of the value obtained just before starting the drug application.

2.6. Statistical analysis

All data are expressed as means ± S.E.M. One-way analysis of variance was conducted to evaluate the one-way layout data. Time-dependent data were subjected to two-way analysis of variance to detect any significant differences between the groups. The $[K^+]_e$ data were divided into three phases so that each phase of the triphasic increase in $[K^+]_e$ (i.e. the early [0–8 min], plateau [10–16 min] and late [18–30 min] phases) could be analyzed separately. The pH and ischemic contracture data obtained from just after the onset of ischemia until the cessation of ischemia were also analyzed.

If a significant difference was found, Bonferroni's post hoc test was conducted to determine which group showed the significant difference. Differences with a probability value of less than 0.05 were considered statistically significant.

IC_{50} values for D-*cis* and L-*cis* diltiazem (Fig. 4) were estimated by using dose–response curves fitted by the Hill equation: changes in developed tension (%) = $100/(1+(IC_{50}/[D]^{n_H})$, where IC_{50} is the concentration at the half-blockade, $[D]$ is the drug concentration and n_H is the Hill coefficient.

2.7. Chemicals

Diltiazem HCl (D-*cis* isomer) and L-*cis* diltiazem were provided by Tanabe Seiyaku, Osaka, Japan. These compounds were dissolved in the normal or modified $[K^+]_e$ KHS. Valinomycin (purity: >97%) was purchased from Sigma (St. Louis, MO, USA) or Fluka (Buchs, Switzerland), tridodecylamine was from Fluka or Tokyo Chemical Industry (Tokyo, Japan), and heparin Na was from Sigma. All other drugs and chemicals used were purchased from commercial sources.

3. Results

3.1. Effects of L-*cis* and D-*cis* diltiazem on $[K^+]_e$ elevation, acidosis and ischemic contractures during ischemia

Table 1 shows the effects of L-*cis* and D-*cis* diltiazem on LVDP and HR before inducing ischemia. No differences in these parameters between the treatment and control groups were observed at baseline (before adding the drugs).

A low dose (1 μM) of L-*cis* diltiazem had no effect on either LVDP or HR. High doses (10 and 30 μM) significantly reduced LVDP while they had a weaker action on HR. In contrast, 3 μM of the D-*cis* isomer potently inhibited both LVDP and HR.

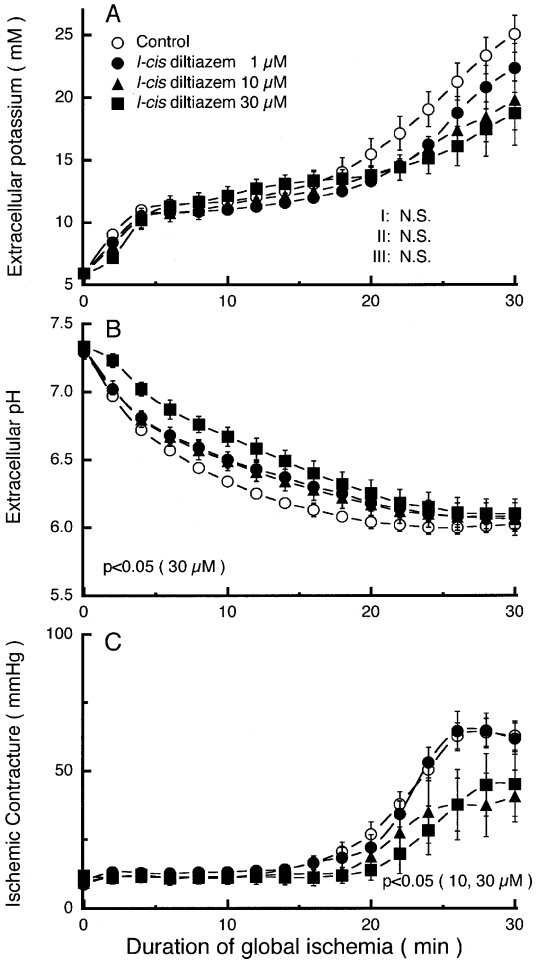

Fig. 1. Effects of L-*cis* diltiazem (1, 10 and 30 μM) on extracellular potassium accumulation (A), acidosis (B) and LVEDP (C) during 30 min of global ischemia in isolated perfused guinea pig hearts. Each point represents the means ± S.E.M. of the number of experiments shown in Table 1.

