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# Mechanical and electrophysiological effects of 8-oxoberberine (JKL1073A) on atrial tissue

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**1** The effects of 8-oxoberberine (JKL1073A) on contractions and electrophysiological characteristics of atrial tissues were examined.

**2** In driven left atria of the rat JKL1073A (10-100 $\mu$M) increased twitch tension dose-dependently. In spontaneously beating right atria, JKL1073A increased twitch tension but decreased beating rate slightly.

**3** The positive inotropic and the negative chronotropic effect of 30 $\mu$M JKL1073A was not affected by prazosin (1 $\mu$M), propranolol (1 $\mu$M) and 3-isobutyl-1-methyl-xanthine (10 $\mu$M) but significantly suppressed by 4-aminopyridine (2 mM 4-AP).

**4** Current-clamp study revealed that JKL1073A prolonged rat atrial action potential duration (APD). This prolongation of APD by JKL1073A was decreased by pretreating the cells with 2 mM 4-AP. Voltage-clamp study showed that JKL1073A inhibited the integral of the transient outward current ($I_{to}$) dose-dependently with a $K_D$ value of $3.66 \pm 0.93$ $\mu$M in rat atrial myocytes. The equilibrium dissociation constant ($K_d$) for JKL1073A bindings to open state $I_{to}$ was $0.50 \pm 0.08$ $\mu$M. The suppression of $I_{to}$ by 3 $\mu$M JKL1073A was accompanied by shortening of its inactivation time constant from $52.5 \pm 0.9$ ms to $16.8 \pm 0.7$ ms. $V_{0.5}$ for the steady-state inactivation curve of $I_{to}$ was shifted from $-25.7 \pm 3.3$ mV to $-34.8 \pm 3.2$ mV.

**5** In human atrial cells, similar inhibition of $I_{to}$ and prolongation of APD by JKL1073A was found. The $K_D$ value of JKL1073A for inhibition of the integral of $I_{to}$ in human atrial cells is $4.03 \pm 0.02$ $\mu$M. The $K_d$ for bindings to open state $I_{to}$ is 0.5 $\mu$M.

**6** Currents through $K_1$ channels of rat and human atrial myocytes were not inhibited by JKL1073A at concentrations up to 10 $\mu$M.

**7** These results indicate that JKL1073A exerts a positive inotropic effect by inhibition of $I_{to}$. JKL1073A inhibit $I_{to}$ by binding to open state channels or shifting of the steady-state inactivation curve of $I_{to}$.

**Keywords:** 8-Oxoberberine; positive inotropic action; transient outward current; $K_1$ channel; potassium channel blocker

## Introduction

Fast and irregular impulse conduction to the ventricle is the most threatening factor of atrial flutter and atrial fibrillation. Cardioversion performed by electrical d.c. shock should be the initial treatment of choice. If electrical conversion is not possible, medical cardioversion can be attempted with drugs that inhibit the sodium inward current and/or prolong the cardiac action potentials. A successful conversion of atrial fibrillation of recent onset was obtained in 60 to 95% with class I drugs (Bianconi *et al.*, 1989; Suttorp *et al.*, 1990) and of 40 to 60% with amiodarone (Antman *et al.*, 1990). To prevent recurrence of atrial fibrillation after cardioversion, quinidine is the most widely used drug which maintains sinus rhythm one year after cardioversion in up to 54% of patients (Coplen *et al.*, 1990). Class III antiarrhythmic agents such as amiodarone and sotalol can also successfully maintain sinus rhythm after cardioversion (Horowitz *et al.*, 1985; Antman *et al.*, 1990; Juul-Möller *et al.*, 1990). Traditionally digoxin is the mostly commonly used drug, sometimes in combination with $\beta$-adrenoceptor blockers or calcium channel antagonists to decrease AV nodal conduction (Lewis *et al.*, 1987; Steinberg *et al.*, 1987). The increase of arrhythmias by digoxin and the negative intropic effect of $\beta$-adrenoceptor blockers and calcium channel antagonists limit the usage of these drugs.

Berberine is an alkaloid found in many medicinal plants of the genera *Berberis* and *Coptis*. These medicinal plants have been used for centuries as a folk medicine in treatment of jaundice, dysentery, diarrhoea, hypertension and other diseases (Sabir & Bhide, 1971; Swabb *et al.*, 1981; Tai *et al.*, 1981; Ludan, 1988). Important cardiovascular effects of berberine have also been reported: berberine induced a vasodilator effect in smooth muscle (Chiou *et al.*, 1991; Bova *et al.*, 1992) and has been reported to exert both positive inotropic and negative chronotropic actions on guinea-pig right atria (Shaffer, 1993). JKL1073A, a synthesized berberine derivative (Figure 1), was able to convert the ouabain-induced arrhythmia in guinea-pig isolated atria and ischaemia-reperfusion-induced arrhythmia in rat isolated whole hearts to normal rhythms (unpublished observations). In addition to the antiarrhythmic activity, JKL1073A, like berberine, exerted positive inotropic actions and negative chronotropic actions on rat atrial preparations.

To characterize the mechanism underlying the anti-

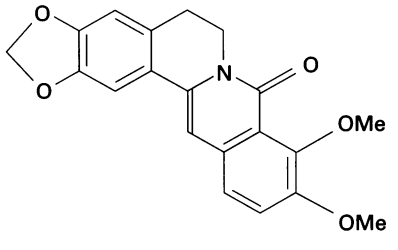


**Figure 1** Chemical structure of 8-oxoberberine (JKL1073A).

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arrhythmic activity and positive inotropic action, the electrophysiological effects of JKL1073A on rat and human atrial cells were examined.

## Methods

### *Mechanical response*

The heart was excised from pentobarbitone-anaesthetized adult male rats (Wistar Kyoto strain, weighing 250–230 g). Right and left atria were dissected from the hearts and placed in an organ bath containing 10 ml HEPES-buffered Tyrode solution, gassed with 100% $O_2$ at 37±0.2°C. To obtain the optimal developed twitch tension, a preload of 0.5–1.0 g was used. Contractions of spontaneous beating right atria and of electrically driven left atria were recorded isometrically by connecting one end of the preparation to a force displacement transducer (BG 25, Gould, Cleveland, OH, U.S.A.). The left atria were stimulated by rectangular pulses of 1 ms duration at supramaximal intensity via an isolated Grass SD9 stimulator (Grass Instruments Co., Quincy, MA, U.S.A.) (Su *et al.*, 1994a).

### *Isolation of rat atrial myocytes*

Rat single cells were isolated by procedures previously described (Mitra & Morad, 1985; Su *et al.*, 1993). Briefly, the hearts were excised from rats and retrogradely perfused in a Langendorff apparatus with $Ca^{2+}$-free HEPES-buffered Tyrode solution. The perfusate was oxygenated and maintained at 37±0.2°C. After 5 min, the perfusate was changed to the same solution containing 0.5 mg $ml^{-1}$ collagenase (type I, Sigma Chemical Co., St. Louis, MO, U.S.A.) and 0.1 mg $ml^{-1}$ protease (type XIV, Sigma). After 20–30 min digestion, the residual enzaymatic solution was cleared with 0.2 mM $Ca^{2+}$-HEPES-buffered Tyrode solution. Thereafter, the atria were separated from the ventricles, the cells were dispersed and stored in 0.2 mM $Ca^{2+}$-HEPES-buffered Tyrode solution at room temperature (25–27°C) for later use.

