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Electrophysiological mechanisms for antiarrhythmic efficacy and positive inotropy of liriodenine, a natural aporphine alkaloid from *Fissistigma glaucescens*

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1 The antiarrhythmic potential and electromechanical effects of liriodenine, an aporphine alkaloid isolated from the plant, *Fissistigma glaucescens*, were examined.

2 In the Langendorff perfused (with constant pressure) rat heart, at a concentration of 0.3 to 3 μM, liriodenine was able to convert a polymorphic ventricular tachyrhythmia induced by the ischaemia-reperfusion ($EC_{50}=0.3$ μM).

3 In isolated atrial and ventricular muscle, liriodenine increased the contractile force and slowed the spontaneous beating of the right atrium.

4 The liriodenine-induced positive inotropy was markedly attenuated by a transient outward K^+ channel blocker, 4-aminopyridine (4-AP) but was not significantly affected by prazosin, propranolol, verapamil or carbachol.

5 In rat isolated ventricular myocytes, liriodenine prolonged action potential duration and decreased the maximal upstroke velocity of phase 0 depolarization ($\dot{V}_{max}$) and resting membrane potential in a concentration-dependent manner. The action potential amplitude was not significantly changed.

6 Whole-cell voltage clamp study revealed that liriodenine blocked the Na^+ channel (I_{Na}) concentration-dependently ($IC_{50}=0.7$ μM) and caused a leftward shift of its steady-state inactivation curve. However, its recovery rate from the inactivated state was not affected. The L-type Ca^{2+} currents (I_{Ca}) were also decreased, but to a lesser degree ($IC_{50}=2.5$ μM, maximal inhibition = 35%).

7 Liriodenine inhibited the 4-AP-sensitive transient outward current (I_{to}) ($IC_{50}=2.8$ μM) and moderately accelerated its rate of decay. The block of I_{to} was not associated with changes in the voltage-dependence of the steady-state inactivation curve or in the process of recovery from inactivation of the current. Liriodenine also reduced the amplitude of a slowly inactivating, steady-state outward current (I_{ss}) ($IC_{50}=1.9$ μM). These effects were consistent with its prolonging effect on action potential duration. The inwardly rectifying background K^+ current (I_{K1}), was also decreased but to a less degree.

8 Compared to quinidine, liriodenine exerted a stronger degree of block on I_{Na}, comparable degree of block on I_{K1}, and lesser extent of block on I_{Ca} and I_{to}.

9 It is concluded that, through inhibition of Na^+ and the I_{to} channel, liriodenine can suppress ventricular arrhythmias induced by myocardial ischaemia reperfusion. The positive inotropic effect can be explained by inhibition of the I_{to} channel and the subsequent prolongation of action potential duration. These results provide a satisfactory therapeutic potential for the treatment of cardiac arrhythmias.

Keywords: Liriodenine; cardiac arrhythmia; positive inotropy; Na^+, Ca^{2+} and K^+ currents; cardiac myocytes; quinidine; *Fissistigma glaucescens*

Introduction

Medicinal plants have been used as traditional remedies in oriental countries over hundreds of years. In a large scale screening test, several aporphine alkaloids were isolated and found to be active in the cardiovascular system (Teng *et al.*, 1991; Su *et al.*, 1994; Young *et al.*, 1994). Recently, we found that liriodenine (Figure 1), an aporphine derivative isolated from the plant *Fissistigma glaucescens*, possessed selective M_3 muscarinic receptor antagonistic activity in guinea-pigs (Lin *et al.*, 1994a) and in canine tracheal smooth muscle cells (Lin *et al.*, 1994b). It is ten times more potent for smooth muscle M_3 muscarinic receptors than for the cardiac M_2 muscarinic receptors that mediate negative inotropic and chronotropic effects. This compound was thereafter studied for antiarrhythmic and electromechanical effects in our laboratory. In the present study, we show that liriodenine has potent antiarrhythmic and positive inotropic activities. The mechanism of these activities in rat cardiac muscle and myocytes was investigated.

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Methods

Solutions

The normal Tyrode solution contained (in mM): NaCl 137.0, KCl 5.4, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.33, dextrose 11.0 and $CaCl_2$ 1.8. The HEPES-buffered Tyrode solution contained (in mM): NaCl 137.0, KCl 5.4, $CaCl_2$ 1.8, $MgSO_4$ 1.22, KH_2PO_4 1.2, dextrose 22, HEPES 6.0, titrated to pH 7.4 with NaOH. The internal pipette filling solution contained (in mM): KCl 120.0, NaCl 10.0, MgATP 5.0, K_2EGTA 5.0, HEPES 10.0, titrated to pH 7.4 with KOH. The Cs^+-containing pipette filling solution contained (in mM): CsCl 130.0,

NaCl 10.0, EGTA 5.0, tetraethylammonium (TEA) chloride 15.0, cyclic AMP 0.03, dextrose 5.0, HEPES 10.0, titrated to pH 7.4 with CsOH.

Ischaemia-reperfusion-induced arrhythmias

Adult male Wistar-Kyoto (WKY) rats, weighing 200–300 g, were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.) and given heparin (300 units kg^{-1}, i.p.).

The Langendorff-perfused heart model with constant perfusion pressure instead of constant flow was used (Curtis & Hearse, 1989). The hearts were excised immediately and immersed in ice-cold perfusion medium. Isolated hearts were then mounted on a Langendorff apparatus and perfused via the aorta with normal Tyrode solution. The medium was gassed with 95% O_2:5% CO_2 at 37°C and pH 7.4. The electrograms were recorded from a low atrial and a ventricular recording electrode and were continuously displayed on a Gould RS3400 recorder (Gould Inc., Cleveland, Ohio, U.S.A.) at 5 mm s^{-1} chart speed and a HP oscilloscope at 100 mm s^{-1} sweep speed. A traction type coronary occluder consisting of a silk suture thread through a polyethylene guide cannula was used for coronary occlusion. The left anterior descending coronary artery was ligated for 20 min before the release of the ligature. The establishment of ischaemia and reperfusion were ascertained by the amount of coronary effluent. A successful occlusion was confirmed by 40–50% reduction in coronary flow as compared with pre-ischaemic values. The antiarrhythmic effect of the compound was tested after arrhythmias had been induced by reperfusion and persisted longer than 5 min.

Mechanical response

Right atria, left atria, and right ventricular strips were dissected out and were suspended by silk threads in organ baths contained normal Tyrode solution (10 ml) maintained at 37°C and aerated with a mixture of 95% O_2:5% CO_2. Contractions of spontaneously beating right atria and of electrically driven left atria and right ventricular strips were measured by connecting one end of the preparation to a force displacement transducer (Type BG, Gould Inc.) by a fine silk thread and tension was recorded on a Gould recorder. To obtain the maximum developed tension, an optimal preload (0.5–1.0 g) was used. Both left atria and right ventricular strips were stimulated at a frequency of 2 Hz by rectangular pulses 1 ms in duration at about 2 fold threshold intensity via an isolated Grass stimulator (Grass Instruments Co., Quincy, MA, U.S.A.). The preparations were equilibrated for 30 min and cumulative concentration-responses to liriodenine were obtained. At the end of each experiment, the maximal developed tension was determined in each muscle by administration of isoprenaline (3×10^{-8}–1×10^{-7} M) after washout of all drugs for 30 min. The concentration of isoprenaline was increased stepwise until the maximal response was obtained. The response to liriodenine was expressed as a percentage of the maximal response to isoprenaline determined in the same muscle.

Isolation of ventricular myocytes

Rat ventricular myocytes were isolated according to the procedure described by Mitra & Morad (1985). Whole hearts from adult rats were perfused retrogradely via the aorta until free of blood using a Langendorff apparatus with a HEPES-buffered Tyrode solution containing 1.8 mM Ca^{2+}. Hearts were then perfused for 5 min with a nominally Ca^{2+}-free HEPES-Tyrode solution, followed by 20 min perfusion with the same solution containing protease (Type XIV, Sigma Chemical Co., St. Louis, Mo., U.S.A.) 0.08 mg ml^{-1} and collagenase (Type II, Sigma) 0.3 mg ml^{-1}. After brief perfusion with a 0.2 mM Ca^{2+}-containing Tyrode solution, the hearts were removed and the cells were dispersed by gentle mechanical agitation. This procedure yielded approximately 60–70% rod-shaped Ca^{2+}-tolerant cells.

Electrophysiological study

Single ventricular myocytes were placed in a recording chamber (1 ml volume) attached to an inverted microscope (Nikon, Diaphot, Japan) and perfused with control external solution. All the experiments were performed at room temperature (25–27°C). Whole-cell membrane voltages and currents were recorded by the patch clamp method (Hamill *et al.*, 1981). Patch electrodes were made from borosilicate glass (WPI Inc., Sarasota, FL, U.S.A.), and had tip resistances of 2–5 MΩ when filled with pipette solution. A Dagan 8900 patch/whole-cell clamp amplifier fitted with 100 MΩ feedback resistor in the headstage was used to clamp the myocytes. Action potentials were elicited by intracellularly applied stimuli (4 ms duration of 1 nA) through the microelectrode. The maximal rate of rise of the action potential upstroke (V_{max}) was obtained from electronic differentiation of the action potential. The formation of a high resistance seal (5–10 GΩ) was monitored by applying a 1 nA current from a digital pulse generator. The total series resistance for the pathway between pipette interior and cell membrane was estimated from the cell capacitance and capacitance decay time constant. It was possible to compensate electronically for 60–80% of the voltage drop across the electrode produced by the current flow. Cell capacitance was measured for each cell by integrating the current transient in response to a 10-mV depolarization. Voltage and membrane current were displayed on a storage oscilloscope (Model 511A, Tektronix Inc., Beaverton, OR, U.S.A.) and photographed for subsequent analysis or acquired using a pCLAMP data acquisition and analysis system.