Table 1
Effects of L-*cis* and D-*cis* diltiazem on preischemic cardiac functional parameters in the guinea pig

Drug	Concentration (μM)	*n*	Before drug	After drug
LVDP			(mm Hg)	(mm Hg)
Control		8	81.9 ± 3.4	74.7 ± 3.8
L-*cis*	1	5	80.7 ± 6.2	69.1 ± 8.0
	10	5	80.0 ± 7.5	55.0 ± 3.9 *
	30	5	76.2 ± 2.9	29.6 ± 4.2 *
D-*cis*	1	6	75.4 ± 3.1	60.1 ± 4.7
	3	5	85.5 ± 3.2	40.5 ± 3.7 *
Heart rate			(beats/min)	(beats/min)
Control		8	231.5 ± 7.9	233.0 ± 9.7
L-*cis*	1	5	231.6 ± 12.0	225.2 ± 10.9
	10	5	235.6 ± 10.1	219.8 ± 12.5
	30	5	241.2 ± 8.4	200.2 ± 14.1
D-*cis*	1	6	236.2 ± 13.1	179.0 ± 15.7 *
	3	5	231.6 ± 8.6	164.4 ± 8.6 *

Before drug administration, values for all the parameters did not differ between the treatment and control groups. All values are expressed as means ± S.E.M.

* Significantly different from the control at $p < 0.05$.

Figs. 1 and 2 show the time courses of the changes in $[K^+]_e$ (elevation), acidosis (pH_e decline) and ischemic contractures. In the control group, the increase in $[K^+]_e$ during the 30-min ischemic period showed a triphasic pattern, consisting of an early phase (< 8 min), a plateau phase (10–16 min) and a late phase (18–30 min) (Figs. 1A and 2A). The pH_e decreased rapidly during the early period (< 14 min), followed by a more gradual decline to a plateau at approximately pH 6.1 (Figs. 1B and 2B). Ischemic contractures assessed by measurement of the LVEDP during the ischemic period started to increase 15 min after the cessation of perfusion and reached approximately 70 mm Hg at the end of the ischemic period (Figs. 1C and 2C).

L-*cis* Diltiazem (1 and 10 μM) did not affect either the early or plateau phase of the increase in $[K^+]_e$ (Fig. 1A). However, the late phase of the $[K^+]_e$ increase was slightly

