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Electrophysiological basis for the antiarrhythmic action and positive inotropy of HA-7, a furoquinoline alkaloid derivative, in rat heart

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1 HA-7, a new synthetic derivative of furoquinoline alkaloid, increased the contractile force of right ventricular strips and effectively suppressed the ischaemia-reperfusion induced polymorphic ventricular tachyrhythmias in adult rat heart ($EC_{50}=2.8$ μM).

2 In rat ventricular myocytes, HA-7 concentration-dependently prolonged the action potential duration (APD) and decreased the maximal rate of rise of the action potential upstroke ($\dot{V}_{max}$). The action potential amplitude and resting membrane potential were also reduced, but to a smaller extent. The prolongation of APD by HA-7 was prevented by pretreating the cells with 1 mM 4-AP.

3 Voltage clamp experiments revealed that HA-7 decreased the maximal current amplitude of I_{Na} ($IC_{50}=4.1$ μM) and caused a negative shift of its steady-state inactivation curve and slowed its rate of recovery from inactivation. The use-dependent inhibition of I_{Na} by HA-7 was enhanced at a higher stimulation rate. The L-type Ca^{2+} current (I_{Ca}) was also reduced, but to a lesser degree ($IC_{50}=5.3$ μM, maximal inhibition = 31.8%).

4 This agent also influenced the time- and voltage-dependent K^+ currents. The prolongation of APD was associated with an inhibition of a 4-AP sensitive transient outward K^+ current (I_{to}) ($IC_{50}=2.9$ μM) and a slowly inactivating, steady-state outward current (I_{ss}) ($IC_{50}=2.5$ μM). The inhibition of I_{to} by HA-7 was associated with an acceleration of its time constant of inactivation. HA-7 suppressed I_{to} in a time-dependent manner and caused a significant negative shift of the voltage-dependent steady-state inactivation curve but did not affect its rate of recovery from inactivation.

5 At higher concentrations, the inward rectifier K^+ current (I_{K1}) was also inhibited but to a less extent. Its slope conductance after 3, 10 and 30 μM HA-7 was decreased by 24 ± 4%, 41 ± 5% and 54 ± 8%, respectively.

6 We conclude that HA-7 predominantly blocks I_{to} and Na^+ channels and that it also weakly blocks Ca^{2+} and I_{K1} channels. These changes alter the electrophysiological properties of the heart and terminate the ischaemia reperfusion induced ventricular arrhythmia. The significant I_{to} inhibition and minimal I_{Ca} suppression may afford an opportunity to develop an effective antiarrhythmic agent linked with positive inotropy.

Keywords: HA-7; furoquinoline derivative; cardiac arrhythmia; positive inotropy; action potential; Na^+, Ca^{2+} and K^+ currents; cardiac myocytes

Introduction

Medicinal plants have been used as traditional remedies in Asia for hundreds of years. The furoquinoline alkaloids are well known in nature and occur almost exclusively in the Rutaceae family. Most of these alkaloids have been found to possess some interesting pharmacological activities. Some of them, such as dictamine, isolated from *Dictamus dasycarpus* Turcz, causes vasorelaxation of rat aorta by suppressing the Ca^{2+} influx through Ca^{2+} channels (Yu *et al*., 1992). Skimmianine and kokusaginine (isolated from *Evodia merrillii*) were found to have selective 5-HT_2 receptor antagonistic activity (Cheng *et al*., 1994). Acrophylline and acrophyllidine, another two furoquinolines isolated from *Acronychia halophylla*, were demonstrated to possess significant antiallergic activity (Huang *et al*., 1995). Recently, in a large scale screening test, we found that HA-7 (Figure 1a), a newly synthesized furoquinoline derivative, can increase the contractile force in isolated rat ventricular strips (Figure 1b and c). In this study antiarrhythmic efficacy and electrophysiological actions of HA-7 have been evaluated by measuring its effects on the ionic currents of cardiac myocytes. The antiarrhythmic efficacy was demonstrated by its ability to convert ventricular arrhythmias which were induced by ischaemia-reperfusion of the isolated heart. We concluded that HA-7 is a useful class I and class III antiarrhythmic agent with a satisfactory antiarrhythmic potential.