During measurement of Na^+ (I_{Na}) and L-type Ca^{2+} current (I_{Ca}), the K^+ currents were blocked by addition of 2–4 mM Cs^+ to the bathing medium and internal dialysis of the cells with Cs^+-containing pipette solution. The maximal I_{Na} elicited from adult myocytes in normal Tyrode solution was usually larger than 20 nA which would result in significant voltage clamp artefacts. Therefore, I_{Na} was studied in low Na^+ Tyrode solution (80 mM NaCl was substituted with N-methyl-D-glucamine) and dialysis of the cell with Na^+ (10 mM) containing Cs^+ pipette solution. Under these conditions the I_{Na} elicited was smaller than 5 nA, and the estimated voltage error attributed to uncompensated series resistance would be lower than 5 mV. For measurement of I_{Ca}, the I_{Na} inactivated by stepping the membrane potential to −40 mV, I_{Ca} was then activated by a second depolarization to 0 mV from a holding potential of −80 mV. During measurement of the K^+ outward currents, contamination by the I_{Na} and I_{Ca} was prevented by addition of 30 μM tetrodotoxin (TTX) and 1 mM Co^{2+}, respectively, to the bathing medium. In this condition, 160 ms depolarization of membrane potential to a level positive to −40 mV resulted in a generation of a transient outward K^+ current (I_{to}) and followed by a steady-state outward current (I_{ss}).

Drugs

Liriodenine was isolated from the plant *Fissistigma glaucescens* as previously described (Lu *et al.*, 1985). The following drugs were used: (−)-isoprenaline hydrochloride, prazosin hydrochloride, propranolol hydrochloride, (±)-verapamil hydrochloride, carbachol, 4-aminopyridine (4-AP), quinidine, 3-isobutyl-1-methylxanthine (IBMX) and tetrodotoxin (TTX) were purchased from Sigma Chemical Co. Liriodenine, IBMX and quinidine were dissolved in dimethylsulphoxide (DMSO). The final concentration of DMSO in the bathing solution did not exceed 0.1% and had no effect on the muscle contraction and electrophysiological parameters.

Data analysis and statistics

All values are presented as means ± s.e. A repeated-measures analysis of variance and Student's paired or unpaired *t* test were applied where appropriate, and values of $P<0.05$ were

considered significant. Concentration-response curves were fit by the Hill equation:

$$\text{Inhibition of current (\%)} = 100/[1 + (IC_{50}/D)^{n}{}_{H}]$$

where D is the drug concentration, IC_{50} is the concentration of drug for half-maximal block, and n_H is the Hill coefficient. The inactivation curves of I_{Na} or I_{to} were fitted by the Boltzmann equation:

$$I/I_{max} = 1/\{1 + \exp[(V_m - V_h)/s]\}$$

where V_m is the conditioning potential, V_h is the potential at which the normalized current equals 0.5, and s is the slope factor.

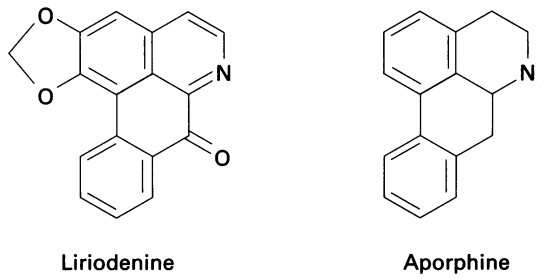

Figure 1 Chemical structures of liriodenine and aporphine.

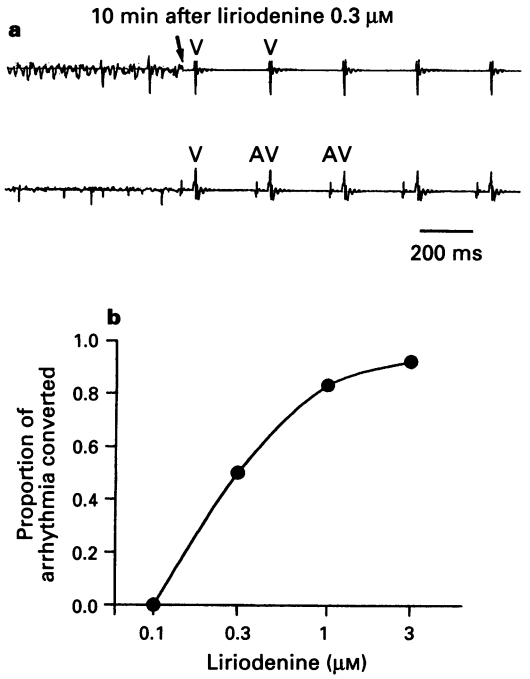

Figure 2 (a) Antiarrhythmic effect of liriodenine on Langendorff perfused rat heart. A polymorphic ventricular tachyrhythmia induced by ischaemia-reperfusion was converted to normal sinus rhythm 10 min after 0.3 μM liriodenine. Upper and lower panel show the electrogram recorded from the ventricle and the lower right atrium. Simultaneous recording of atrial (A) and ventricular depolarization (V) is shown. The paper speed was 100 mm s^{-1}. (b) Concentration-response curve for the antiarrhythmic effect of liriodenine. The proportion of arrhythmias converted to normal sinus rhythm by liriodenine is plotted against the drug concentration.

Results

Effects on ischaemia-reperfusion-induced arrhythmias

At a concentration of 0.3 to 3 μM, liriodenine was able to convert a polymorphic ventricular tachyrhythmia induced by the ischaemia-reperfusion experiment model (Figure 2a). Out of twelve episodes of ventricular tachyrhythmia induced by ischaemia-reperfusion, liriodenine at 0.3 μM converted the tachyrhythmias to normal sinus rhythm in six instances, 1 μM converted four of the remaining six episodes and 3 μM converted one of the other two episodes. For the one episode of refractory tachyrhythmia, when 10 μM of liriodenine was applied, complete atrioventricular block occurred. The concentration-response curve for arrhythmia conversion showed an EC_{50} of 0.3 μM (Figure 2b). No new tachyrhythmias upon perfusion of liriodenine for about 1 h were observed during the experiment.

Effects on atrial rate and cardiac contractility

In the rat spontaneously beating right atria, driven left atria and right ventricular strips, liriodenine increased the contractile force in a concentration-dependent manner (Figure 3a

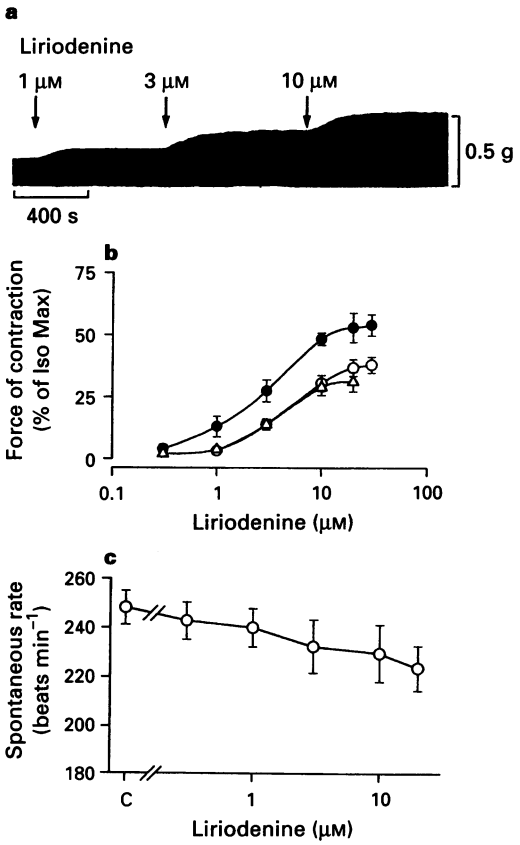

Figure 3 Effects of liriodenine on the contractile force and spontaneously beating rate in rat cardiac tissues. (a) Representative tracing shows the liriodenine-induced positive inotropic effect in a right ventricular strip. (b) Cumulative concentration-response curves for the positive inotropic effect of liriodenine in isolated rat right atria (RA; ○), left atria (LA; ●) and right ventricular strips (RV; △). All values represent means ± s.e. Ordinate scale, force of contraction is expressed as the percentage of the maximal response to isoprenaline (Iso). Abscissa scale, logarithmic concentrations of liriodenine. The basal force and maximal response to Iso in individual experimental groups were 0.11 ± 0.02 and 0.38 ± 0.07 g ($n = 14$) in RA, 0.13 ± 0.02 and 0.53 ± 0.05 g ($n = 14$) in LA, and 0.36 ± 0.06 and 1.49 ± 0.15 g ($n = 15$) in RV, respectively. (c) Effect of liriodenine on heart rate in spontaneously beating right atria. The averaged predrug control values (C) was 248 ± 7 beats min^{-1}.

Table 1 Influences of prazosin, propranolol, 4-aminopyridine (4-AP), verapamil and carbachol on the positive inotropic effect of liriodenine in rat right ventricular strips stimulated at 2 Hz

		EC_{50} (μM)		Maximal force of contraction (% of Iso Max)	
Drug treatment	n	*Before*	*Treatment*	*Before*	*Treatment*
Prazosin (1 μM)	6	3.6±0.9	3.3±0.5	39±10	36±8
Propranolol (2 μM)	7	4.8±1.0	5.8±0.8	35±4	37±10
4-AP (1mM)	5	6.3±1.0	6.8±1.0	24±3	8±2**
Verapamil (0.2 μM)	9	4.2±0.5	5.5±0.5	31±4	30±7
Carbachol (0.3 μM)	7	4.1±0.6	4.6±0.5	23±4	21±3

Values are means±s.e. *n* is number of experiments. EC_{50} is the concentration that produces 50% of the maximal effect of liriodenine. Iso Max, maximum response to isoprenaline. $^{**}P<0.01$ as compared with the respective control. The addition of prazosin, propranolol or carbachol failed to alter the basal force of contraction, whereas 0.2 μM verapamil decreased developed tension by 45±3% ($n = 9$), and 1 mM 4-AP increased it by 38±5% ($n = 5$).