### *Isolation of human atrial myocytes*

The specimens were obtained from donor hearts which were not transplanted. These donors had died of head injuries in accidents. Single atrial cells were isolated by a procedure slightly modified from those described previously (Escande *et al.*, 1987). Briefly, the specimens were placed in chilled oxygenated $Ca^{2+}$-free HEPES-buffered solution and transported from the operating room to the laboratory. After washout of blood and calcium from the tissues by the same solution, chunks of these tissues were incubated in a similar solution (35±0.2°C) containing 400 iu $ml^{-1}$ collagenase (type I, Sigma) and 4 iu $ml^{-1}$ protease (type XXVII, Sigma) for 30 min. Thereafter, the partial digested atrial tissues were transferred to fresh enzymatic solution containing 400 iu $ml^{-1}$ collagenase. Continuous microscopic examination of the enzymatic solution was performed to assess the number and the quality of isolated cells. The isolated cells were washed and then stored in 0.2 mM $Ca^{2+}$-HEPES-buffered Tyrode solution at room temperature for later use.

### *Whole cell recording*

Transmembrane voltages and currents were evaluated by the whole-cell patch clamp method (Hamill *et al.*, 1981) with a Dagan model 8900 patch clamp amplifier. All experiments were performed at room temperature. Action potentials were elicited by intracellularly applied suprathreshold stimuli of 4 ms duration through a heat-polished patch electrode with resistance between 1 to 2 MΩ when filled with potassium pipette solution. The maximum rate of rise of the action potential upstroke ($\dot{V}_{max}$) was obtained from electronic differentiation of the action potential. Electrode junction potentials (5 to 10 mV) were measured and nulled before impalement of the cell. The formation of a high resistance seal was monitored by applying 1 nA current from a digital pulse generator (M-100; Medical System, Greenvale, NY, U.S.A.). A high resistance seal (5–10 GΩ) was obtained before the disruption of the membrane patch. The cells were dialyzed with the pipette solution for 5–10 min to reach a state of equilibrium after disruption of the membrane patch. The total series resistance for the pathway between the pipette interior and the cell membrane was estimated from the cell capacitance and capacitance current decay. The capacitive transient during step changes in potential was partially compensated with analog circuitry. Series resistance after maximal compensation (60–80%) was usually less than 1 MΩ. The maximal expected voltage drop across the uncompensated series resistance was less than 8 mV for the largest current recorded and therefore was not corrected. During measurement of potassium outward current, the contamination of the sodium inward current ($I_{Na}$) and calcium inward current ($I_{Ca}$) were prevented by adding 10 μM tetrodotoxin (TTX) and 0.5 mM $CoCl_2$. Under these conditions, depolarization of membrane potentials to levels positive to −40 mV from −80 mV resulted in a rapid activation of transient outward current ($I_{to}$) which then decayed exponentially to a steady state. Inward rectifying current ($I_{K1}$) was measured by hyperpolarization of membrane potential to levels more negative than −80 mV. The delayed outward current was found to be very small in human and rat atrial cells (Escande *et al.*, 1987; Wang *et al.*, 1993; Chi *et al.*, 1994) and has been considered to play only a minor role in the regulation of the action potential in both human and rat atrial cells.

### *Mathematical analysis*

The membrane potential at which half of the ionic channels were inactivated was obtained by fitting the normalized inactivation curves to the Boltzmann equation:

$$I/I_{max} = 1/\{1 + \exp[(V - V_{0.5})/\kappa]\}$$

where $V_{0.5}$ is half inactivation voltage and $\kappa$ is the slope factor for voltage-dependence.

The time course of recovery of ionic channels from their inactivation state was studied by sequential increase of the time interval between twin pulse depolarizations. The time constant of recovery was obtained after fitting the recovered fraction of ionic current to the following equation:

$$I_{testpulse}/I_{prepulse} = 1 - [A_1 \cdot \exp(-t/\tau) + A_0]$$

where $A_1$ is the time-dependent coefficient and $A_0$ is the time-independent coefficient. The interpulse interval (ms) is expressed as t, and $\tau$ is the time constant of recovery (ms).

The half-maximal concentration ($K_D$) of JKL1073A to inhibit transient outward current was obtained by the Hill's equation:

$$\int I_{to(drug)}/\int I_{to(control)} = 1/[1 + (D/K_D)^{n_H}]$$

where D is the concentration of JKL1073A and $n_H$ is the Hill coefficient.

The rate of block of open state $I_{to}$ channel by different concentrations of JKL1073A was determined from the following equations:

$$B = B_{max} \cdot (1 - e^{(-K_{+1}[D]+K_{-1}) \cdot t})$$

where $B_{max}$ is maximum block at drug concentration [D]; $B$ equals the amount of block at time $t$; $K_{+1}$ and $K_{-1}$ are the association and dissociation rate constants for JKL1073A, respectively.

$$B = B_{max}(1 - e^{-t/\tau}) + C$$

Where C equals the instantaneous (or time-independent inhibition of $I_{to}$) and $B_{max}$ is the normalized component time-dependent inhibition of $I_{to}$ which develops with a time constant $\tau$.

### Solutions

Two basic solutions were used with the following composition in mM: (1) HEPES-buffered Tyrode solution: NaCl 137, KCl 5.4, $MgCl_2$ 1.1, $CaCl_2$ 1.8, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid] (HEPES) 10, titrated with NaOH to pH 7.4; (2) potassium solution for filling the suction pipettes: KCl 120, NaCl 10, MgATP 5, EGTA 5, HEPES 10, titrated with KOH to pH 7.4.

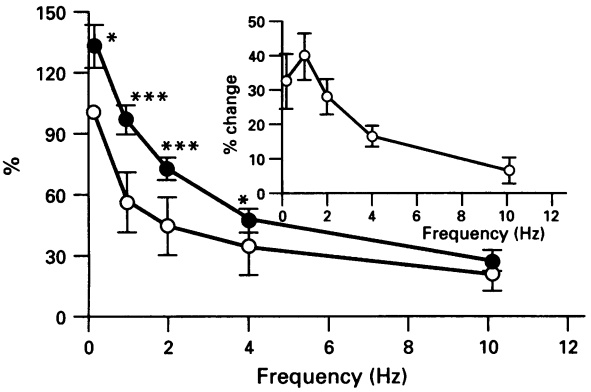



**Figure 2** Comparison of the change in contractions in rat left atria driven at different rates from 0.2 Hz to 10 Hz obtained before (○) and after 5 min exposure to 30 μM JKL1073A (●) ($n=9$). The magnitude of twitch tension in control preparations driven at 0.2 Hz is expressed as 100%. Inset shows the percentage increase of twitch tension at each driving frequency.