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Methods

Contraction experiments

Adult male Wistar-Kyoto (WKY) rats, weighing 200–300 g, were sacrificed after being anesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.), and their hearts were removed quickly. Right ventricular strips (2×5 mm) were dissected and mounted vertically in 10 ml organ baths containing normal Tyrode solution, gassed with 95% O_2 and 5% CO_2 and maintained at 36 ± 0.5°C. Contractions of electrically driven right ventricular strips were measured by connecting one end of the preparations using a fine silk thread to a force displacement transducer (Type BG 25, Gould Inc., Cleveland, Ohio, U.S.A.) and recorded on a Gould RS3400 pen recorder. Preparations were stimulated at a frequency of 2 Hz through bipolar platinum electrodes with rectangular pulses (1 ms duration, twice

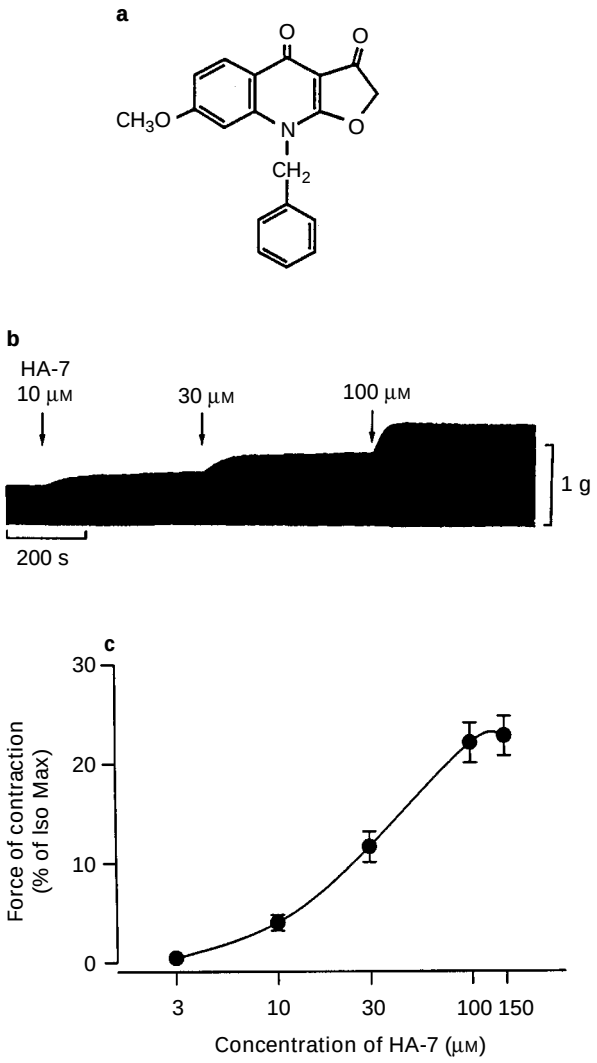


Figure 1 (a) Chemical structure of *N*-benzyl-7-methoxy-2,3,4,9-tetrahydrofuro[2,3-*b*]quinoline-3,4-dione (HA-7). (b) Representative tracing shows the effect of HA-7 on isometric contractions of rat right ventricular strip. (c) Concentration–response curve for the positive inotropy of HA-7 in rat right ventricular strips. Ordinate scale, force of contraction is expressed as the percentage of the maximal response to isoproterenol (Iso). Abscissa scale, logarithmic concentrations of HA-7. All values represent means ± s.e.means. The basal force and maximal response to Iso were 0.33 ± 0.02 and 1.35 ± 0.12 g ($n = 13$), respectively.

threshold strength) delivered from a Grass S8800 stimulator (Grass Instruments Co., Quincy, MA, U.S.A.). The preparations were equilibrated at an optimal preload of 0.5–1 g for 1 h before experiments started and cumulative concentration–responses to HA-7 were obtained. At the end of each experiment, the maximal developed tension was determined in each muscle by administration of isoproterenol (3×10^{-8} to 1×10^{-7} M) after washout of HA-7 for 30 min. The response to HA-7 was expressed as a percentage of the maximal response to isoproterenol determined in the same muscle.

Single cell isolation

Single cells from adult rats were isolated according to the experimental procedure described previously (Mitra & Morad, 1985). Briefly, the excised heart was cannulated and retrogradely perfused with nominally 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 10–20 min digestion, the residual enzymatic solution was removed by more than 5 min perfusion with 0.2 mM Ca^{2+}-containing Tyrode solution. Thereafter, the ventricles were separated from the atria, dispersed and stored in 0.2 mM Ca^{2+}-containing Tyrode solution for later use. This procedure yielded approximately 60–70% rod shaped Ca^{2+}-tolerant cells.