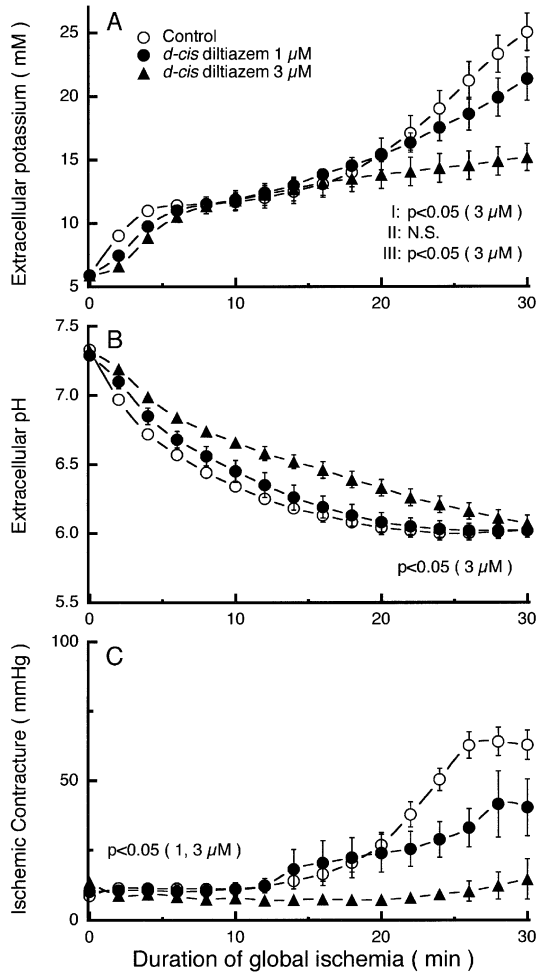

Fig. 2. Effects of diltiazem (D-*cis* conformation, 1 and 3 μM) on extracellular potassium accumulation (A), acidosis (B) and LVEDP (C) during 30 min of global ischemia in isolated perfused guinea pig hearts. Each point represents the means ± S.E.M. of the number of experiments shown in Table 1.

inhibited by the 10-μM dose. A higher concentration (30 μM) caused no further inhibition of the late phase of the $[K^+]_e$ increase although it seemed to delay the early phase of the increase in $[K^+]_e$ (Fig. 1A). The L-*cis* isomer (1 and 10 μM) produced only slight inhibition of the pH_e decrease, and a higher concentration (30 μM) inhibited the pH decrease significantly (Fig. 1B). At the 10-μM dose level, this isomer significantly inhibited ischemic contractures (by about half) although a higher concentration (30 μM) caused no further inhibition of ischemic contractures (Fig. 1C).

The effects of the D-*cis* isomer on $[K^+]_e$, acidosis and ischemic contractures are shown in Fig. 2. In contrast to the L-*cis* isomer, D-*cis* diltiazem delayed the early phase of the increase in $[K^+]_e$ in a concentration-dependent manner even at low doses (1 and 3 μM) (Fig. 2A). The late phase of the $[K^+]_e$ increase was also attenuated by this isomer in a concentration-dependent manner. Indeed, a 3-μM dose evoked complete inhibition. The D-*cis* isomer (1 μM) produced marginal inhibition of the pH_e decrease, while 3 μM significantly inhibited this reduction (Fig. 2B). Furthermore, 1 μM of the D-*cis* isomer reduced ischemic contractures by half, while 3 μM completely abolished them (Fig. 2C).

These results indicate that 10 μM of L-*cis* diltiazem is close to its maximally effective concentration, and increased doses are unlikely to match the maximum effects of the D-*cis* isomer. The potency of 10 μM of the L-*cis* isomer appears to be similar to that of 1 μM of the D-*cis* isomers in terms of their effects on $[K^+]_e$, pH_e and ischemic contractures (Figs. 1 and 2, see Discussion).