Table 2 Effects of liriodenine on the action potential parameters in rat ventricular myocytes driven at 1 Hz

		Liriodenine (μM)			
	Control	*0.3*	*1*	*3*	*Washout*
RMP (mV)	−76.3±0.7	−74.9±0.7	−74.0±1.1	−70.7±2.5*	−74.6±3.2
APA (mV)	107.8±3.7	107.0±6.1	109.0±4.8	102.0±5.8	106.5±5.6
$\dot{V}_{max}$ (Vs^{-1})	230.4±5.5	146.7±34.6*	133.2±36.3*	113.6±36.4**	205.3±26.4
APD_{50} (ms)	16.9±3.4	42.7±8.2**	111.0±20.9***	189.9±36.8***	28.5±18.6
APD_{90} (ms)	36.3±5.1	77.3±8.9***	164.1±32.3***	291.3±45.6***	52.8±16.6

Values are expressed as means±s.e. of 7 cells. RMP, resting membrane potential; APA, action potential amplitude; $\dot{V}_{max}$, maximal rate of rise of the action potential upstroke; APD_{50} and APD_{90}, action potential duration measured at 50% and 90% repolarization, respectively. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared with the respective control.

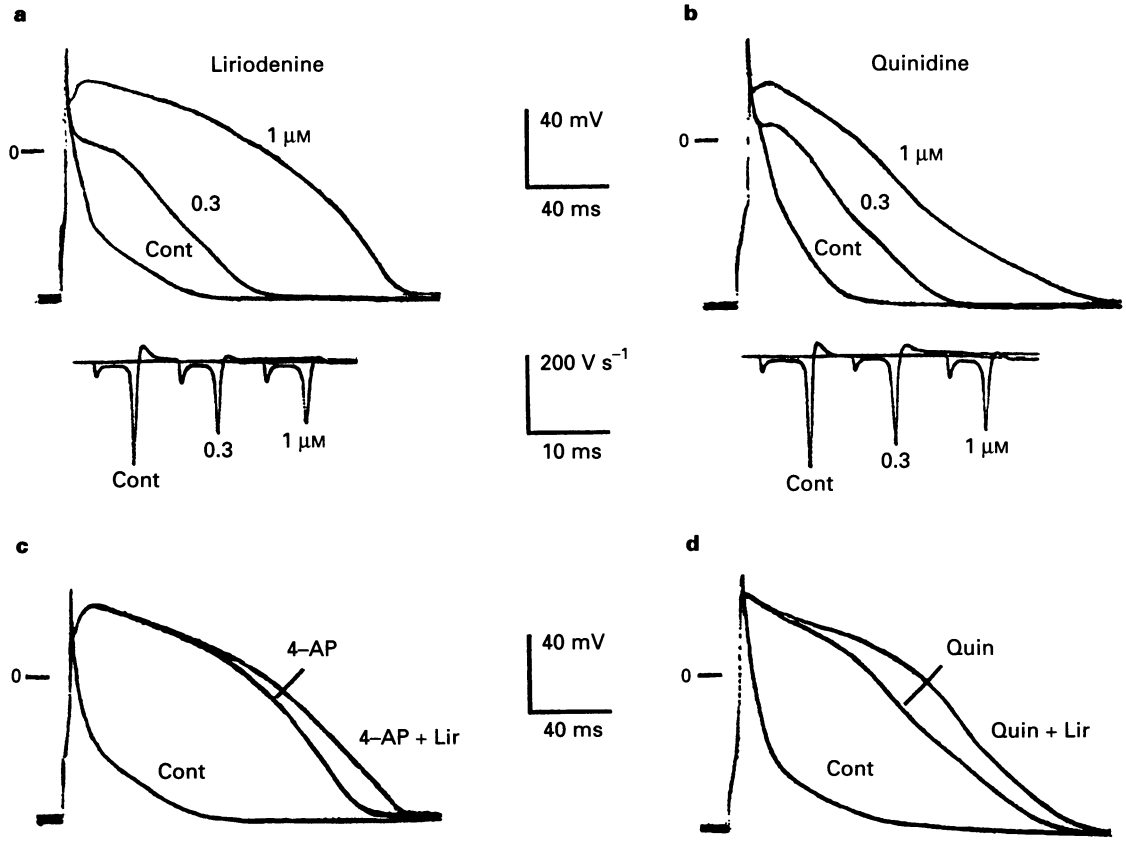

Figure 4 (a) and (b) Comparative effects of liriodenine and quinidine on the action potentials and $\dot{V}_{max}$ elicited in rat ventricular myocytes stimulated at a frequency of 1 Hz. After recording of the control action potential (Cont), the cells were superfused either with liriodenine (a) or quinidine (b) at increasing concentrations of 0.3 and 1 μM. (c) Effect of 4-AP on the action potential waveform induced by liriodenine (Lir). An action potential was first recorded in control 'external' solution (Cont). External solution containing 1 mM 4-AP was then added, followed by a solution containing 1 mM 4-AP and 1 μM liriodenine. (d) Effect of quinidine (Quin) on the action potential waveform induced by liriodenine. Action potentials were recorded in control solution, then 1 μM quinidine, and finally in 1 μM quinidine and liriodenine. The stimulation frequency was 1 Hz in both panel (c) and (d).

and b). The threshold concentration was approximately 0.3 μM. The maximal response was achieved at concentrations between 10 and 20 μM. The EC_{50} values for right atria, left atria, and right ventricular strips were calculated to be 3.8±0.5 (n=14), 3.1±0.6 (n=14), and 5.5±0.7 μM (n=15), respectively. In spontaneously beating right atria, the positive inotropic effect was accompanied by a modest decrease of heart rate (Figure 3c). The positive inotropic effect of liriodenine on ventricular strips was not antagonized by prazosin (1 μM), propranolol (2 μM) or verapamil (0.2 μM) but was significantly suppressed by 4-AP (1 mM) with a prominent reduction of the maximum inotropic response (Table 1). The positive inotropy of liriodenine was not altered by pretreatment with 0.3 μM carbachol (Table 1). However, the same concentration of carbachol significantly attenuated the inotropic effect of a cyclic-AMP phosphodiesterase inhibitor, IBMX (not shown).

Effects on action potentials of isolated cardiomyocytes

Representative action potentials before and after liriodenine in a rat ventricular myocyte driven at 1 Hz are illustrated in Figure 4a. As shown in Figure 4a and Table 2, liriodenine prolonged the early and the late phases of repolarization in a concentration-dependent manner. Liriodenine also produced a concentration-dependent depression of the maximal upstroke velocity (V_{max}). At higher concentrations (e.g., 3 μM), liriodenine slightly reduced resting membrane potential. The effect of liriodenine could be reversed by washout, although a period of at least 5–10 min was needed. Quinidine prolonged the ventricular APD (Figure 4b) as in the previous study (Clark *et al.*, 1995). For 8 cells tested with 1 μM quinidine, APD_{50} was prolonged from 12.6±6.4 to 78.1±17.5 ms, whereas APD_{90} was prolonged from 41.2±6.6 to 138.6±27.0 ms. The V_{max} was reduced from 226.5±11.3 to 165.7±32.4 V s^{-1}. The lengthening of the action potential in rat ventricular myocytes by liriodenine suggested that part of its action may have resulted from inhibition of the outward currents in these cells. To test this possibility, we therefore examine the effects of liriodenine on action potentials of myocytes pretreated with 4-AP or quinidine. Figure 4c shows the effect of 1 mM 4-AP on the action potential of one ventricular myocyte. It can be seen that the prolonging effect of 1 mM 4-AP was comparable to that of 1 μM liriodenine. Addition of liriodenine (1 μM) to the 4-AP-treated myocyte produced little additional effect (Figure 4c). Similarly, the prolonging effect of liriodenine on the action potential was also attenuated by 1 μM quinidine (Figure 4d). Similar effects of 4-AP and quinidine on the action of liriodenine on the action potential were also observed in another 4 and 5 cells, respectively.

Effects on inward Na^+ currents (I_{Na})

The mean capacitance of the rat ventricular cells in this study was 186.5±21.4 pF (n=20). Figure 5a and b show the effect of 0.3 μM liriodenine on the current-voltage (I-V) relationship for