### Drugs and chemicals

JKL1073A was synthesized from berberine (purchased from Aldrich Co., Milwaukee, WI, U.S.A.). JKL1073A was purified by column chromatography packed with silica gel followed with recrystallization using a mixed ethyl acetate-hexane solvent. The structure of JKL1073A was confirmed by n.m.r., IR and MS spectra. Other chemicals were bought from Sigma Chemical Company (St. Louis, MO, U.S.A.). All drugs except JKL1073A were dissolved in distilled water or suitable buffer solution. JKL1073A was prepared as a stock solution in dimethylsulphoxide (DMSO). The stock solution was diluted by the bathing solution to a given concentration. In control experiments, DMSO (up to 0.1%) alone had no discernible effect on the mechanical and electrophysiological parameters.

### Statistics

Results are expressed as mean ± s.e.mean. Comparison of mean values among groups was done by a repeated-measure

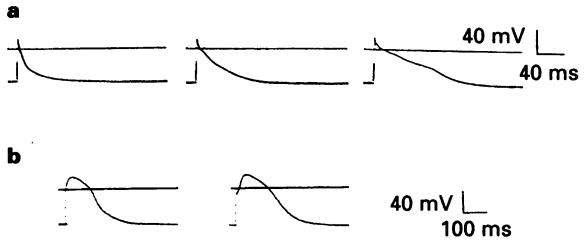



**Figure 3** Effects of JKL1073A on action potentials of rat and human atrial cells. The atrial cells were deiven at 0.2 Hz (a). Action potentials of the rat atrial cell were obtained before (left) and after 5 min exposure to 1 μM (middle) and 3 μM (right) JKL1073A. In (b) action potentials were obtained before (left) and after 5 min exposure to 3 μM JKL1073A (right).

**Table 1** Effects of JKL1073A on contractions and spontaneous beating rate in rat atria

| | | *JKL1073A/control × 100* | | |
|---|---|---|---|---|
| | | 10 μM | 30 μM | 100 μM |
| Right atrium | Spontaneously beating rate | 98.4 ± 0.9 (15)[c] | 93.9 ± 1.2 (15)[c] | 86.8 ± 2.5 (12)[c] |
| | Contractile force | 110.1 ± 2.0 (14)[c] | 143.1 ± 6.9 (15)[a] | 224.2 ± 36.8 (12)[b] |
| Left atrium | Contractile force | 109.1 ± 4.8 (11) | 142.5 ± 12.2 (11)[a] | 237.4 ± 36.8 (8)[b] |

Data are presented as a percentage of control and as means ± s.e.mean. Figures in parentheses indicate the number of preparations. The control values of contractile force in right atria and left atria were 0.45 ± 0.06 g and 0.40 ± 0.05 g, respectively. The control value of spontaneous beating rate in right atria was 242.8 ± 8.2 beats $min^{-1}$.
[a]$P<0.01$, [b]$P<0.001$, [c]$P<0.0001$ as compared with the control data.

**Table 2** Effects of JKL1073A on contractions and spontaneous beating rate in rat atria after treatment with prazosin, IBMX, 4-aminopyridine, propranolol

| | | *Pretreatment with 30 μM JKL1073A* | | | |
|---|---|---|---|---|---|
| | | *Prazosin* (1 μM) | *IBMX* (10 μM) | *4-AP* (2 mM) | *Propranolol* (1 μM) |
| RA | Beating rate | 94.1 ± 1.8 (221.0 ± 8.9) | 89.8 ± 4.5 (332.0 ± 18.6) | 94.1 ± 1.2 (213.8 ± 14.7) | 91.4 ± 3.1 (245.0 ± 7.9) |
| | Contractile force | 154.6 ± 15.6 (0.36 ± 0.09) | 142.9 ± 15 (0.32 ± 0.05) | 118.0 ± 11.7** (0.40 ± 0.07) | 171.1 ± 16.2 (0.32 ± 0.10) |
| LA | Contractile force | 156.2 ± 3.0 (0.37 ± 0.06) | 128.4 ± 4.6 (0.55 ± 0.08) | 118.6 ± 10.8** (0.40 ± 0.08) | 175.2 ± 44.9 (0.28 ± 0.09) |

Data are presented as a percentage of control (before exposure to JKL1073A) and expressed as mean ± s.e.mean ($n$ = 4–15). Values in parentheses indicate the control values of contractile force (g) and spontaneous beating rate (beats $min^{-1}$). **$P<0.01$ as compared with the data for 30 μM JKL1073A-treated group (see Table 1). RA: right atria; LA: left atria.

**Table 3** Effects of JKL1073A on parameters of action potential in rat atrial myocytes

| | | *Control* | *JKL1073A* (1 μM) | *JKL1073A* (3 μM) |
|---|---|---|---|---|
| 0.2 Hz ($n$ = 10) | APA (mV) | 105.2±12.9 | 103.8±12.8 | 101.6±13.8 |
| | $\dot{V}_{max}$ ($Vs^{-1}$) | 69.3±12.1 | 65.6±11.2 | 68.6±11.2 |
| | RMP (mV) | −70.8±5.6 | −67.8±5.8 | −66.0±6.1 |
| | $APD_{50}$ (ms) | 31.4±6.9 | 47.7±8.7* | 83.2±12.5*** |
| | $APD_{90}$ (ms) | 72.6±15.0 | 119.4±33.6** | 186.9±35.0*** |
| 1 Hz ($n$ = 9) | APA(mV) | 80.9±2.7 | 84.8±2.8 | 87.4±5.4 |
| | $\dot{V}_{max}$ ($Vs^{-1}$) | 110.0±8.1 | 99.6±6.4 | 90.7±4.8* |
| | RMP (mV) | −63.8±4.1 | −62.8±4.1 | −62.2±5.0 |
| | $APD_{50}$ (ms) | 57.3±11.7 | 88.8±21.8 | 111.7±25.0* |
| | $APD_{90}$ (ms) | 80.9±14.9 | 136.6±31.6* | 162.9±32.5*** |

Data are expressed as the means±s.e.mean; APA: peak amplitude of action potential; $\dot{V}_{max}$: the maximal rate of upstroke of action potential. $APD_{50}$: action potential duration measured at 50% repolarization; $APD_{90}$: action potential measured at 90% repolarization; *$P<0.05$; **$P<0.01$; ***$P<0.001$ as compared with control group.

**Table 4** Effects of 4-AP on the changes in action potential parameters induced by JKL1073A in rat atrial myocytes

| | | *Pretreatment with 2 mM 4-AP* | *JKL1073A* (1 μM) | *JKL1073A* (3 μM) |
|---|---|---|---|---|
| 0.2 Hz ($n$ = 13) | APA (mV) | 107.8±5.7 | 99.2±7.7 | 111.7±5.3 |
| | RMP (mV) | −68.1±2.6 | −60.9±5.0 | −61.7±4.7 |
| | $APD_{50}$ (ms) | 88.2±23.4 | 88.9±13.3 | 122.7±18.9 |
| | $APD_{90}$ (ms) | 132.9±33.5 | 136.9±16.5 | 197.7±20.3 |
| 1 Hz ($n$ = 9) | APA (mV) | 108.2±7.9 | 103.8±8.7 | 108.8±6.7 |
| | RMP (mV) | −66.2±4.5 | −59.7±7.2 | −60.0±6.8 |
| | $APD_{50}$ (ms) | 67.1±29.5 | 69.1±16.7 | 69.4±19.5 |
| | $APD_{90}$ (ms) | 105.6±36.6 | 115.6±21.4 | 118.8±26.2 |

Data are expressed as the means±s.e.mean; APA: peak amplitude of action potential; $APD_{50}$: action potential duration measured at 50% repolarization; $APD_{90}$: action potential measured at 90% repolarization; *$P<0.05$ as compared with the group receiving pretreatment with 2 mM 4-AP.