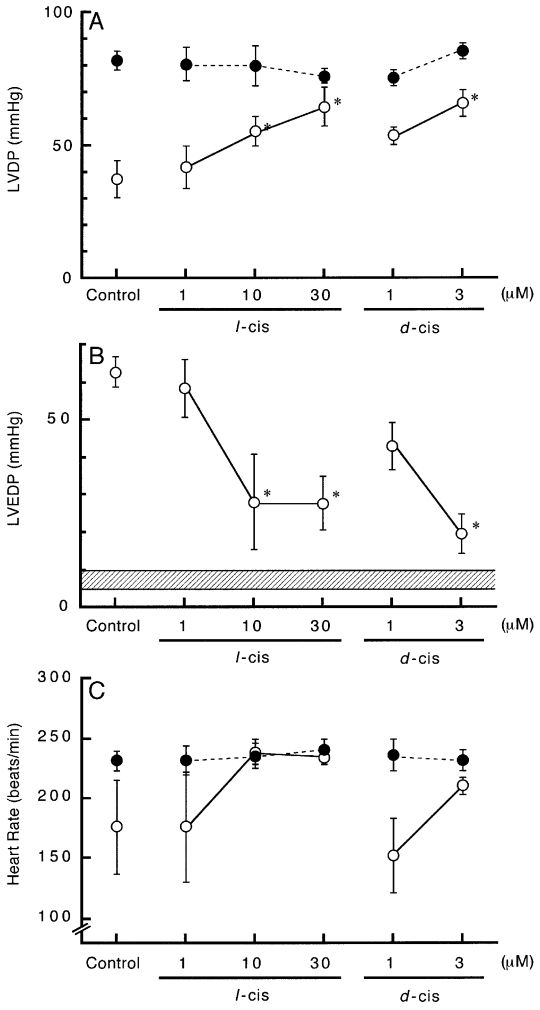

Fig. 3. Effects of L-*cis* and D-*cis* diltiazem on the postischemic recovery of LVDP (A), LVEDP (B) and HR (C) after 30 min of reperfusion. After the 30-min ischemic period, the hearts were reperfused with drug-free KHS, and each parameter was measured after 30 min of reperfusion (open circles). Closed circles in (A) and (C) represent the baseline LVDP and HR values obtained in preischemic hearts perfused with drug-free KHS as shown Table 1. LVEDP was adjusted to 5–10 mm Hg before inducing ischemia (hatched area in (B)). All values are expressed as means ± S.E.M., * significantly different from the control (open circles) at $p < 0.05$.

3.2. Effects on functional recovery after reperfusion

The effects of L-*cis* and D-*cis* diltiazem on postischemic cardiac functional parameters are shown in Fig. 3. After the 30-min ischemic period, the hearts were reperfused with drug-free KHS and the postischemic functional parameters were measured after reperfusion for 30 min. In control hearts, LVEDP was elevated to 62.8 mm Hg while LVDP and HR were suppressed to 46% and 76%, respectively, of their initial values (closed circles). These changes represent the cardiac dysfunction usually observed after ischemia/reperfusion.

Both diltiazem isomers (L-*cis*, 10–30 μM; D-*cis*, 3 μM) improved the postischemic function in the guinea pig hearts. Indeed, Fig. 3 shows that each parameter recovered almost to its baseline value. Doses of 10 and 30 μM of the L-*cis* isomer restored LVDP to 73% and 85% of its initial value, respectively (Fig. 3A). The effect of D-*cis* diltiazem (1 μM) on LVDP recovery was similar to that of the L-*cis* isomer (10 μM). These concentrations were also equipotent in terms of their effects on $[K^+]_e$, pH and ischemic contractures (Section 3.1). However, it is notable that 10 μM of the L-*cis* isomer produced greater recovery of LVEDP than 1 μM of the D-*cis* isomer (Fig. 3B), even though these concentrations were otherwise equipotent. Furthermore, 10 μM of the L-*cis* isomer produced a marked recovery of HR (Fig. 3C), whereas 1 μM of the D-*cis* isomer had no effect on HR. The HR values in the presence of these isomers (L-*cis*, 10 μM; D-*cis*, 1 μM) were significantly different ($p < 0.05$).

3.3. Negative inotropic effects of L-cis and D-cis diltiazem at various $[K^+]_e$

The effects of various $[K^+]_e$ on the negative inotropic effects of the L-*cis* and D-*cis* isomers are shown in Fig. 4.