Electrophysiological recording

A small aliquot of dissociated cells was placed in a 1 ml chamber mounted on the stage of an inverted microscope (Nikon, Diaphot, Japan). Cells were bathed at room temperature (25–27°C) in a normal Tyrode solution. Transmembrane voltages and currents were recorded by using the whole-cell patch-clamp technique (Hamill *et al*., 1981). Patch pipettes were made from borosilicate glass capillaries (outer diameter 1.5 mm) using a two-stage vertical puller and were fire-polished. The microelectrode resistance was 2–5 MΩ when filled with pipette solution. Action potentials and membrane currents were recorded using a Dagan 8900 patch/whole cell clamp amplifier (Dagan Corp., Minneapolis, MN, U.S.A.). Electrode junction potentials (5–10 mV) were measured and nulled before suction was applied. A high resistance seal (5–10 GΩ) was obtained before the disruption of the membrane patch and was monitored by applying 10–100 pA current from a digital pulse generator (Model S-95, Medical Systems Co., Greenvalle, NY, U.S.A.). The cells were dialyzed with the pipette solution for 5–10 min to reach equilibrium after disruption of the membrane patch. To record action potentials, the cells were paced through the recording pipette by use of suprathreshold depolarizing stimuli 3–5 ms in duration. The maximal rate of rise of the action potential upstroke ($\dot{V}_{max}$) was obtained by electronic differentiation of the action potential. In voltage-clamp experiments, pipette capacitance and series resistance were compensated to minimize the duration of capacitive current. About 60–80% of the series resistance was compensated. Capacitance of the cell was measured by calculating the total charge movement of the capacitative transient in response to a 5 mV hyperpolarizing pulse. The mean capacitance of the rat ventricular cells was 123.5 ± 15.8 pF ($n = 20$). During the measurement of K^+ currents in rat ventricular cells, contamination by Na^+ and Ca^{2+} inward current was prevented by adding TTX (30 μM) and Co^{2+} (1 mM) to the bathing solution, respectively. For measurement of Ca^{2+} and Na^+ inward currents of rat ventricular cells, the K^+ currents were blocked by adding CsCl (2 mM) 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. Under this condition, the voltage control of the membrane potential was not satisfactory. Therefore, I_{Na} was studied in low Na^+ Tyrode solution (80 mM NaCl was replaced by *N*-methyl-D-glucamine) and dialysis of the cell with Na^+ (10 mM) containing Cs^+ pipette solution. Action potentials and membrane currents were displayed on a Tektronix 511A storage oscilloscope (Tektronix Inc., Beaverton, OR, U.S.A.). Oscilloscope tracings were photographed with a Polaroid camera. Most of the recordings were stored in a video cassette recorder using PCM converter system (A.R. Vetter Co., Rebersburg. PA, U.S.A.). The data were reproduced and were analysed using a pCLAMP software (Version 5.5, AXON Instruments Inc., Foster City, CA, U.S.A.).

Induction and conversion of ventricular arrhythmia

A Langendorff-perfused heart model with constant perfusion pressure instead of constant flow was used (Curtis & Hearse, 1989). The rat 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 experiment protocol

was not started until at least 30 min after isolation of the heart. The atrial and ventricular electrograms were obtained by placing recording electrodes on the epicardium of right atrium and right ventricular apex, respectively. The electrograms were continuously displayed on a Gould pen recorder at 5 mm s^{-1} chart speed and a HP oscilloscope (Hewlett Packard, 54503A) at 100 mm s^{-1} sweep speed. A traction-type coronary occluder consisting of a silk suture threaded 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 was 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 and persisted for at least 5 min.

Solutions and drugs

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, $CaCl_2$ 1.8 and dextrose 11.0. The HEPES-buffered Tyrode solution contained (in mM): NaCl 137.0, KCl 5.4, KH_2PO_4 1.2, $MgSO_4$ 1.22, $CaCl_2$ 1.8, dextrose 22.0, and 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, EGTA 5.0, and HEPES 10.0, adjusted to pH 7.2 with KOH. The Cs^+-containing pipette filling solution contained (in mM): CsCl 130.0, EGTA 5.0, tetraethylammonium (TEA) chloride 15.0, dextrose 5.0 and HEPES 10.0, adjusted to pH 7.2 with CsOH. *N*-benzyl-7-methoxy-2,3,4,9-tetrahydrofuro[2,3-*b*]quinoline-3,4-dione (HA-7) was synthesized by Dr S.C. Kuo. (−)Isoproterenol hydrochloride, 4-aminopyridine (4-AP) and tetrodotoxin (TTX) were purchased from Sigma Chemical Co. HA-7 was dissolved in dimethylsulphoxide (DMSO). In control experiments, DMSO (up to 0.1%) alone produced no significant effect on muscle contractions and electrophysiological parameters of the cells.

Data analysis and statistics

All values are presented as the means ± s.e. Student's *t* test was used to compare test and control values and values of $P<0.05$ were considered significant. Concentration-response curves were fitted by the Hill equation:

$$\text{Inhibition of current (\%)} = 100/[1 + (IC_{50}/C)^{n_H}],$$

where C is the drug concentration, IC_{50} is the concentration of drug for half-maximal block, and n_H, 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 potential of prepulse, V_h is the potential at which the normalized current equals 0.5 and s is the slope factor.