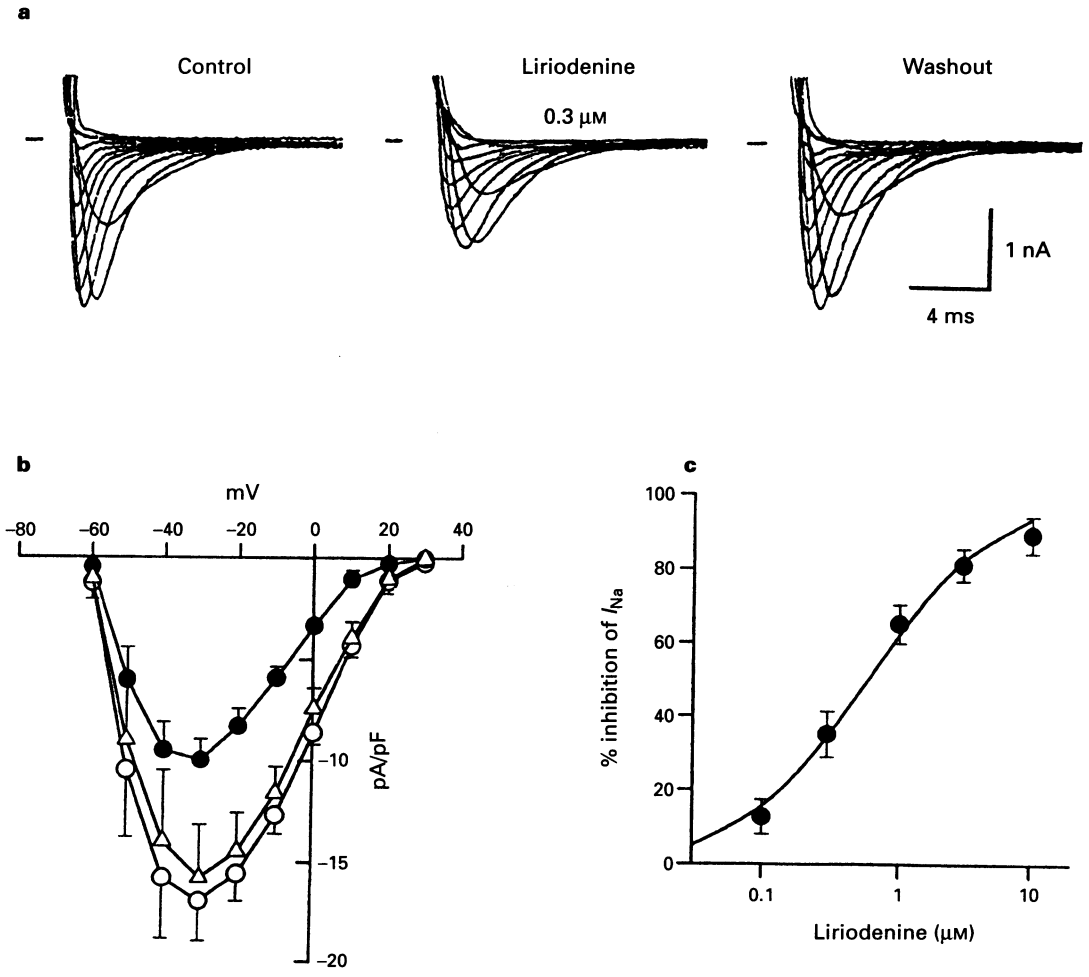

Figure 5 Effects of liriodenine on the I_{Na} in rat ventricular myocyte. (a) The original current traces elicited by depolarizing pulses applied at 10 mV increments from −60 mV to +30 mV from a holding potential of −80 mV in the absence (left), after application of liriodenine 0.3 μM (middle) and 9 min after washout (right). (b) The I-V relationship for I_{Na} in the absence (○), in the presence of liriodenine 0.3 μM (●) and washout (△). Each data point represents mean±s.e. from 5 cells. (c) Concentration-dependent inhibition of liriodenine on I_{Na}. Percentage inhibition of peak I_{Na} at −20 mV corresponding to the control value were plotted against drug concentration. Each data point indicates mean±s.e. (n=9). The continuous line was drawn by fitting to the Hill equation.

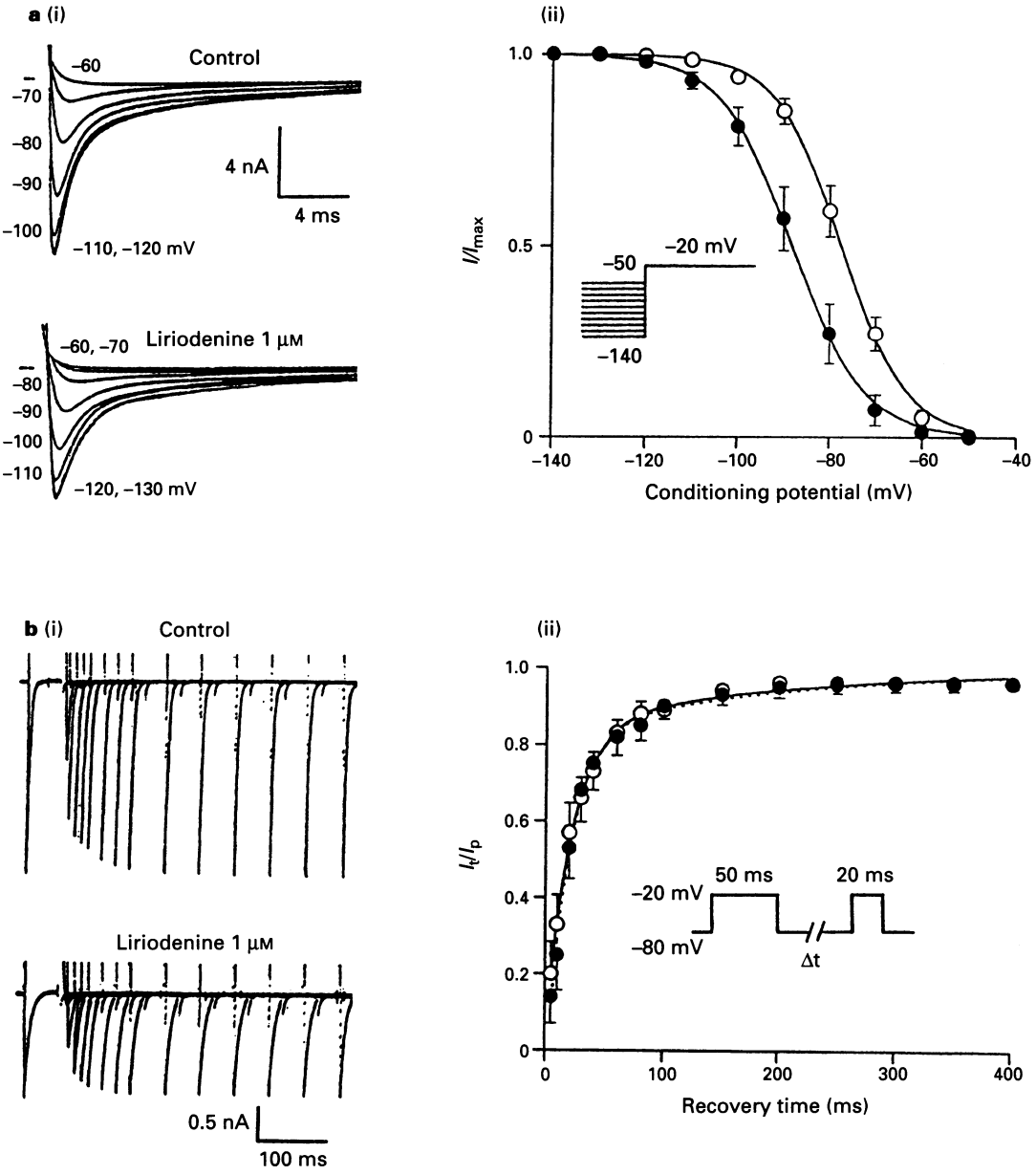

Figure 6 (a) Effects of liriodenine on the steady-state inactivation curve for I_{Na}. The pulse protocol is illustrated in the inset of panel (a(ii)). A 500 ms conditioning pulse was applied from the holding potential of −80 mV to various potentials ranging from −140 to −50 mV. Each conditioning pulse was followed by a 20 ms test pulse to −20 mV. Test current traces under control conditions (upper) and after 5 min superfusion with 1 μM liriodenine (lower) are shown in panel (i). Panel (ii) shows steady-state inactivation curves for I_{Na} were obtained by normalizing the current amplitudes obtained by the test pulse to the maximal value in each condition. (○) Before liriodenine application; (●) in the presence of liriodenine (1 μM). Each symbol shows mean±s.e. ($n=7$). The line drawn through the data points was the best fit to the Boltzmann equation. (b) Effects of liriodenine on the recovery of I_{Na} from inactivation. The pulse protocol is shown in the inset of panel (b(ii)). The holding potential was −80 mV. A 50-ms prepulse from −80 to −20 mV was followed after various recovery times by a 20 ms test pulse to −20 mV. The currents shown were recorded from the same myocyte in control and 1 μM liriodenine. In panel (b(ii)), averaged (means±s.e.) recovery data plotted from 7 different cells. Test pulse current amplitude (I_t), normalized to prepulse current amplitude (I_p), is plotted against the recovery time in the absence (○) and presence of 1 μM liriodenine (●). Continuous line (control) and dotted line (liriodenine) show best-fits of double exponential functions.

Table 3 Kinetic parameters of steady-state inactivation and recovery from inactivated state of Na channel under the influence of liriodenine and quinidine

		Control	*Liriodenine (μM)*				*Control*	*Quinidine (μM)*		
	n		*0.3*	*1*	*3*	n		*0.3*	*1*	*3*
V_h (–mV)	7	78±2	81±2	88±3**	97±4**	5	75±4	83±4	91±6*	98±6**
s (mV)	7	6.6±0.1	6.5±0.3	6.9±0.3	6.1±0.3	5	6.4±0.5	7.2±0.6	7.7±0.5	7.9±0.5
τ_f (ms)	7	16±4	19±5	17±4	18±4	6	15±5	18±5	26±8	69±23*
τ_s (ms)	7	172±19	186±25	197±23	205±24	6	128±35	243±62	431±21***	519±80***

V_h and s indicate half-maximal inactivation voltage and slope factor; τ_f and τ_s, fast and slow time constant for Na channel recovery from inactivated state. Values are means±s.e. n is number of experiments. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ as compared with the respective control.

I_{Na}. The membrane was held at −80 mV, and depolarizing pulses were applied to various potentials for 20 ms. Liriodenine (0.3 μM) inhibited the peak I_{Na}, but the voltage-dependence of *I-V* relationship was not significantly changed (Figure 5b). The depressant effect of liriodenine on peak I_{Na} was concentration-dependent and is summarized in Figure 5c. The data points in the concentration-response curve are well fitted by the Hill equation and the IC_{50} value for liriodenine on I_{Na} was 0.7±0.1 μM and n_H was 1.02±0.10 ($n=9$). Quinidine (1, 3, 10, and 20 μM) also depressed peak I_{Na} by 22±3, 52±4, 74±2, and 93±1% ($n=7$) respectively. The calculated IC_{50} value for quinidine was 3.1±0.3 μM ($n_H=1.15\pm0.08$) ($P<0.001$, liriodenine vs quinidine).