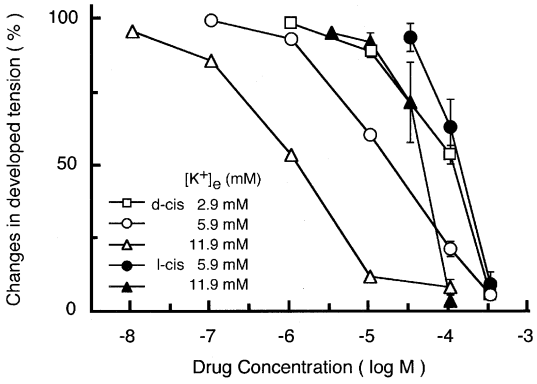

Fig. 4. Comparison of the negative inotropic effects of L-*cis* and D-*cis* diltiazem on isolated guinea pig atria. Each point represents the means ± S.E.M. of five experiments. IC_{50} values (μM) for L-*cis* diltiazem were 135 ± 23 in 5.9 mM $[K^+]_e$ and 35.9 ± 5.8 in 11.9 mM $[K^+]_e$. The values (μM) for the D-*cis* isomer were 127 ± 18 in 2.9 mM $[K^+]_e$, 21.3 ± 2.7 in 5.9 mM $[K^+]_e$ and 1.04 ± 0.07 in 11.9 mM $[K^+]_e$. No negative inotropic effects were observed with doses of up to 3×10^{-4} M of L-*cis* diltiazem at a $[K^+]_e$ of 2.9 mM.

The IC_{50} values calculated from the concentration–tension curves are shown in the legends. The IC_{50} value for the L-*cis* isomer only increased fourfold when $[K^+]_e$ was raised from 5.9 to 11.9 mM, whereas the IC_{50} value for D-*cis* diltiazem showed a 20-fold increase over this $[K^+]_e$ range.

4. Discussion

4.1. Difference in functional recovery after reperfusion by two diltiazem isomers at equipotent doses for ischemic parameters

The results of the present study demonstrate that at concentrations up to 10 μM, L-*cis* diltiazem produces only limited attenuation of the late phase of the increase in $[K^+]_e$ and of the decrease in pH seen during ischemia. The degree of inhibition of $[K^+]_e$ and pH changes produced by 10 μM of the L-*cis* isomer was similar to that evoked by a lower dose (1 μM) of the D-*cis* isomer. The effect of the L-*cis* isomer on ischemic contractures was more pronounced than that on $[K^+]_e$: at 10 μM, ischemic contractures were almost halved, the effect being equivalent to that of the D-*cis* isomer at 1 μM. Taken together, under the present experimental condition, the potencies of 10 μM L-*cis* and 1 μM D-*cis* diltiazem appear to be similar in terms of their effects on $[K^+]_e$, pH and ischemic contractures (Figs. 1 and 2).

The novel finding of the present study is that L-*cis* diltiazem can specifically improve postischemic function in addition to the modest action on $[K^+]_e$ and pH during ischemia in guinea pig hearts. Analysis of the various ischemic and postischemic parameters for L-*cis* diltiazem indicated that 10 μM is close to its maximally effective concentration and that higher doses are unlikely to match the maximum effects of the D-*cis* isomer (Figs. 1 and 3). However, in spite of its lower potency, an interesting feature of the L-*cis* isomer (10 μM) is that when compared with the D-*cis* isomer (1 μM), it produces a greater postischemic recovery of LVEDP and HR after reperfusion at doses with equipotent effects on the other parameters described above.

A possible cause of the increase in LVEDP is the Na^+ accumulation due to the blockade of the Na^+–K^+ pump and/or the activation of Na^+-permeable channels and subsequent Ca^{2+} overloading via the Na^+–Ca^{2+} exchange during reperfusion. A previous study demonstrated that L-*cis* diltiazem can inhibit the Na^+ and subsequent Ca^{2+} overloading caused by veratridine-induced inhibition of the Na^+ channel inactivation in rat myocytes (Itogawa et al., 1996). These results lead us to speculate that the blocking of the Na^+ channel or Na^+–Ca^{2+} exchange may contribute to the cardioprotective effect of the L-*cis* isomer. Recently, Hashimoto et al. (2000) found that this isomer inhibits the voltage-dependent Na^+ channel as well as nonselective cation channels activated by lysophosphatidylcholine, which is known to accumulate and induce Ca^{2+} overloading in ischemic hearts (Magishi et al., 1996). Thus, it is plausible

that the inhibitory actions of L-*cis* diltiazem on cation conductance may explain its effectiveness in improving some aspects of postischemic functional recovery after reperfusion.