Results

Effects of HA-7 on action potentials of rat ventricular myocytes

The representative changes in action potential configuration produced by HA-7 in rat ventricular cells stimulated at 1.0 Hz are shown in Figure 2, and the mean data are summarized in Table 1A. The most striking effect was the marked concentration-dependent prolongation of APD_{50} and APD_{90}. At the same time, the action potential amplitude and maximal rate of depolarization ($\dot{V}_{max}$) were decreased. HA-7 produced very little change (<2.5 mV) in the resting membrane potential. The prolongation of action potential reached a steady state in less than 3 min and was reversible after washout of the drug. Table 1B shows the effects of 4-AP on HA-7-induced changes in action potential repolarization. Exposure of the cell to 4-AP (1 mM) produced a marked prolongation of the action potential; addition of HA-7 to the 4-AP containing solution produced only a very small additional effect.

Effects of HA-7 on inward Na^+ current (I_{Na})

The effect of HA-7 on I_{Na} is shown in Figure 3. I_{Na} was elicited by a clamp from −80 mV to −20 mV. HA-7 clearly decreased the I_{Na} amplitude concentration-dependently in all cells tested (Figure 3a). The concentration–response curve for the inhi-

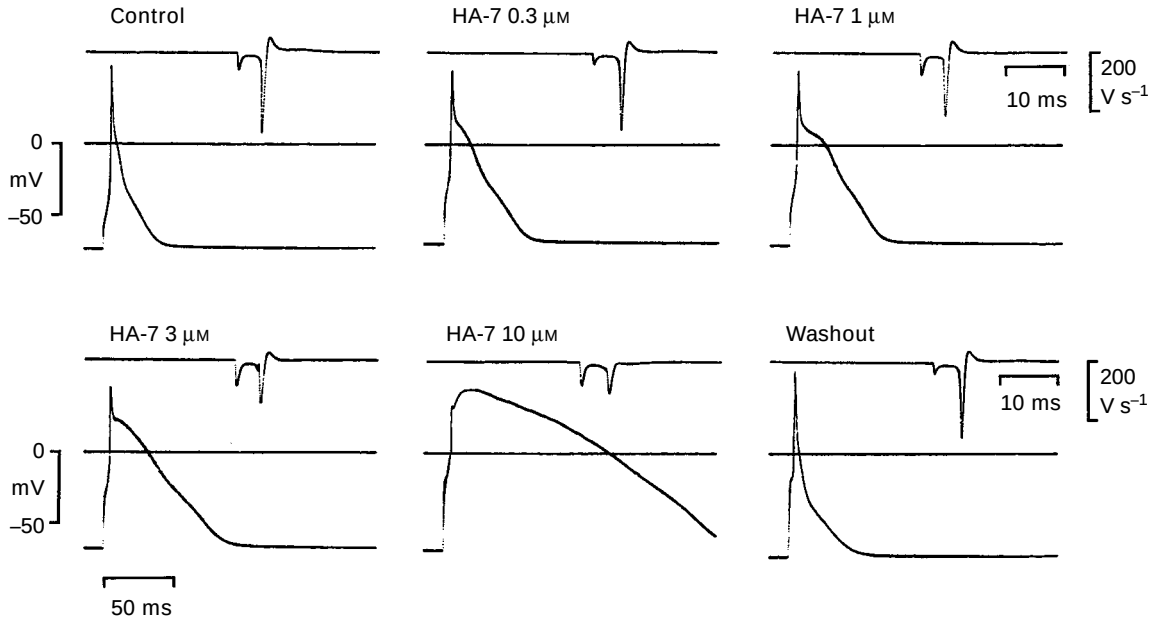


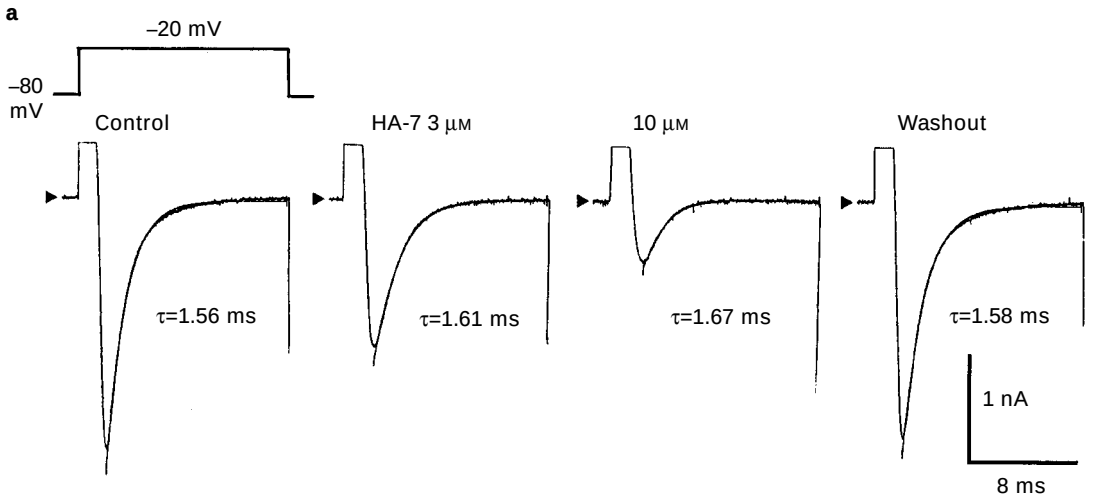
Figure 2 Effects of HA-7 on action potentials recorded in a rat ventricular myocyte. Stimulation frequency was 1 Hz. At each panel, the top trace shows $\dot{V}_{max}$ of the action potential (the second downward deflection) and the bottom is action potentials.