The effect of liriodenine on the steady-state inactivation curve for I_{Na} was studied by use of the double-pulse protocol as shown in the inset of Figure 6a(ii). A 20-ms test pulse to −20 mV following a 500-ms prepulse to various potential levels ranging from −140 to −50 mV in 10-mV steps. The inactivation curves were obtained by normalizing the test current amplitudes by taking the maximum value under each condition as unity. The curves were fitted to the Boltzmann equation. Liriodenine caused a parallel shift toward the negative potentials with no change in the slope factor. The averaged data of liriodenine and quinidine on the inactivation kinetic parameters are depicted in Table 3.

The speed of the dissociation of liriodenine from inactivated Na^+ channels was then assessed by a double-pulse protocol (see inset of Figure 6b(ii)). A 50-ms prepulse was first applied from −80 to −20 mV, which was followed by a 20-ms test pulse after various interpulse intervals ranging from 5 to 400 ms. The recovered fraction of I_{Na} was plotted against the recovery time (Figure 6b(ii)). Under control conditions, I_{Na} recovered rapidly, and the recovery can be described by the sum of two exponentials, one with a fast time constant (τ_f) and the other with a slow time constant (τ_s). During exposure to 1 μM liriodenine, the I_{Na} recovery rate was not significantly affected. The averaged data from the experiments examining the effect of liriodenine and quinidine on the recovery time constants are summarized in Table 3.

Effects on inward Ca^{2+} current (I_{Ca})

L-type Ca^{2+} currents were elicited by a series of 120 ms depolarizing pulses from a holding potential of −80 mV after ap-

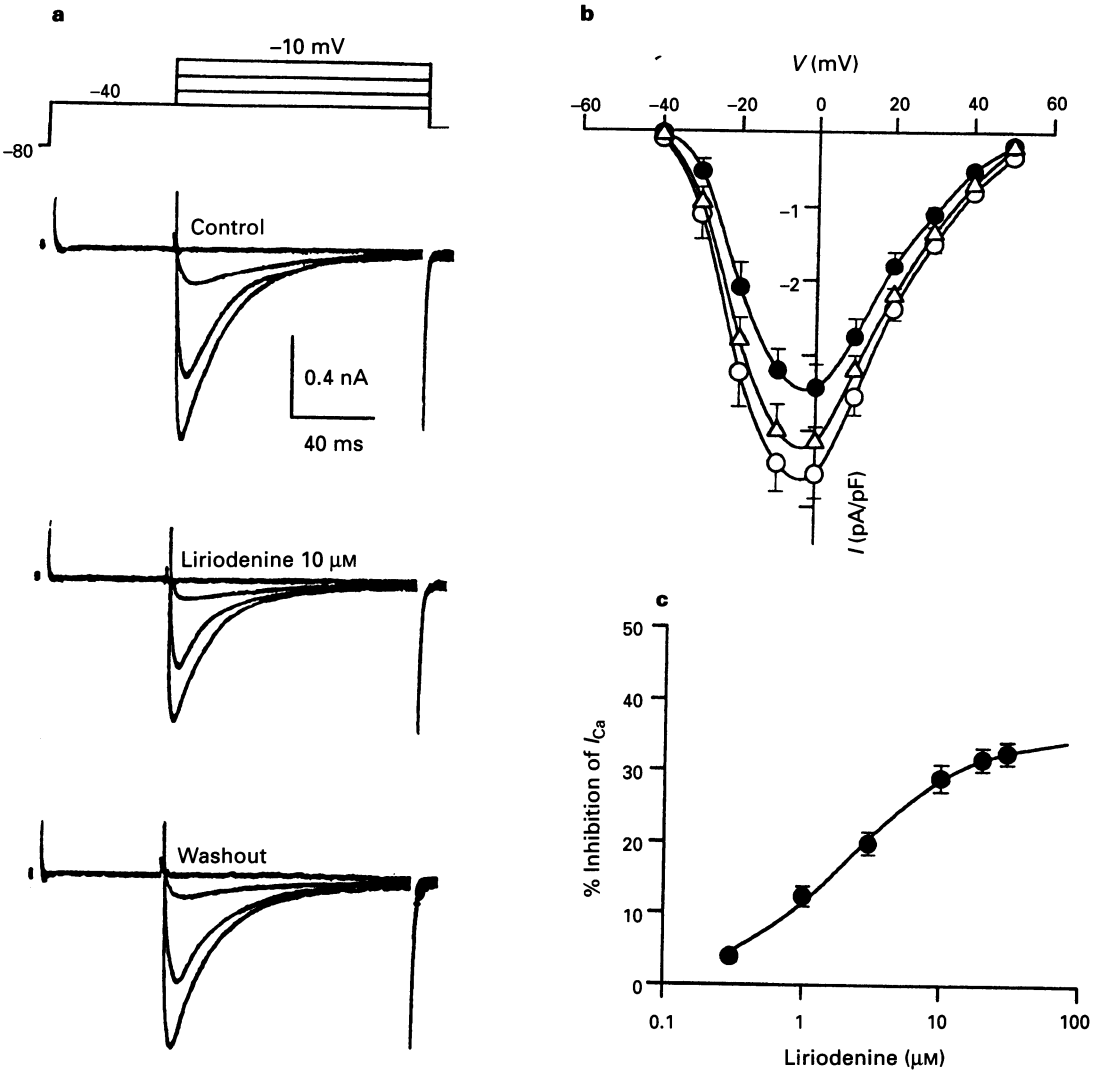

Figure 7 Effects of liriodenine on I_{Ca}. (a) Families of Ca^{2+} current traces produced by a series of depolarizing pulses (120 ms, ranged from −40 to −10 mV) from a holding potential of −80 mV (protocol see upper panel) in the absence and presence of 10 μM liriodenine and following 8 min washout of the drug. The cell was superfused with a normal Tyrode solution containing 30 μM tetrodotoxin (TTX). Test pulses were preceded by a 60 ms prepulse to −40 mV to ensure complete inactivation of I_{Na}. (b) *I-V* curves for I_{Ca} in the absence (○), in the presence of 10 μM liriodenine (●) and washout (△). Means±s.e. from 6 cells. (c) Concentration-response curve of liriodenine on I_{Ca}. Percentage inhibition of peak I_{Ca} at 0 mV corresponding to the control value were plotted against drug concentration. Each point is mean±s.e. for 8 cells at each concentration. Continuous line was best fit to the Hill equation: inhibition of $I_{Ca}(\%)=E_{max}/[1+(IC_{50}/D)n_H]$, where E_{max} is the maximal attainable percent decrease, IC_{50} is the concentration produced half-maximal block, and D is the drug concentration.

plication of a 60 ms prepulse to −40 mV to inactivate Na^+ and T-type Ca^{2+} channels. Cells were bathed in 30 μM TTX to ensure complete elimination of the Na^+ current. With regular depolarizing clamp steps, the peak amplitude of I_{Ca} in control cells declined spontaneously ('rundown') after rupture of the membrane patch. The rundown phenomenon was more prominent during the initial 3–9 min access of the patch pipette to the interior of the cell. When I_{Ca} (evoked at 0 mV) was normalized to the value at 1 min after disruption of membrane patch (= 100%), it decreased to 92±2, 87±3, 82±3, 76±3, 72±4, and 71±4% (n=12) during the subsequent 3, 6, 9, 12, and 15 min, respectively. Therefore, experiments were performed only on those cells with stable Ca^{2+} currents 10 min after cell rupture. Figure 7a shows a representative family of I_{Ca}. The I-V curves for I_{Ca} are shown in Figure 7b. Liriodenine (10 μM) inhibited the amplitude of the voltage-dependent I_{Ca} at any command-voltage steps and this effect was partially reversible after washout. The concentration-response curve of the effect of liriodenine on I_{Ca} is shown in Figure 7c. Average control peak current density at 0 mV was 4.8±0.6 pA/pF (n=8). Peak I_{Ca} decreased by 12±1, 20±2, 29±3, and 32±4% after 1, 3, 10, and 20 μM liriodenine (n=8); and by 17±3, 33±4, 62±4, and 72±5% respectively (n=7) after quinidine (P<0.001, liriodenine vs quinidine). The calculated IC_{50} values for liriodenine and quinidine were 2.5±0.4 μM (maximal inhibition = 35±2%, n_H = 1.05±0.06) and 5.6±0.9 μM (maximal inhibition = 93±5%, n_H = 1.10±0.13) respectively.

Effects on outward K^+ currents in rat ventricular myocytes

The outward currents activated by depolarizing steps in rat ventricular myocytes consist of a rapidly activating and inactivating transient outward component, I_{to}, and a slowly inactivating, steady-state component, I_{ss} (Apkon & Nerbonne, 1991). Figure 8a shows a family of outward K^+ current traces obtained following a series of 160 ms depolarizing pulses from a holding potential of −80 mV in the absence of, after 5 min exposure to, and after 8 min washout of 3 μM liriodenine. Liriodenine depressed both the peak and the steady-state current amplitude and accelerated the inactivation velocity of I_{to}. Figure 8b shows the I-V relationship of I_{to} obtained from 6 cells. The concentration-dependence of inhibition of outward K^+ currents by liriodenine and quinidine are shown in Figure 9a and b. Inhibition of I_{to} was quantified by measuring the integral of the inactivating component of the outward current, whereas the inhibition of I_{ss} was estimated from the reduction in amplitude of the sustained current component measured at the end of a 160 ms depolarizing pulse. The concentration-response curves for the inhibitions of I_{to} and I_{ss} by liriodenine and quinidine are shown in Figure 9c and d. The calculated IC_{50} values of liriodenine and quinidine in inhibiting the I_{to} integral were 2.8±0.5 μM (n_H = 1.00±0.06, n=8) and 0.5±0.2 μM (n_H = 0.90±0.08, n=7) (P<0.001, liriodenine vs quinidine), respectively. Liriodenine and quinidine also re-