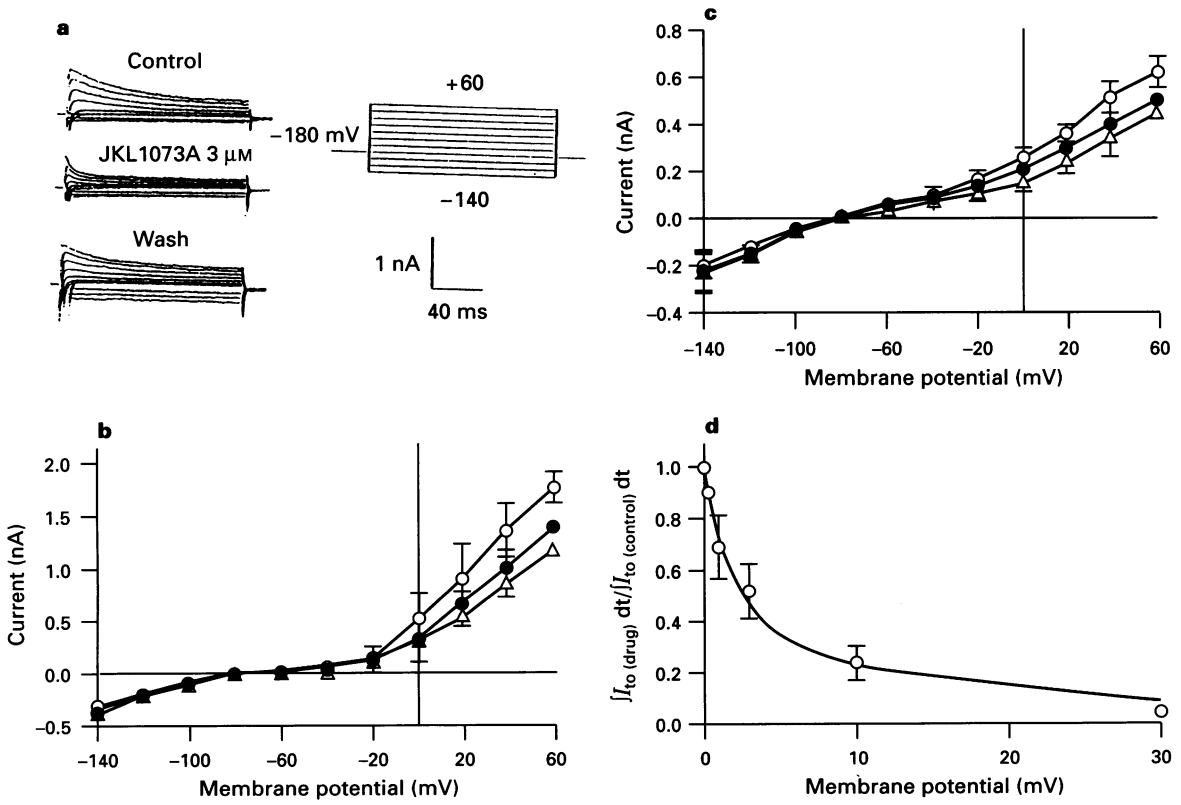



**Figure 4** Effects of JKL1073A on potassium currents in rat atrial myocytes. (a) Current traces showing the effect of JKL1073A on outward and inward potassium currents elicited by a series of 180 ms depolarizing or hyperpolarizing pulses from −80 mV. (b) The $I$-$V$ relationship of potassium currents measured at the peak of the voltage pulses before (○) and after exposure to JKL1073A (●: 1 μM; △: 3 μM; $n$=6). (c) The $I$-$V$ relationship of potassium currents measured at the end of the voltage pulse. (d) Dose-dependent inhibition of JKL1073A on $I_{to}$ in rat atrial myocytes. The integral of $I_{to}$ in the presence of JKL1073A is expressed as a percentage of the integral of $I_{to}$ in the absence of drug.

analysis of variance (ANOVA). Paired or unpaired Student's $t$ test was used to compare the difference between the test and control. Probability ($P$) values less than 0.05 were considered to be significant.

## Results

### *Effects of JKL1073A on isometric contractions in rat atria*

In rat right atria, JKL1073A (10–100 μM) increased twitch tension dose-dependently but reduced the spontaneous beating rate slightly (Table 1). In the driven left atria, JKL1073A (10–100 μM) also increased twitch tension dose-dependently (Table 1). The positive inotropic effect and the negative inotropic effect were reversible after washout of JKL1073A. The positive inotropic effect of JKL1073A (30 μM) was unaffected by 1 μM prazosin, 1 μM propranolol or 10 μM 3-isobutyl-1-methylxanthine (IBMX) but significantly decreased by 2 mM 4-aminopyridine (4-AP) in right and left atria (Table 2). The slight negative chronotropic effect in right atria was affected neither by agents stated above (Table 2) nor by 1 μM atropine (data not shown). Pretreatment with 30 μM JKL1073A did not affect either the positive inotropic effect of isoprenaline ($10^{-10}$–$10^{-5}$ M) in left atria and also did not affect the negative chronotropic effect of carbachol ($10^{-10}$–$10^{-5}$ M) in rat right atria (data not shown).

When the positive inotropic effect was examined in left atria driven at frequencies ranging from 0.2 to 10 Hz, JKL1073A was found to increase twitch tension more in atria driven at frequency lower than 4 Hz but less in atria driven at 4 to 10 Hz (Figure 2).

### *Effects on action potential of atrial myocytes*

In rat atrial cells driven at 0.2 Hz and 1 Hz, the action potential duration (APD) was significantly prolonged by JKL1073A dose-dependently (Figure 3, Table 3). The percentage increase of $APD_{50}$ (APD measured at 50% repolarization) were 65.3±21.2%, 203.2±48.1% by 1 μM and 3 μM JKL1073A, respectively, in cells driven at 0.2 Hz. The percentage increases in $APD_{50}$ were 69.8±38.1%, 112.8±52.0% by 1 μM and 3 μM JKL1073A, respectively, in cells driven at 1 Hz. The percentage increases of $APD_{90}$ (APD measured at 90% repolarization) were 66.8±14.4%, 165.1±24.5% by 1 μM and 3 μM JKL1073A, respectively, in cells driven at 0.2 Hz. The percentage increases of $APD_{90}$ were 80.0±39.7%, 120.0±44.1% by 1 μM and 3 μM JKL1073A, respectively, in cells driven at 1 Hz. The resting potential and the action potential amplitude were not significantly affected by 3 μM JKL1073A; however, the maximal rate of the action potential upstroke ($V_{max}$) of atrial cells driven at 1 Hz was significantly reduced by 3 μM JKL1073A (Table 3). The prolongation of APD by JKL1073A was also observed in human atrial cells. A typical record was shown in Figure 3b.