bition of HA-7 on I_{Na} is shown in Figure 3b. Under control conditions, the mean I_{Na} density was 22.6±1.4 pA/pF (n=13). The data points are fitted according to the Hill equation with an IC_{50} for HA-7 on I_{Na} is 4.1±0.5 μM and n_H, 1.02±0.05 (n=13). HA-7 did not significantly modify the inactivation rate of I_{Na}. The decay of I_{Na} was well fitted to the monoex-

Table 1 Effects of HA-7 on the action potential parameters in the absence (A) and presence of 1 mM 4-AP (B) in rat ventricular myocytes driven at 1 Hz

	RMP (mV)	*APA* (mV)	$\dot{V}_{max}$ (Vs^{-1})	APD_{50} (ms)	APD_{90} (ms)
A (n=8)					
Control	−82.0±0.9	129.5±3.3	240.6±9.1	12.1±1.6	42.9±5.3
HA-7 1 μM	−81.8±1.5	125.6±4.8	212.3±20.0	26.8±4.8**	73.3±12.6*
3 μM	−80.4±1.6	118.1±6.1	168.9±26.2*	67.1±10.6***	131.0±14.4***
10 μM	−79.5±1.6	119.1±3.5*	133.1±22.5***	136.4±19.2***	215.4±19.0***
Washout	−82.3±1.4	122.3±6.1	225.0±19.5	17.6±8.2	58.6±12.4
B (n=6)					
Control	−81.3±2.2	102.7±5.1	218.3±20.7	12.5±5.8	36.7±7.3
4-AP	−77.0±3.2	104.3±2.7	185.2±28.6	137.8±20.4	215.8±30.3
4-AP+HA-7 1 μM	−77.3±2.4	101.7±3.7	154.8±23.3	140.5±21.7	220.5±32.5
4-AP+HA-7 3 μM	−76.4±3.1	90.8±3.1##	106.3±18.4#	148.6±19.8	222.8±23.3

Values are means±s.e. 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, ***P<0.001 compared to respective control. #P<0.05 and ##P<0.01 compared to 4-AP alone.



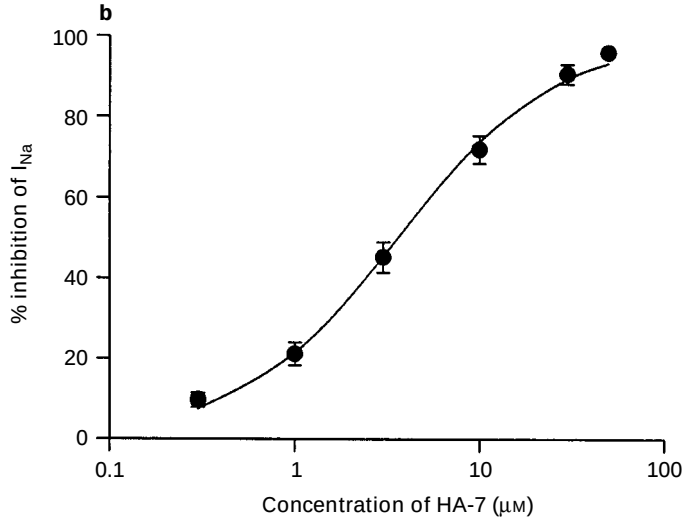


Figure 3 Effects of HA-7 on I_{Na}. (a) Sample traces obtained in one myocyte in the absence, in the presence of 3 and 10 μM HA-7 and 8 min after washout. The cell was held at −80 mV and step to −20 mV for 15 ms to evoke I_{Na}. Test pulses were delivered at a rate of 0.1 Hz. The line drawn through the decay phase of the current traces were fitted from a monoexponential equation. Time constant (τ) of decay are shown as labels. Arrow head in each panel indicates zero current level. (b) Concentration-dependent block of I_{Na} by HA-7. Percentage inhibition of the amplitude of peak I_{Na} by HA-7 was plotted against drug concentration. Each symbol is means±s.e.mean for 13 cells at each concentration. The continuous line was drawn according to the fitting of the Hill equation.

ponential function (Figure 3a). The average time constant (τ_d) of the decay phase in control and in the presence of HA-7 are depicted in Table 2.