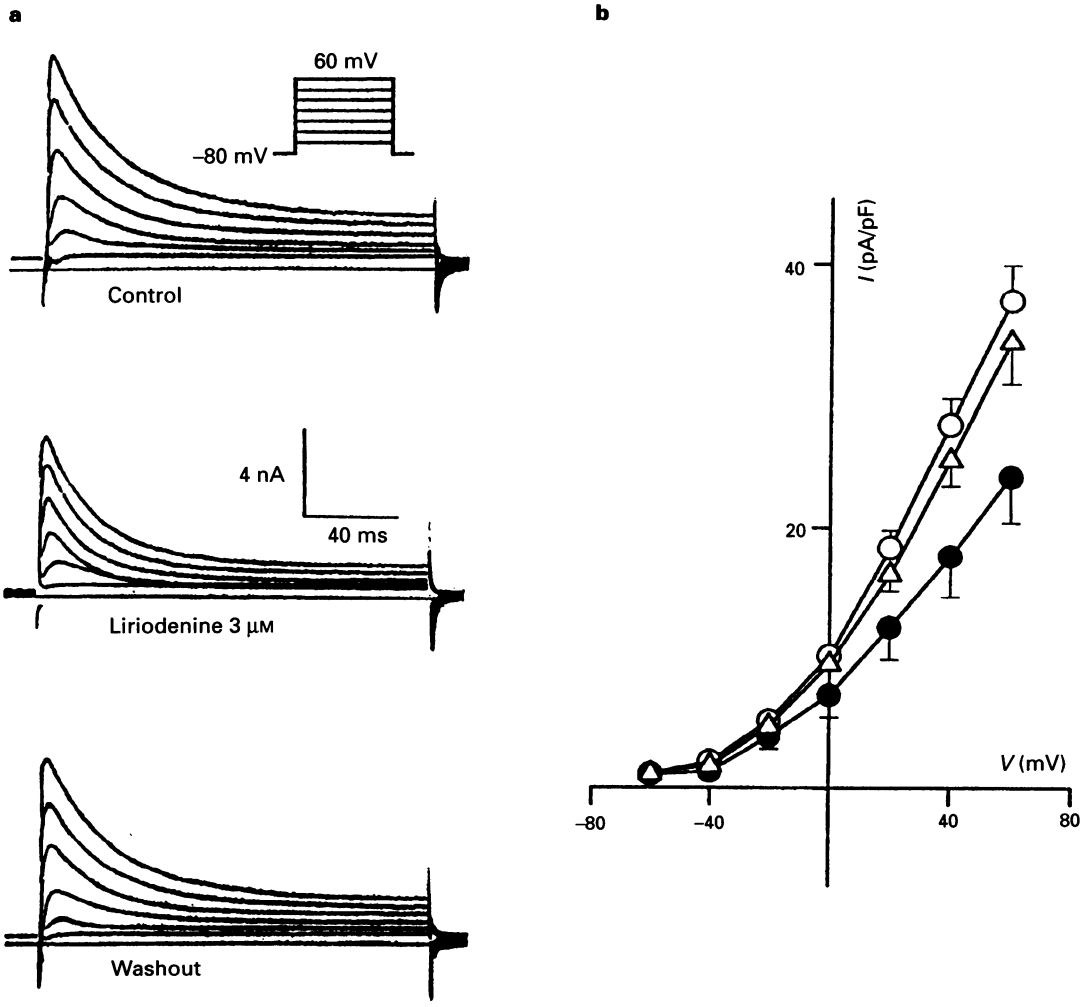

Figure 8 Effects of liriodenine on outward K^+ currents in rat ventricular cells. (a) Families of outward current traces elicited by a series of 160 ms long depolarizing pulses from a holding potential of −80 mV in the absence and presence of 3 μM liriodenine, and following 8 min washout of the drug. Cells were bathed in 30 μM TTX and 1 mM Co^{2+} (to block I_{Na} and I_{Ca}). Horizontal line indicates zero-current level. (b) I-V curves of I_{to} in the absence (○) and presence of 3 μM liriodenine (●), and after washout (△). Each data point represents mean±s.e. from 6 cells.

duced the magnitude of I_{ss} with IC_{50} values of 1.9 ± 0.3 μM ($n_H = 0.80 \pm 0.04$, $n = 8$) and 0.4 ± 0.1 μM ($n_H = 0.81 \pm 0.09$, $n = 7$) ($P < 0.001$, liriodenine vs quinidine), respectively.

Effect of liriodenine on the inactivation time course, voltage-dependence of steady-state inactivation and recovery from inactivation of I_{to}

The prominent concentration-dependent effects of liriodenine and quinidine on the rate of inactivation of I_{to} are shown in Figure 9a and b. The inactivation of I_{to} in the control conditions was well fitted by a curve describing a single exponential decay. Liriodenine caused a modest increase in the rate of current decay which was still well fitted by a single exponential function (Figure 9a, Table 4). In contrast, quinidine significantly increased the rate of current decay which was well described by a biexponential function comprised of a fast and a slow component (Figure 9b, Table 4). The voltage-dependence of steady-state inactivation of I_{to} was evaluated by using a standard two-pulse protocol (200 ms prepulse to varying potentials followed by a 200 ms test pulse to +50 mV) in the absence and presence of 3 μM liriodenine (Figure 10a). As

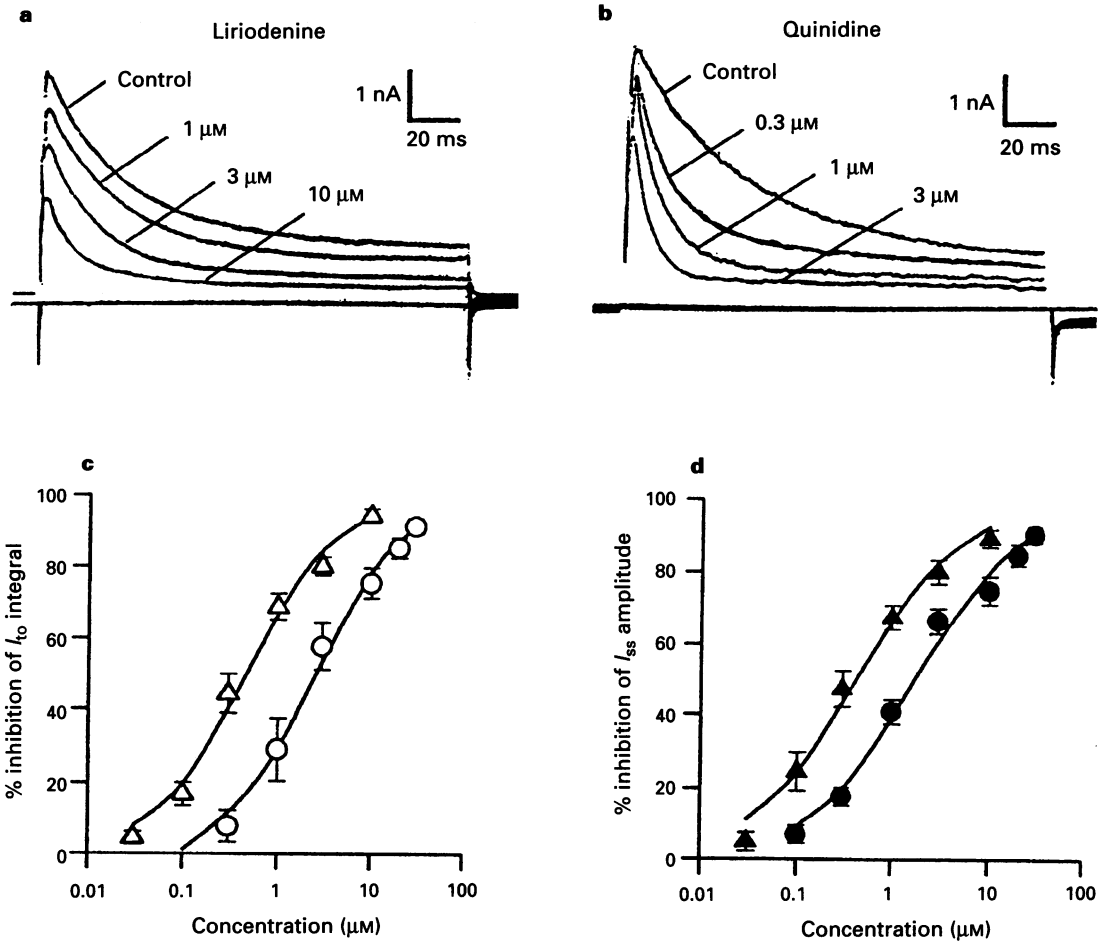

Figure 9 Inhibition of K^+ currents by liriodenine and quinidine as a function of drug concentration. (a) and (b), Superimposed current traces elicited by 160 ms step depolarization from −80 mV to +40 mV in control conditions and in the presence of liriodenine and quinidine, respectively. (c) Concentration-response curve for the effect of liriodenine (○, $n = 8$) and quinidine (△, $n = 7$) on the integral of the inactivating component of the outward current (I_{to}). Continuous lines were drawn according to the fitting of Hill equation. (d) Concentration-response curves for inhibition of the amplitude of the steady-state outward current (I_{ss}) by liriodenine (●, $n = 8$) and quinidine (▲, $n = 7$).