The prolongation of APD by JKL1073A was decreased in rat atrial cells pretreated with 2 mM 4-AP (Table 4). The percentage increases in $APD_{50}$ were about 1% and 39% by 1 μM and 3 μM JKL1073A, respectively, in cells driven at

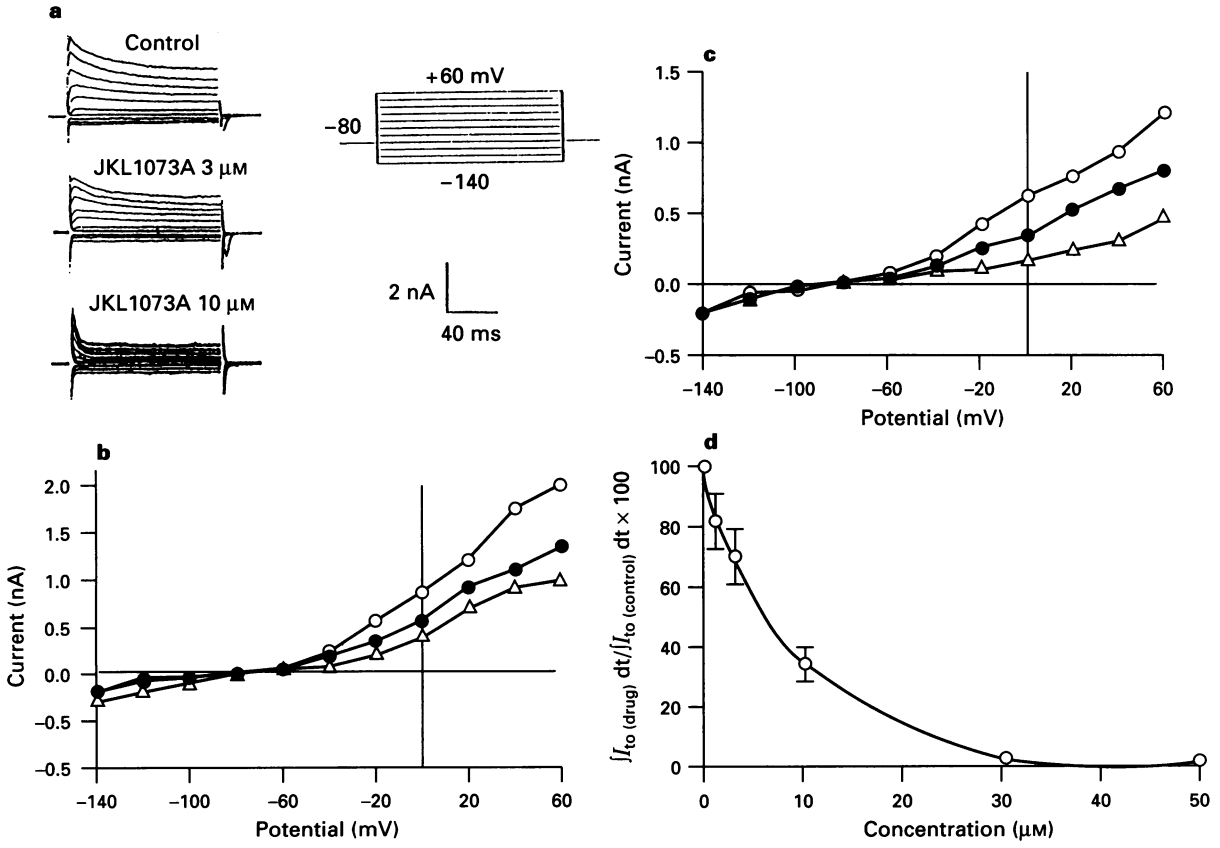



**Figure 5** Effects of JKL1073A on potassium currents in human atrial myocytes. (a) Current traces showing the effect of JKL1073A on outward and inward potassium currents elicited by a series of 180 ms depolarizing or hyperpolarizing pulses from −80 mV. (b) The $I$-$V$ relationship of potassium currents measured at the peak of the voltage pulses before (○) and after exposure to JKL1073A (●: 3 μM; △: 10 μM; $n=5$). (c) The $I$-$V$ relationship of potassium currents measured at the end of the voltage pulse. (d) Dose-dependent inhibition of JKL1073A on $I_{to}$ in human atrial myocytes. The integral of $I_{to}$ in the presence of JKL1073A is expressed as percentage of the integral of $I_{to}$ in the absence of drug ($n=6$).

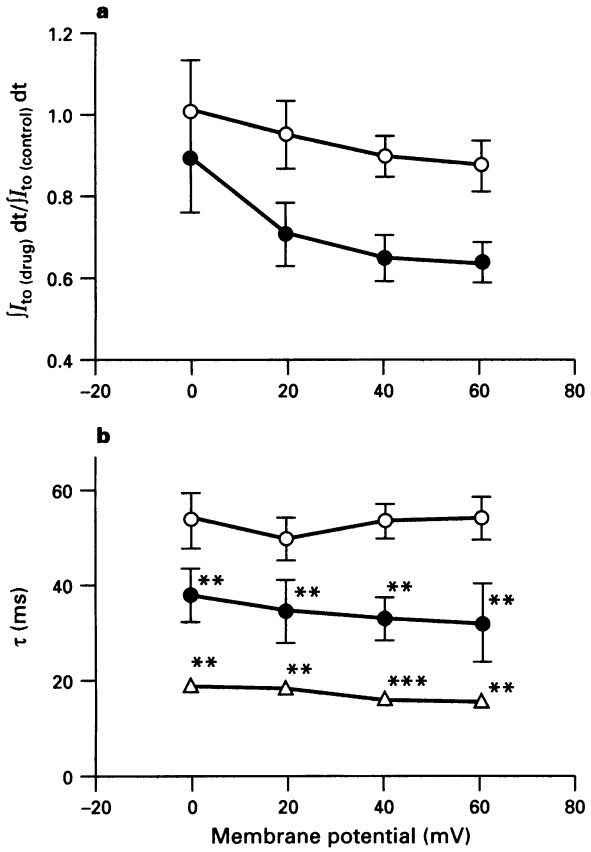



**Figure 6** Inhibition of $I_{to}$ by JKL1073A in rat atrial myocytes. (a) Relations between JKL1073A-induced reduction of $I_{to}$ and membrane potentials (○: 1 μM JKL1073A; ●: 3 μM JKL1073A; $n=6$). (b) The inactivation time constant (τ) and its relations to membrane potentials before (○) and after exposure to 1 μM (●) and 3 μM (△) JKL1073A; $n=6$.

0.2 Hz. The percentage increases in $APD_{50}$ were about 3% and 4% by 1 μM and 3 μM JKL1073A, respectively, in cells driven at 1 Hz. The percentage increases in $APD_{90}$ were about 3% and 49% by 1 μM and 3 μM JKL1073A, respectively, in cells driven at 0.2 Hz. The percentage increases in $APD_{90}$ were about 10%, 13% by 1 μM and 3 μM JKL1073A, respectively, in cells driven at 1 Hz.