Frequency-dependent block of I_{Na}

Frequency-dependent effects of HA-7 on I_{Na} were evaluated by applying 15 consecutive depolarizing pulses. As shown in Figure 4a, I_{Na} was activated by depolarization from a holding potential of -80 mV to -20 mV for 15 ms at various stimulation frequencies. The fifteenth current traces of I_{Na} elicited at different stimulation frequencies of 0.2–20 Hz were superimposed (Figure 4a). At a constant stimulation frequency of 0.2 Hz, the amplitude of I_{Na} elicited by the fifteenth stimulation was almost the same as that elicited by the first stimulation. Therefore, the steady-state amplitude of I_{Na} was maximal

Table 2 Kinetic parameters of steady-state inactivation and recovery from inactivated state of Na^+ channel under the influence of HA-7

	n	*Control*	*HA-7 (μM)* *1*	*3*	*10*
τ_d (ms)	8	1.58±0.11	1.67±0.14	1.69±0.19	1.73±0.17
V_h (−mV)	8	81.4±1.9	83.1±2.1	88.5±1.6**	91.0±2.1**
s (mV)	8	6.2±0.4	6.2±0.3	6.2±0.4	6.3±0.3
τ_f (ms)	7	21.4±2.4	25.5±2.1	42.9±8.1*	59.0±8.7**
τ_s (ms)	7	197.5±45.5	234.2±69.6	284.9±43.5	342.8±59.0
$A_f/(A_f+A_s)$	7	0.78±0.04	0.77±0.08	0.81±0.06	0.80±0.09

τ_d indicates the time constant for decay of I_{Na}; 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 inactivation state; $A_f/(A_f+A_s)$, proportion of fast recovery component of I_{Na}. Values are means±s.e. *n* is number of experiments. $*P<0.05$, $**P<0.01$ compared to the respective control.