Table 4 Kinetic parameters of I_{to} inactivation and the voltage-dependence of steady-state inactivation and recovery from inactivated state of I_{to} channel under the influence of liriodenine and quinidine

		Control	*Liriodenine* (μM)				*Control*	*Quinidine* (μM)		
	n		*0.3*	*1*	*3*	n		*0.3*	*1*	*3*
τ_1 (ms)	8	30±2	27±2	22±1#	18±3#	9	31±4	8±1#	5±1#	2±1#
τ_2 (ms)								85±11	84±9	52±10
V_h (-mV)	6	56±1	55±2	57±2	55±1	6	55±3	52±1	52±2	51±1
s (mV)	6	4.5±0.5	5.1±0.6	4.6±0.8	5.2±0.7	6	5.2±0.5	5.4±0.6	5.7±0.3	5.8±0.3
τ_f (ms)	6	20±3	20±3	21±3	23±3	7	23±2	19±4	18±2	17±2*
τ_s (ms)								502±41	649±145	739±167

τ_1 indicates the time constant for decay of I_{to}; τ_1 and τ_2 in the presence of quinidine represent the time constant for the fast and slow decaying components of I_{to}, respectively. V_h and s indicate half-maximal inactivation voltage and slope factor. τ_f indicates the time constant for I_{to} channel recovery from inactivated state; τ_f and τ_s in the presence of quinidine represent fast and slow time constant for I_{to} channel recovery. Values are means±s.e. n is number of experiments. *$P < 0.05$, #$P < 0.001$ as compared with the respective control.

shown by graphs in Figure 10a and Table 4, liriodenine, as well as quinidine had no significant effect on the steady-state inactivation of I_{to}. The effect of liriodenine on the recovery from inactivation of I_{to} was studied by use of a paired-pulse protocol in which prepulse and test pulse (−80 mV to +50 mV for 200 ms) were applied at a variable time interval (Figure 10b). The time course of I_{to} recovery from inactivation in the absence of drug was well fitted by a single exponential function. Liriodenine had no effect on the recovery of I_{to} from inactivation (Figure 10b and Table 4). In contrast, the time course of I_{to} recovery in cells exposed to quinidine was prolonged, with a slow, second phase of recovery following a rapid, initial phase. The time course was well fitted by a biexponential function (Table 4).

Effects on inward rectifying K^+ currents (I_{K1})

Currents through the I_{K1} were elicited by serial hyperpolarization after a prepulse to −20 mV to deactivate fully I_{K1} and inactivate I_{Na}. Cumulative application of liriodenine depressed I_{K1}, concentration-dependently, and I_{K1} partially recovered during washout for 8 min (Figure 11a). Figure 11b shows *I-V* curves obtained from 8 cells in the absence and presence of 3, 10, and 30 μM liriodenine. The mean value of slope conductance measured between the membrane potential −70 to −140 mV was 0.29±0.03 nS/pF ($n=8$) in the control condition. The slope conductance was suppressed by 19±6, 40±7, and 65±5% ($n=8$) after 3, 10, and 30 μM liriodenine, the corresponding value after quinidine were 26±8, 48±5, and 64±7% ($n=7$) respectively ($P=0.52$, liriodenine vs quinidine).

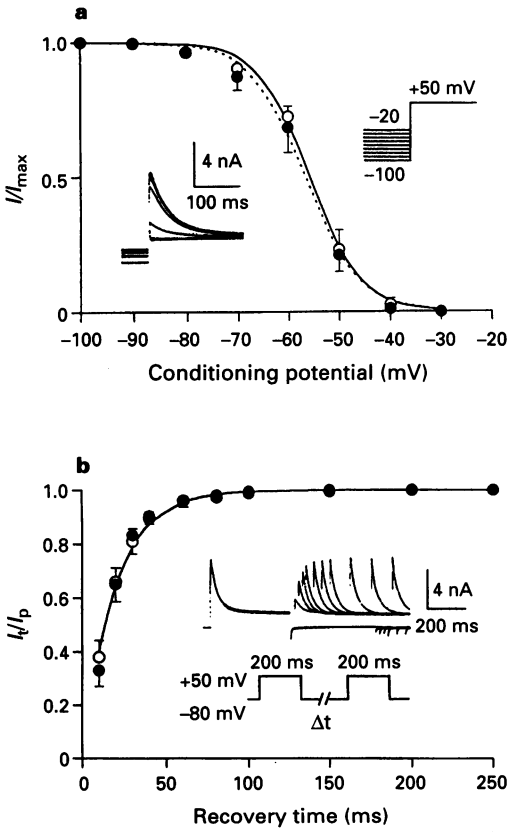

Figure 10 (a) Steady-state inactivation curve of I_{to} before (○) and after 3 μM liriodenine (●). The steady-state inactivation curve was obtained by 2 steps voltage pulses (shown in the text and inset of (a)). Typical predrug superimposed current traces are shown in inset. Dashed line and continuous line drawn through the data points were the best fit to the Boltzmann equation before and after liriodenine. Data points are means±s.e. ($n=6$). (b) Effect of liriodenine on I_{to} recovery from inactivation. The pulse protocol is shown in the text and inset. An example of recovery of I_{to} from inactivation in control conditions is shown in the inset. Plot of test pulse current amplitude (expressed as a proportion of the prepulse current amplitude minus the steady-state current at the end of the pulse) against the interpulse interval in the absence (○) and presence of 3 μM liriodenine (●). Data points are means±s.e. ($n=6$). The curves are best fit of a single exponential function.

Discussion

This study has shown that liriodenine, an aporphine alkaloid isolated from *Fissistigma glaucescens*, possesses potent antiarrhythmic effect. The antiarrhythmic action of liriodenine appears to be mediated through blockade mainly of the Na and I_{to} channels. Through the I_{to} inhibition, liriodenine could prolong the action potential duration of the cardiomyocytes and account for the positive inotropy of this agent. The electrophysiological effects of liriodenine were similar to those of quinidine, but were different from it in the selectivity of ion channel blockade.

Mechanism of positive inotropy

The positive inotropic effect of liriodenine, assessed by the study of phasic mechanical activity, was not antagonized by prazosin or propranolol. Therefore, this positive inotropy was not due to activation of α- or β-adrenoceptors. In mammalian ventricular myocardium, muscarinic receptor activation selectively inhibits the positive inotropic effect which is associated with cyclic AMP accumulation but the cyclic AMP-independent inotropism is barely affected (Endoh, 1979). The positive inotropy of liriodenine was not attenuated by carbachol suggesting that the inotropic effect of liriodenine is not mediated by the cyclic AMP-dependent pathway. In the heart, APD may be prolonged by an increase of inward current, e.g. Na^+ or Ca^{2+} current, and/or a decrease of K^+ outward currents, either of which is expected to increase the force of contraction. Since the positive inotropic effect of liriodenine was not attenuated by verapamil, the activation of the L-type Ca^{2+} channel can be excluded. The fact that the effect of liriodenine was markedly attenuated in the presence of 1 mM 4-AP suggests that this agent may act through the same mechanism as 4-AP which blocks K^+ channels and hence prolong the APD. This speculation is further supported by its inhibition of I_{to} and prolongation of APD in normal ventricular myocytes and insignificant prolongation of APD in cells pretreated with 4-AP. The similarity between the concentrations of liriodenine needed for positive inotropy and K^+ outward current blockade (i.e., I_{to} and I_{ss}) suggest that inhibition of both currents contributes to the positive inotropy. In general, the majority of the positive inotropy resulting from AP prolongation is caused by increased SR Ca^{2+} loading and release. The increase in SR Ca^{2+} loading is due to enhanced Ca^{2+} entry via the Ca^{2+} channel during which myocytes remain in the depolarized state and a delay in Ca^{2+} extrusion by the Na^+-Ca^{2+} exchange during the slower repolarization (Bouchard *et al.*, 1993; 1995).

Mechanism of antiarrhythmic action of liriodenine

It is well-established that transient coronary artery occlusion often leads to malignant ventricular arrhythmias during the ischaemic and reperfusion periods (Penkoske *et al.*, 1978; Janse & Kléber, 1981; Ferrier *et al.*, 1985). The mechanism(s) underlying the genesis of these lethal arrhythmia (i.e., ventricular tachycardia and ventricular fibrillation) are complex and remain controversial (Manning & Hearse, 1984; Ferrier *et al.*, 1985). Recent studies provide evidence that re-entry and oscillatory after-potential are two major mechanisms responsible for the genesis of reperfusion arrhythmia (Ferrier *et al.*, 1985; Pogwizd & Corr, 1987). Liriodenine reduced action potential upstroke velocity in a concentration-dependent manner. The effect on action potential upstroke is well-correlated with the inhibition of I_{Na} by liriodenine. These results suggest that liriodenine may

exert antiarrhythmic activity by suppression of oscillatory afterpotentials or extrasystole via blocking the Na^+ channels like most of the Class I antiarrhythmic agents (Colatsky, 1982; Sanchez-Chapula *et al.*, 1983; Vaughan Williams, 1984).

Blockade of the myocardial repolarizing K^+ current, resulting in a prolongation of action potential duration and an increase in refractoriness (i.e., Class III electrophysiological activity) has received considerable attention as a potential antiarrhythmic mechanism (Vaugham Williams, 1984; Singh & Nademanee, 1985; Gwilt *et al.*, 1991; Sanguinetti, 1992). The role of the transient outward current in the genesis of ventricular arrhythmia and as a target for Class III antiarrhythmic drugs has also been suggested (Beatch *et al.*, 1991; Sanguinetti, 1992; Näbauer *et al.*, 1993). An increase in the electrophysiological heterogeneity between the epicardium and endocardium has been well demonstrated as one of the prominent ischaemia and reperfusion-induced alterations (Ruffy *et al.*, 1979; Kimura *et al.*, 1986). The large epicardial I_{to} may contribute to this electrical inhomogeneity and to the genesis of ventricular arrhythmias via a phase 2 re-entry me-