### *Effects of JKL1073A on $I_{to}$ and $I_{K1}$ of atrial cells*

Figures 4 and 5 show traces of ionic currents in rat and human atrial cells elicited by depolarization and hyperpolarization pulses. Application of a depolarization pulse to potentials more positive than −40 mV resulted in the generation of a transient outward current which then decayed to a steady-state level. The steady-state component remained at a constant level for more than 1 s during the depolarization step. The *I-V* relationship shows that the peak transient outward current ($I_{to}$) and steady state outward current ($I_{180}$, current magnitude recorded at the end of 180 ms depolarization) were both significantly suppressed at potentials positive to −20 mV. The inhibition of $I_{to}$ by JKL1073A was associated with an acceleration of its rate of inactivation (Figure 6) and a shifting of the voltage-dependent steady state inactivation curve of $I_{to}$. $V_{0.5}$ of the steady state inactivation curve of $I_{to}$ was shifted by 1 μM JKL1073A from $-25.7\pm3.3$ mV to $-34.8\pm3.2$ mV ($P<0.05$; $n=6$; Figure 7). In addition to the left shift of the half inactivation potential, the recovery of $I_{to}$ from inactivation was retarded by 1 μM JKL1073A with a prolongation of recovery time constant from $18.0\pm4.8$ ms to $36.2\pm10.5$ ms ($P<0.05$; $n=5$). The extent of inhibition of $I_{to}$ by 3 μM JKL1073A seems to be more prominent at potentials more positive than 0 mV (Figure 6). The residual percentage of peak $I_{to}$ and its steady state current were $80.5\pm6.3\%$ and $81.7\pm7.2\%$ in 1 μM JKL1073A-treated cells, and $72.4\pm8.4\%$ and $74.1\pm7.7\%$ in 3 μM JKL1073A-treated cells ($IC_{50}$ for inhibition of peak $I_{to}$ was $6.7\pm1.1$ μM and for inhibition of the steady state current was $6.3\pm1.2$ μM).

The inhibition of $I_{K1}$, inward current through inward rectifying potassium channels in rat and human atrial cells was not significantly inhibited by JKL1073A even at concentrations up to 10 μM (Figure 5).

### *Mode of action of JKL1073A on $I_{to}$*

To measure accurately the inhibition of $I_{to}$ by JKL1073A which accelerated the rate of inactivation of $I_{to}$, we decided to use an integral of $I_{to}$ to quantify the inhibition of $I_{to}$ in atrial cells. Figures 4d and 5d show a dose-dependent reduction of the integral of $I_{to}$ by JKL1073A in rat and human atrial cells, respectively. The half concentration for inhibition of the $I_{to}$ integral was calculated to be $3.66\pm0.93$ μM and $4.03\pm0.02$ μM (not statistically different) in rat and human atrial cells, respectively. Closer inspection of the development of inhibition of $I_{to}$, expressing the inhibition of $I_{to}$ in the presence of JKL1073A as a proportion of the current observed in the absence of JKL1073A and plotting it against the time after the start of a depolarizing pulse, revealed that the inhibition consisted of two components (Figures 8 and 9). They were an initial 'instantaneous' component and a following time-dependent component. Both the magnitude of the instantaneous and time-dependent components of inhibition increased with increasing JKL1073A concentration. Nonlinear least-squares analysis of the binding of JKL1073A to open state $I_{to}$ channels provided the following estimates; $K_{+1}=3.32\times10^7\pm0.93\times10^7\ M^{-1}\ s^{-1}$ and $K_{-1}=16.67\ s^{-1}$ ($n=6$). Thus, the estimated equilibrium dissociation constant ($K_d=K_{-1}/K_{+1}$) for time-dependent inhibition by JKL1073A is $0.50\pm0.08$ μM. For comparison, the $IC_{50}$ for inhibition of the instantaneous component is $5.85\pm0.61$ μM. In human atrial cells, the calculated $K_d$ for inhibition of the time-dependent component by JKL1073A is 0.5 μM and the $IC_{50}$ for inhibition of the instantaneous component is 5.1 μM.

## Discussion

The results of the present study show that JKL1073A increased atrial contractility and prolonged atrial action potential duration. The mode of positive inotropic action is different from that of other cardiotonic agents such as cardiac glycosides, sympathomimetic amines, phosphodiesterase inhibitors and Bay K 8644. Cardiac glycosides increase cardiac contractility by inhibition of Na-Ca exchange via suppression of the sodium pump (Lee & Dagostino, 1982; Eisner *et al.*, 1983). Sympathomimetic amines increase cardiac contractility by an increase of L-type calcium current via the activation of β-adrenoceptors (Tsien *et al.*, 1983; Trautwein & Hescheler, 1990). Phosphodiesterase inhibitors increase cardiac contractility by activation of L-type calcium current via accumulation of cyclic AMP (Scholz & Mayer, 1986). Bay K 8644 increases cardiac contractility by activation of L-type Ca channels (Hess *et al.*, 1984; Kokubun & Reuter, 1984). The vasoconstriction and the positive chronotropic action limits its use as a cardiotonic agents. The increase in intracellular $Ca^{2+}$ produced by cardiac glycosides and sympathomimetic amines aggravates the effects of myocardial ischaemia and causes arrhythmia (Kass *et al.*, 1978; Opie & Coetzee, 1988; Saman *et al.*, 1988). In view of the inherent problems with currently available inotropic agents, it was thought worthwhile to develop inotropic agents with negative chronotropic activity. Agents which suppress $K^+$ efflux will prolong action potential duration, decrease heart rate and increase contractile force (Lathrop *et al.*, 1989; 1993; Beregi *et al.*, 1992; Abrahamsson *et al.*, 1993; Su *et al.*, 1990; 1994a). Since the positive inotropic