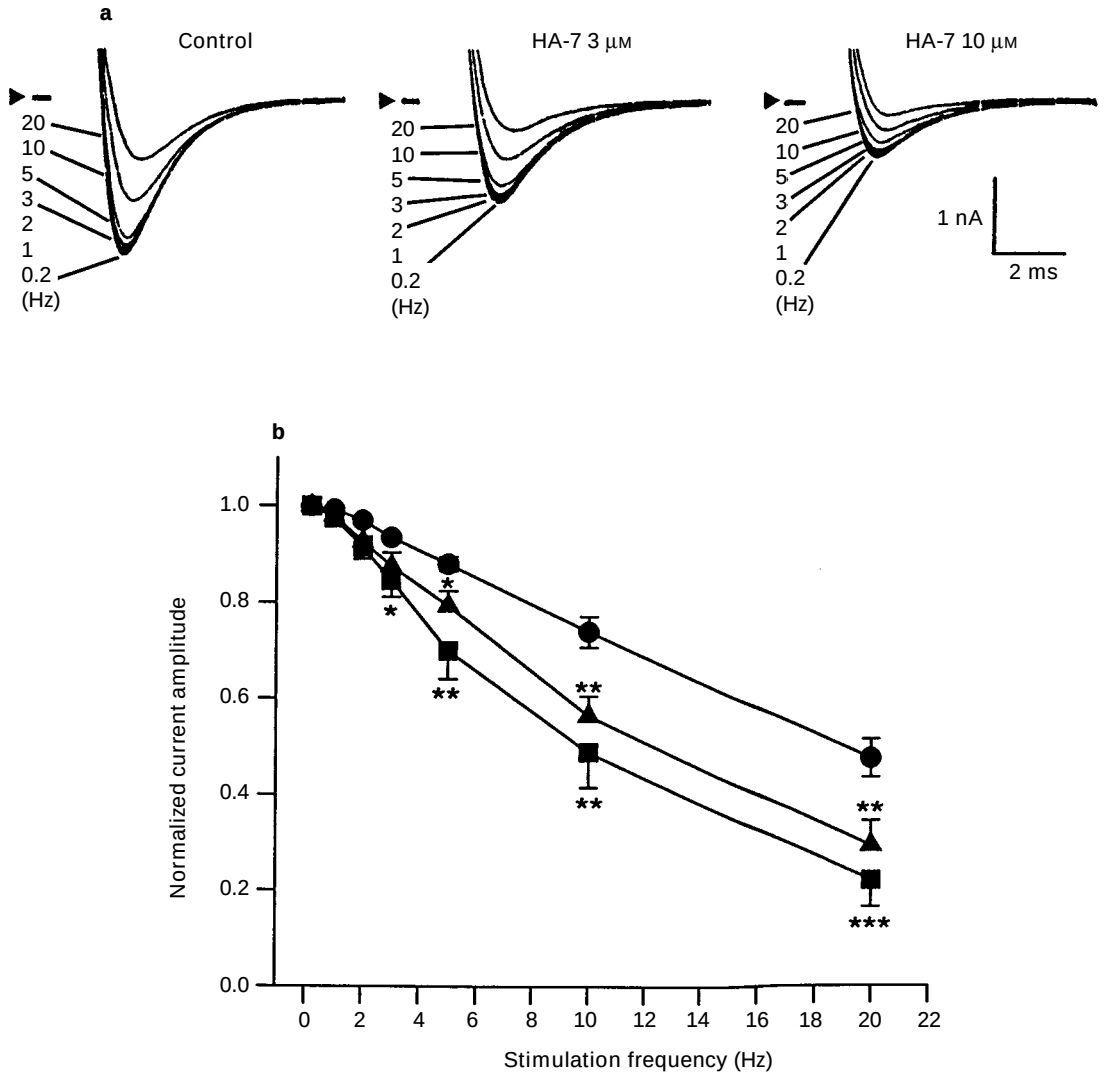


Figure 4 Frequency-dependent block of I_{Na} by HA-7. (a) I_{Na} was elicited by depolarizing pulses to -20 mV from a holding potential of -80 mV. The fifteenth current traces of I_{Na} elicited by stimulation at different frequencies varying from 0.2 to 20 Hz are superimposed. Arrow head in each panel indicates zero current level. (b) The values of I_{Na} elicited by different stimulation frequencies were normalized to the current elicited by stimulation at 0.2 Hz and plotted against the stimulation frequency. (●) control; (▲) 3 μM HA-7; (■) 10 μM HA-7. Means±s.e.means from eight cells. $*P<0.05$, $**P<0.01$, $***P<0.001$, significant difference of the normalized I_{Na} between control and drug-treated group.

when the cells were stimulated at 0.2 Hz. The amplitude of I_{Na} elicited by stimulation at different frequencies was normalized to the amplitude of I_{Na} elicited at 0.2 Hz and was plotted against the stimulation frequencies (Figure 4b). In control condition, the frequency-dependent cumulative inactivation of I_{Na} resulted in a decrease of I_{Na} to 74.0 ± 3.2 and $47.6 \pm 4.0\%$ ($n=8$) when cells were stimulated at 10 and 20 Hz, respectively. The frequency-dependent decrement of I_{Na} under control conditions might be due to the incomplete recovery of I_{Na} from inactivation during the intervals between the repetitive depolarizations, as was reported previously (Gold & Strichartz, 1991). In the presence of 10 μM HA-7 ($n=8$), the normalized I_{Na} was 49.0 ± 7.4 ($P<0.01$ vs control) and $22.1 \pm 5.6\%$ ($P<0.001$ vs control) when cells were stimulated at 10 and 20 Hz, respectively. The magnitude of I_{Na} suppressed by HA-7 was greater at a higher stimulation frequency than at a slower one. Therefore, HA-7 inhibition on I_{Na} was in a frequency-dependent manner.

Effects of HA-7 on steady-state inactivation and recovery from inactivation of I_{Na}

The voltage-dependent steady-state inactivation of I_{Na} was examined by changing the prepulse potentials at levels between