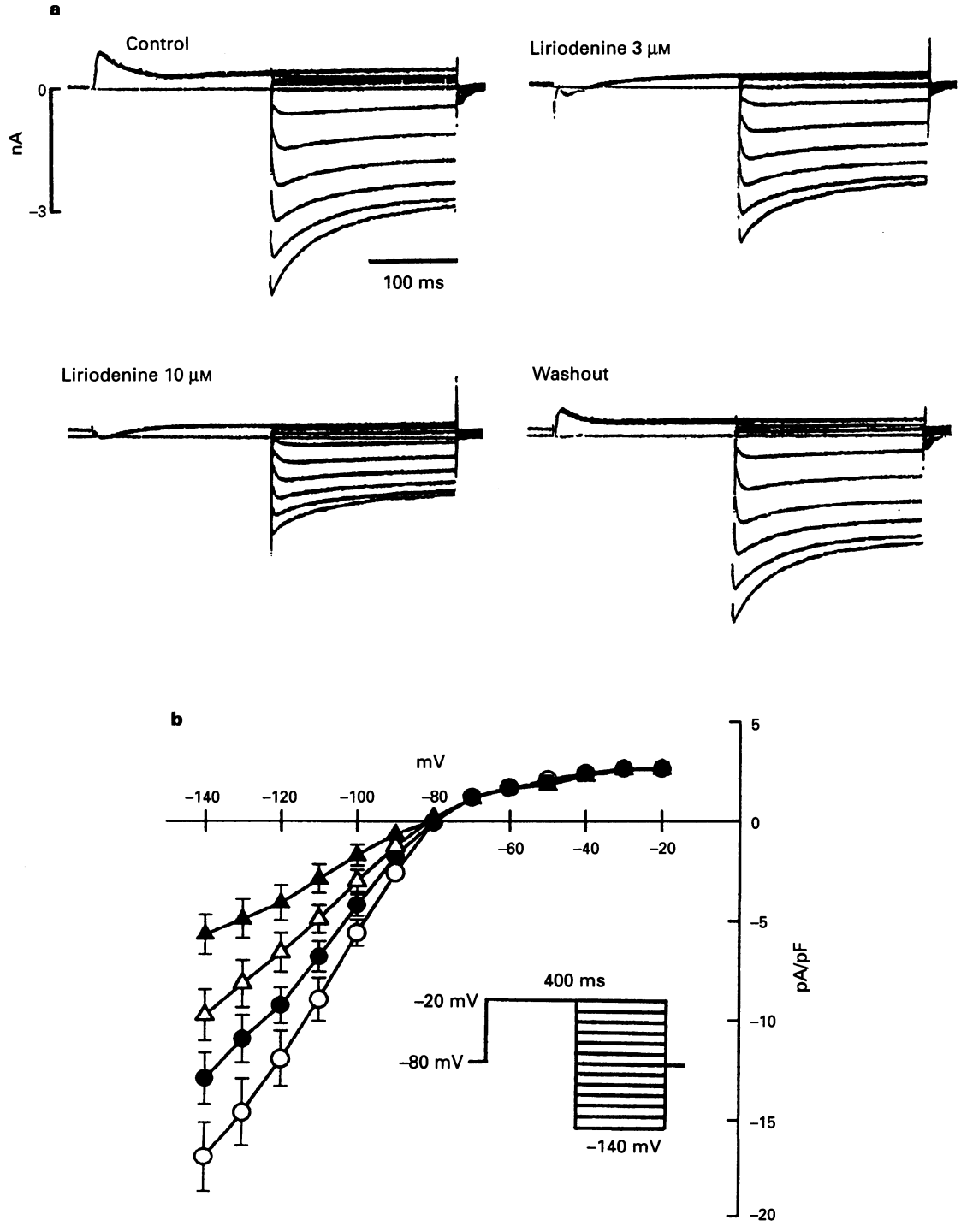

Figure 11 Effects of liriodenine on I_{K1} in rat ventricular cells. (a) Currents were serially elicited (from −20 mV to −140 mV in 10 mV steps) after a prepulse of −20 mV for 200 ms as shown in the inset of (b). Typical current traces before, during application of 3 μM and 10 μM liriodenine, and 8 min after washout were shown. Horizontal line indicates zero-current level. (b) *I-V* curves of I_{K1} in the absence (○) and presence of 3 (●), 10 (△), and 30 μM (▲) liriodenine. I_{K1} was measured at the end of test pulses to various potentials. Each point represents the means ± s.e. of 7 experiments.

chanism (Litovsky & Anzelevitch, 1989; Clark *et al.*, 1993; Di Diego & Antzelevitch, 1994). The finding that blockers of this current such as 4-AP can reverse this extrasystolic activity suggests that compounds which block the I_{to} could exert an antiarrhythmic effect (Di Diego & Anzelevitch, 1994). In the present study, we found that the effects of liriodenine on the action potential of rat ventricular cells are similar to those of 4-AP (Kenyon & Gibbons, 1979), with an inhibition of phase 1 repolarization and an enhancement of plateau level. Voltage clamp experiments demonstrated that the prolongation of APD by liriodenine is mainly attributed to the inhibition of I_{to}. Therefore, it is highly possible that the inhibitory effect of liriodenine on I_{to} is also responsible for the observed antiarrhythmic effects of this drug. The antiarrhythmic effect of liriodenine, as shown in our study, is in a dose range about 2 and 10 fold lower than the inhibition on I_{Na} and I_{to} respectively. At such a low dose-range, the antiarrhythmic effect may be mainly due to its inhibition of I_{Na}, whereas the synergism and the combining inhibition of I_{Na}, I_{to} and I_{ss} contribute to its effects at the higher dose-range.

Mode of channel blocking actions of liriodenine and comparison with quinidine

Use-dependent inhibition of I_{Na}, retardation of the recovery of Na^+ channels from their inactivated state and negative shift of the voltage-dependent inactivation curve of I_{Na} are major mechanisms responsible for the action of most Class I antiarrhythmic agents (Lee *et al.*, 1981; Carmeliet & Saikawa, 1982; Colatsky, 1982; Sanchez-Chapula *et al.*, 1983; Vaughan Williams, 1984). In the present study, liriodenine, as well as quinidine, caused a leftward shift of the steady-state inactivation curve for I_{Na}. This shift suggests that liriodenine and quinidine have a higher affinity for the inactivated Na^+ channels. However, liriodenine did not retard the recovery time course of Na^+ channels from the inactivated state. Whether liriodenine exerts use-dependent inhibition of I_{Na} as do other Class I agents needs further study.

In rat ventricular myocytes, both liriodenine and quinidine block the fast inactivating I_{to} and slowly inactivating steady-state current I_{ss}, but with different potencies; quinidine is approximately 5.5 and 5 fold more potent than liriodenine for the inhibitions on I_{to} and I_{ss} respectively. The inhibition of liriodenine on I_{to} is characterized by a concentration-dependent reduction in peak current and an increase in the rate of current decay. However, as compared to quinidine which caused a prominent biexponential decay process of I_{to}, liriodenine accelerated I_{to} decay to a lesser degree. The accelerated I_{to} decay after liriodenine probably reflects a block of open channels, which is similar to previous observations on the mode of the I_{to} inhibition by quinidine (Imaizumi & Giles, 1987), bupivacaine (Castle, 1990) and SL-1 (Chang *et al.*, 1995). The instantaneous inhibition of I_{to} during depolarization may be partially due to an interaction of liriodenine with the resting state of I_{to} channels. The absence of effect of liriodenine on I_{to} steady-state inactivation curve and the recovery from inactivation process suggests that block does not occur during channel inactivation. On the contrary, quinidine increased the rate of inactivation of the I_{to} and prolonged the recovery of from inactivation although the voltage dependent steady-state inactivation curve of I_{to} was not changed. These quinidine-related effects on I_{to} were similar to previous studies (Slawsky & Castle, 1994; Clark *et al.*, 1995). The influences of liriodenine and quinidine on the kinetics of I_{to} are somewhat different from those on I_{Na}, since they did not produce a detectable shift in the steady-state inactivation curve of I_{to} under these experimental conditions. The I_{to} has also been characterized in pacemaker tissue adjacent to the sinus node region (Giles & Van Ginneken, 1985); it seems reasonable to speculate that the bradycardic effects of liriodenine in spontaneously beating right atria are mediated partly by an inhibition of the I_{to} current.

In rat ventricular myocytes, I_{K1} does not show a negative slope region as compared to that in guinea-pig ventricular myocytes; the decrease of I_{K1} by liriodenine may contribute less to an increase in APD. However, this blockade of I_{K1} seems to be responsible for the slight depolarization of the resting membrane potential.

Significance of our findings

The I_{to} is a major outward repolarizing current in the action potential of several mammalian tissues, including human atrium and ventricle (Escande *et al.*, 1985; Näbauer *et al.*, 1993) and in rabbit and rat ventricular myocytes (Josephson *et al.*, 1984; Giles & Imaizumi, 1988). In addition, the kinetic profile of I_{to} in rat ventricle bears a close resemblance to the I_{to} found in human atria and ventricles (Escande *et al.*, 1985; Näbauer *et al.*, 1993) and molecular biological studies show that I_{to} channels cloned from rat and human hearts share 98% amino acid sequence homology (Tamkun *et al.*, 1991). Although the data presented in this study were generated from studies of K^+ currents in rat ventricular myocytes, it is possible that liriodenine may also affect the I_{to} of human cardiac tissues in a similar fashion. In this respect, the inhibition of I_{to} by liriodenine may render this drug useful as an antiarrhythmic agent.

Most antiarrhythmic drugs have a negative inotropic effect, especially the Na^+ and Ca^{2+} channel blockers (Schlepper, 1989). Quinidine has been reported to cause a negative inotropic effect (Nawrath, 1981). The basis for this depressent effect of quinidine on cardiac force may reside in the ability of high concentrations of quinidine to decrease the slow inward Ca^{2+} current (Nawrath, 1981), despite the marked suppression of K^+ outward currents. Since the I_{to} inhibition by liriodenine is much less than quinidine, the evident liriodenine-induced APD prolongation and inotropism is most likely to be due to its weaker I_{Ca} inhibition than quinidine. These electrophysiological merits of liriodenine will be of potential therapeutic advantage over quinidine, as well as over other conventional Class I antiarrhythmic agents, which have been characterized as cardiodepressant and therefore of limited use in patients with compromised cardiac function (Schlepper, 1989).

In conclusion, we have identified significant antiarrhythmic efficacy for a natural aporphine alkaloid, liriodenine. This drug exerts mixed Class I and III antiarrhythmic properties. In comparison to quinidine, this agent also possesses a potent inotropic effect, suggests that liriodenine may be a promising drug for the treatment of cardiac arrhythmia combined with heart failure.

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