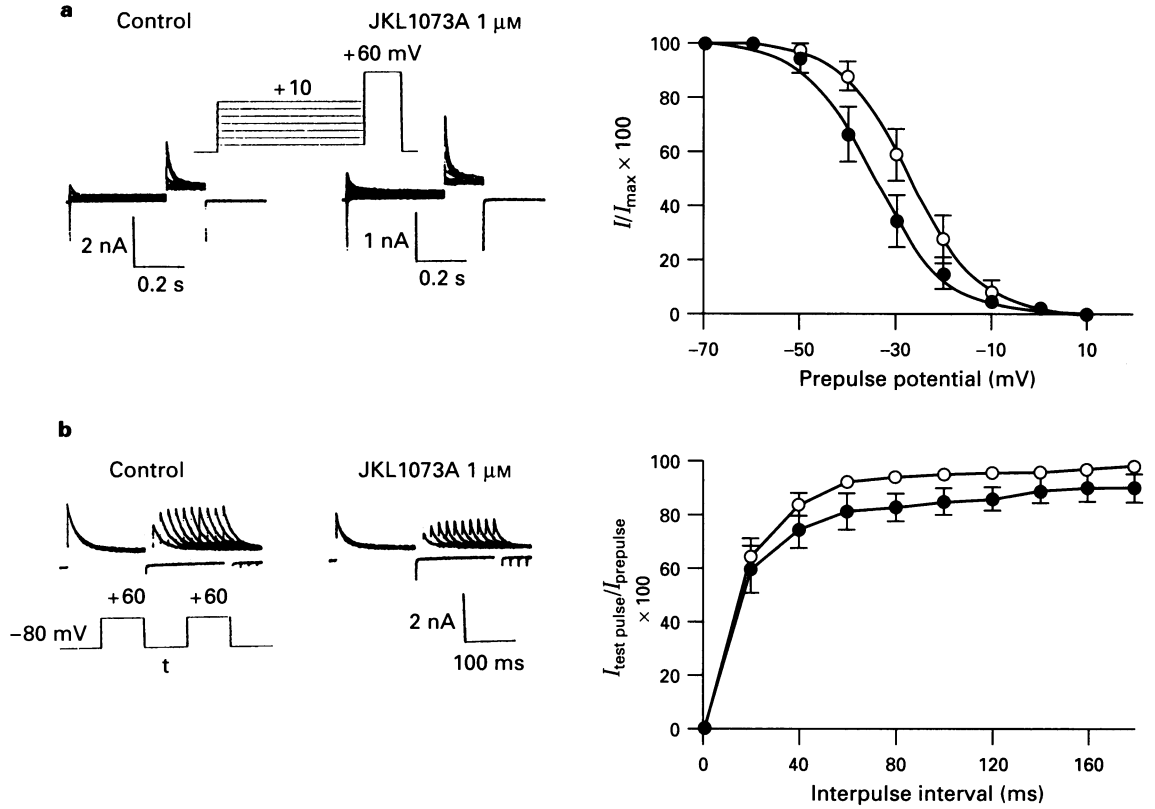



**Figure 7** Effect of JKL1073A on inactivation characters of $I_{to}$ in rat atrial myocytes. (a) Effect of JKL1073A on voltage-dependent steady-state inactivation of $I_{to}$. Left panel: current traces obtained in the absence and presence of JKL1073A after applying the pulse protocol shown in inset of the figure. Right panel: steady-state inactivation curve of $I_{to}$ before (○) and after exposure to 1 μM JKL1073A (●); $n=6$. (b) Effect of JKL1073A on $I_{to}$ recovery from inactivation. Left panels: current traces show the effect of increasing interpulse on the extent of recovery of $I_{to}$ before and after exposure to JKL1073A. Right panel: plot of normalized current amplitude induced by test pulse against the interpulse interval before (○) and after exposure to 1 μM JKL1073A (●); $n=5$.

action of JKL1073A was antagonized neither by prazosin nor by propranolol, this action could not be mediated by activation of α- and β-adrenoceptors. The significant suppression of the positive inotropic action of JKL1073A by pretreatment of the atrial preparations with 4-aminopyridine which is known to inhibit the transient outward potassium current (Castle & Slawsky, 1992; Campbell *et al.*, 1993) suggests that the positive inotropic action of this agent may partially be mediated by inhibition of $I_{to}$. This speculation can be proved by its inhibition of $I_{to}$ and prolongation of atrial action potential duration in normal atrial cells and insignificant prolongation of APD in atrial cells pretreated with 4-AP. Being a structural analogue of berberine, JKL1073A exerted a positive inotropic action at a similar concentration range (10 to 100 μM) to berberine (Shaffer, 1993). Since the positive inotropic action of isoprenaline can be enhanced by agents such as IBMX which are known to inhibit phosphodiesterase, the absence of enhancement of the positive inotropic action of isoprenaline by 30 μM JKL1073A indicates that this concentration does not inhibit the activity of phosphodiesterase. Our experiments proved that JKL1073A exerts a positive inotropic action by a mechanism similar to berberine (Neto, 1993; Shaffer, 1993).

### *Mechanism of the blocking action of JKL1073A and comparison with other compounds*

According to the results of the present study, JKL1073A increased atrial APD via inhibition of $I_{to}$ like class III and class Ia antiarrhythmic agents (Dukes & Morad, 1989; Sanguinetti & Jurkiewicz, 1990; Gwilt *et al.*, 1991; Su *et al.*, 1990; 1994a, b). Since the shape of action potentials are different in various cardiac tissues, the potassium currents responsible for terminating the plateau phase of the action potential are different in cells from different cardiac tissues. In guinea-pig ventricular cells, the current responsible for terminating the plateau action potential is the slowly activated delayed outward current (Matsuura *et al.*, 1987). In rabbit sinoatrial and atrioventricular nodal cells, a transient as well as a delayed outward current are responsible for the repolarization of the action potential (Nakayama & Irisarwa, 1985; Shibaski, 1987). In rat and human atrial cells, the major outward current is a 4-AP-sensitive $I_{to}$ (Escande *et al.*, 1987; Shibata *et al.*, 1989). Our finding regarding the block of $I_{to}$ by JKL1073A includes the following characteristics. (A) There appeared to be a voltage-dependence for the extent of $I_{to}$ integral block by JKL1073A (Figure 6a). (B) JKL1073A induced a dose-dependent but voltage-independent shortening of inactivation time constant of $I_{to}$ (Figure 6b). (C) The fraction of current blocked by the drug increased as a function of time after the start of the depolarization pulse (Figure 8, 9). (D) The steady-state inactivation curve of $I_{to}$ was shifted to the negative potential by JKL1073A (Figure 7). The acceleration of $I_{to}$ inactivation is similar to the phenomenon observed in previous studies of the $I_{to}$ blocking action of tedisamil (Dukes *et al.*, 1990), bupivacaine (Castle, 1990a), propafenone (Duan *et al.*, 1993) and dicentrine (Su *et al.*, 1994a) in rat ventricular cells. The voltage-dependent action of JKL1073A that we observed is similar to previous observations with flecainide (Wang *et al.*, 1995), but different from bupivacaine (Castle, 1990a), tedisamil (Dukes & Morad, 1989), propafenone (Duan *et al.*, 1993), clofilium (Kass & Arena, 1988), and quinidine (Wang *et al.*, 1995). The leftward-shift of the potential-dependent steady-state inactivation curve of $I_{to}$ by JKL1073A is similar to previous observations with dicentrine (Su *et al.*, 1994b), but differs from clofilium (Castle, 1990b), propafenone (Duan *et al.*, 1993), bupivacaine (Castle, 1990a), 2-phenyl-4-oxo-hydro-

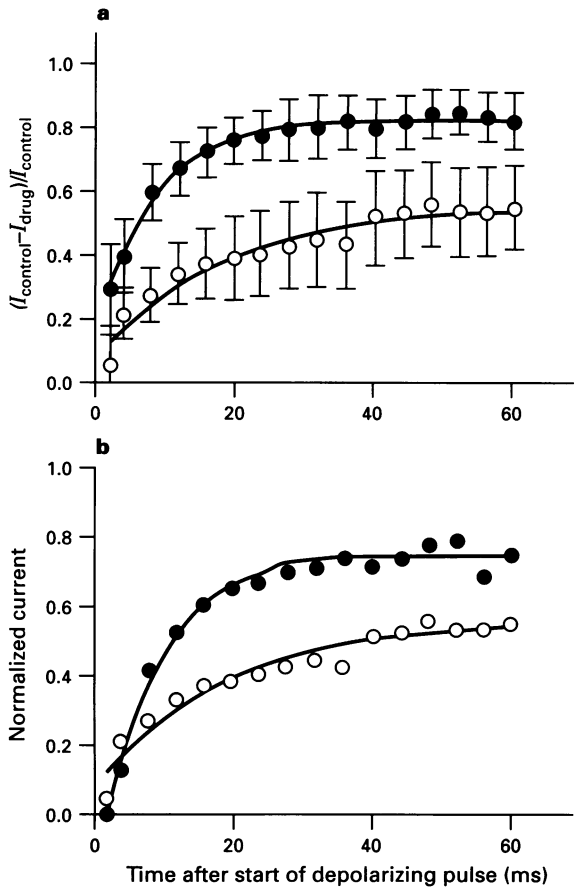



**Figure 8** Kinetics for the inhibition of $I_{to}$ by JKL1073A in rat atrial myocytes. (a) Time course of the development of inhibition of $I_{to}$ by 1 $\mu$M (○) and 3 $\mu$M (●) JKL1073A after a depolarizing pulse to +60 mV from −80 mV (means ± s.e.mean; $n=6$). The JKL1073A-induced reduction of $I_{to}$ is expressed as a percentage of the control current at a given time after the start of the depolarizing pulse (see Methods section, Mathematical analysis). (b) Development of time-dependent inhibition of $I_{to}$ by 1 $\mu$M (○) and 3 $\mu$M (●) JKL1073A. The component of time-dependent inhibition was normalized to the residual current obtained by subtraction of the instantaneous component of inhibition in Figure 8a from the total current.