4.2. Cardioprotection against functional and ionic changes during ischemia by two diltiazem isomers

The early and plateau phases of the $[K^+]_e$ elevation have been shown to occur due to the opening of ATP-sensitive K^+ (K_{ATP}) channels (Bekheit et al., 1990; Wilde et al., 1990; Mitani et al., 1991; Sakamoto et al., 1998). Since K_{ATP} channels have been reported to open in response to a decrease in the concentration of intracellular ATP (Noma and Shibasaki, 1985; Nichols and Lederer, 1990), agents with an inhibitory effect on ATP consumption would be expected to prevent the elevation in $[K^+]_e$. Our previous data have demonstrated that three Ca^{2+} channel blockers (D-*cis* diltiazem, verapamil and nifedipine) with equivalent negative inotropic effects share the property of delaying $[K^+]_e$ elevation (Sato et al., 1999). The present results with the D-*cis* isomer (Fig. 2) confirmed this finding. At the highest dose (30 μM) used in the present study, L-*cis* diltiazem appeared to mimic the effects of Ca^{2+} channel blockers on the $[K^+]_e$ elevation. This suggests that the L-*cis* isom-er possesses similar pharmacological properties to known Ca^{2+} channel blockers.

D-*cis* Diltiazem has been shown to preserve high-energy phosphates (ATP and creatine phosphate) during the early phase of ischemia. It also prolongs the period of glycolytic ATP synthesis, judging from the delay in the saturation of the increase in an anaerobic glycolytic product, lactate (Sato et al., 1999; Sakamoto et al., 1997). These metabolic effects are confirmed by the pH change since the plateau phase of the change in pH can be considered to reflect the cessation of glycolysis (Kingsley et al., 1991) and the declining phase of the pH change is extended by D-*cis* diltiazem (Sakamoto et al., 1997). Despite the weaker effect of L-*cis* diltiazem compared with the D-*cis* isomer in this respect, Sakamoto et al. (2000) demonstrated that both L-*cis* and D-*cis* diltiazem preserve the intracellular ATP content of ischemic and reperfused guinea pig hearts during a study using ^{31}P-nuclear magnetic resonance. Cascio et al. (1990) further reported that the functional and ionic changes that occur during ischemia are closely associated with one another and may be triggered by a common event, e.g., a critical increase in the free intracellular Ca^{2+} concentration due to the depletion of ATP, which would explain the late phase of the $[K^+]_e$ elevation and onset of ischemic contractures.

The preischemic cardiac functional parameters demonstrated the differing pharmacology of the two isomers (Table 1). Unlike the D-*cis* isomer, the L-*cis* isomer had a weaker effect on HR, whereas both isomers potently inhibited LVDP changes. This difference may reveal distinct cardioprotective profiles since maneuvers to decrease both LVDP and HR (e.g., decreasing the external Ca^{2+} concentration and pacing at a lower frequency) can attenuate the late phase of $[K^+]_e$ elevation and the pH decrease (Sato et al., 1999). In addition, the relative potency of these isomers may become dramatically different under quasi-ischemic conditions (Fig. 4). Our previous studies have shown that D-*cis* diltiazem has a greater negative inotropic effect in guinea pig papillary muscle and whole hearts when $[K^+]_e$ is increased to values equivalent to those seen during the plateau phase in ischemia (Sato et al., 1999; Okuyama et al., 1994). In the present study, the shift in the concentration–tension curve was less for the L-*cis* isomer than for the D-*cis* isomer. These differences may account for the weaker action of the L-*cis* isomer on the early and late phases of the $[K^+]_e$ increase, the pH decrease and ischemic contractures.

In conclusion, L-*cis* as well as D-*cis* diltiazem possess cardioprotective actions in ischemic perfused guinea pig hearts. L-*cis* Diltiazem may specifically improve some postischemic functional parameters by a mechanism other than the voltage-dependent Ca^{2+} channel blockade.

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