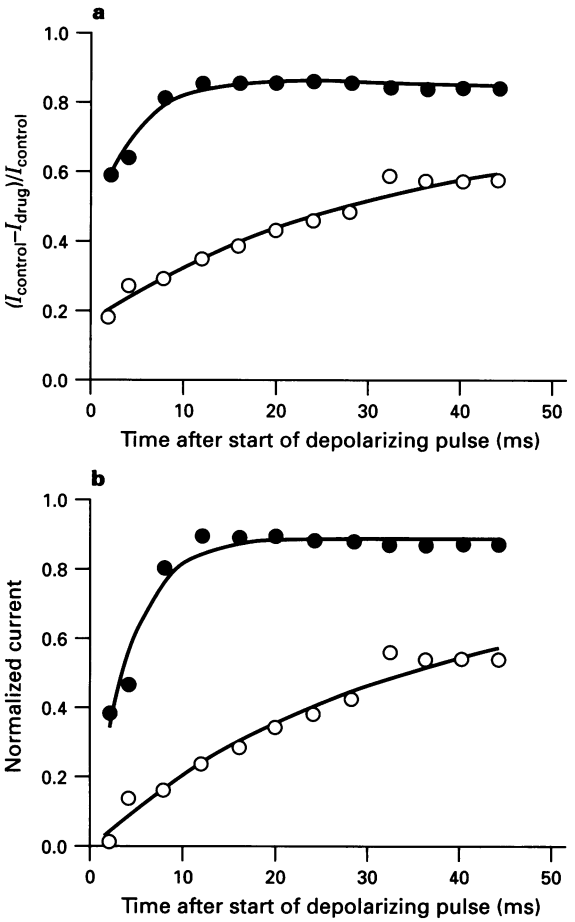
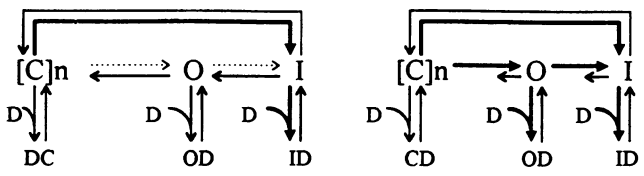



**Figure 9** Kinetics for the inhibition of $I_{to}$ by JKL1073A in human atrial myocytes. (a) Time course of the development of inhibition of $I_{to}$ by 3 $\mu$M (○) and 10 $\mu$M (●) JKL1073A. Data are expressed as described in Figure 8a. (b) Normalized current for the time-dependent inhibition of $I_{to}$ by 3 $\mu$M (○) and 10 $\mu$M (●) JKL1073A.

quinoline (Su *et al.*, 1993) and quinidine (Clark *et al.*, 1995). A biphasic inhibition of $I_{to}$ by JKL1073A was observed in human and rat atrial cells. The instantaneous inhibition that occurs upon depolarization could be contributed to by an interaction with the resting (close) state of the channel or shift of the steady-state inactivation curve of $I_{to}$. Further development of inhibition during depolarization (during channel opening) suggests that JKL1073A also interacts with the open channels. Based on these observations, the inhibition of $I_{to}$ by JKL1073A can be explained in a simple kinetic scheme written as:




Where C, O, I are closed, open and inactivated states of the channel, respectively, and n indicates that there are a series of several closed states leading to an open state (Zagotta & Aldrich, 1990). The agent JKL1073A is expressed as D. On depolarization of the membrane potential from levels more negative than the resting level (e.g. −80 mV) to levels below the threshold potentials (e.g. −40 mV) for activation of $I_{to}$, some channels in close state may change directly into inactivated state (left scheme). The preferential binding of drug to the inactivated state may result in a reduction of channels in the closed state available for activation to the open state. This hypothesis is consistent with the observation of the leftward-shift of $V_{0.5}$ for the steady-state inactivation of $I_{to}$. On depolarization to a level more positive than the threshold potential (e.g. −40 mV), the acceleration of the decline of $I_{to}$ by JKL1073A can occur by specific binding of JKL1073A to channels in the open state (mainly) or inactivated state (right scheme). The retardation of the recovery of $I_{to}$ from the inactivation state can also be explained by binding of JKL1073A to open or inactivated channels.

In addition to $I_{to}$, $I_{K1}$ is also important in the regulation of the resting membrane potential and control of late phase repolarization. The absence of inhibition of $I_{K1}$ is consistent with the observation of little change in the resting membrane potential by JKL1073A.

### *Significance of the inhibition of* $I_{to}$

In rat and human atrial cells, $I_{to}$ is known to be the major outward current responsible for the control of action potential duration (Josephson *et al.*, 1984; Shibata *et al.*, 1989). Therefore, $I_{to}$ suppression would increase the action potential duration and refractory period of the atrial tissues, which then contributes to the suppression of atrial flutter or atrial fibrillation. Since $I_{to}$ is also present in atrioventricular node, Purkinje fibre and rat or human ventricular cells, the inhibition

of $I_{to}$ in these tissues may have clinical implications. This speculation is supported by its effective antagonism of the occlusion-reperfusion ventricular arrhythmia (unpublished observation). Though the inhibition of $I_{to}$ is the most important effect of JKL1073A on rat atrial cells, a weaker but significant suppression of $I_{Na}$ by JKL1073A (6 to 10 $\mu$M) was observed in rat ventricular cells (unpublished observations). The possible inhibition of $I_{Na}$ by 3 $\mu$M JKL1073A can also be proved by the significant inhibition of $V_{max}$ of atrial cells driven at 1 Hz. Therefore the inhibition of $I_{Na}$ by JKL1073A may be of some clinical importance. Partial inhibition of $I_{Na}$ and its contribution to the clinical effect of a potassium channel blocker like amiodarone has also been reported (Manson *et al.*, 1983; Su *et al.*, 1990).

## *Conclusion*

This study has shown that JKL1073A is a positive inotropic agent with strong $I_{to}$ blocking activity and weak $I_{K1}$ blocking activity. The modes of inhibition of $I_{to}$ include an open channel block and leftward shift of the steady-state inactivation curve. The potency of JKL1073A in blocking $I_{to}$ is greater than dicentrine but comparable to quinidine (Su *et al.*, 1994b).

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