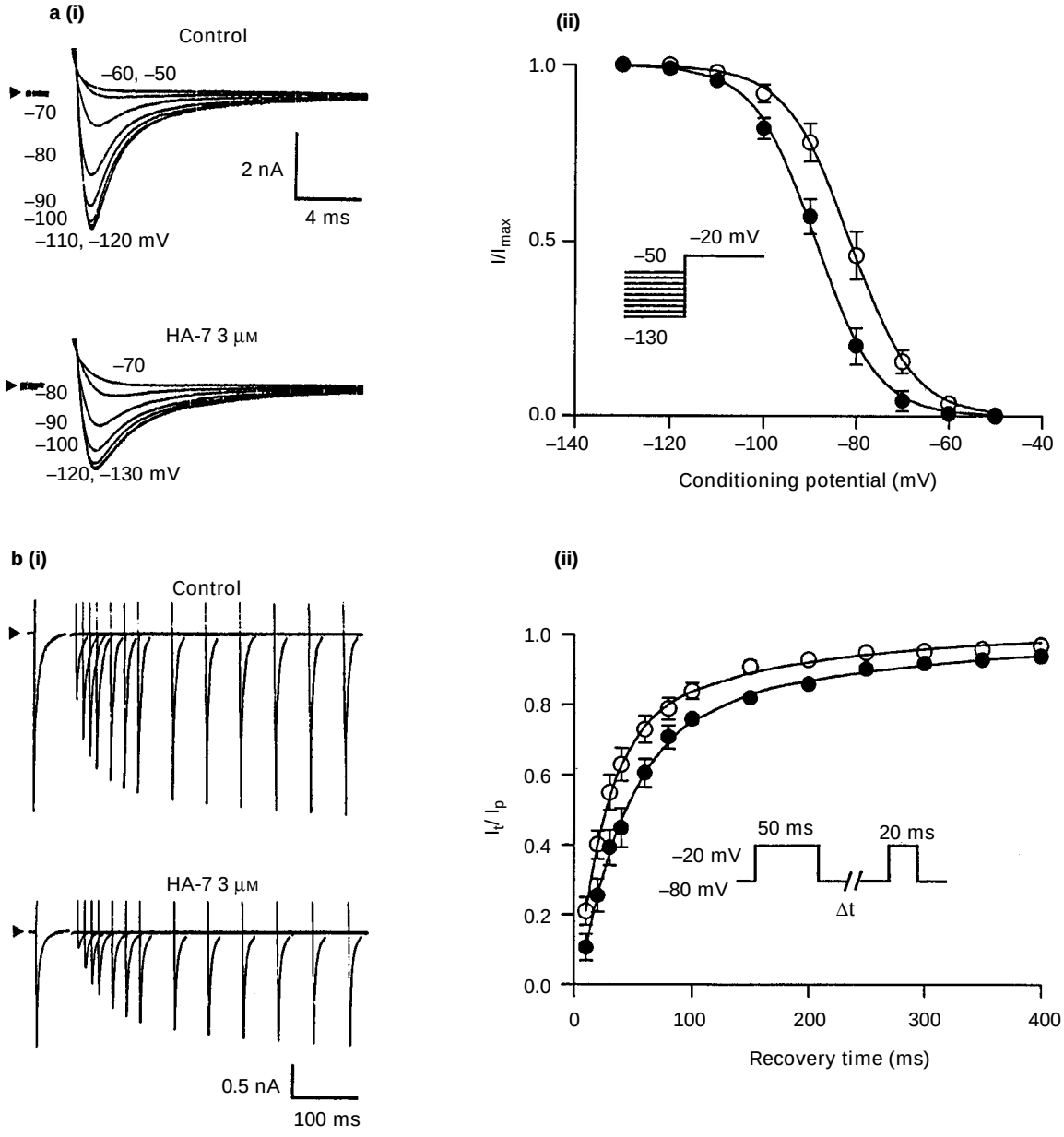


Figure 5 (a) Effects of HA-7 on the steady-state inactivation curve for I_{Na}. Panel (i) shows test current traces under control conditions (upper panel) and after 5 min superfusion with 3 μM HA-7 (lower panel). The pulse protocol is illustrated in the inset of panel (ii). A 100 ms conditioning pulse was applied from the holding potential of -80 mV to various potential ranging from -130 to -50 mV, which was followed by a test pulse to -20 mV. In panel (ii), the steady-state inactivation curves for I_{Na} in the absence (○) and presence of 3 μM HA-7 (●) were obtained by normalizing the test current amplitude to the maximal value in each condition. The data were fitted using the Boltzmann equation. Data points are means ± s.e.mean ($n=8$). (b) Effects of HA-7 on recovery of I_{Na} from inactivation. In panel (i), records of I_{Na} elicited by the prepulse and test pulse were superimposed under control conditions (upper panel) and after 5 min superfusion with 3 μM HA-7 (lower panel), respectively. The double pulse protocol is shown in the inset of panel (ii). A 50 ms prepulse from -80 to -20 mV was followed after various recovery times by a 20 ms test pulse to -20 mV. In panel (ii), I_{Na} elicited during the test pulse (I_t) was normalized to that generated by the prepulse (I_p) and is plotted as a function of the recovery period. (○) Control; (●) in the presence of 3 μM HA-7. The time course of recovery was well fitted to an equation describing the sum of two exponentials. Data points are means ± s.e.means from seven cells.

−130 and −50 mV before depolarization to a test pulse of −20 mV (Figure 5a). The inactivation curves were obtained by normalizing the test current amplitudes by taking the maximum value under each condition as unity (Figure 5a(ii)). The curves were fitted to the Boltzmann equation. The normalized inactivation curve was shifted to more negative potential by HA-7 concentration-dependently. The averaged data of HA-7 on the inactivation kinetic parameters are depicted in Table 2.

The effect of HA-7 on the time-course of I_{Na} recovery from inactivation was then examined by the double-pulse experiments and the results are shown in Figure 5b. The pulse protocol used is shown in Figure 5b(ii). The magnitude of I_{Na} elicited during the second pulse was expressed as a ratio of the current obtained during the first step and plotted against the interpulse duration. In control conditions, even if the time course of I_{Na} recovery was best fitted by the sum of two exponentials, one with a fast time constant (τ_f) and the other with a slow time constant (τ_s). On exposure to 3 μM HA-7, the fraction of fast recovery component was unchanged but the averaged value of τ_f and τ_s were both prolonged even though only τ_f was prolonged to a significant extent (Table 2).

Effects of HA-7 on inward L-type Ca^{2+} current (I_{Ca})

Compared to the marked inhibition of I_{Na}, HA-7 caused a much smaller decrease on I_{Ca} (Figure 6). I_{Ca} was elicited at 0 mV from a holding potential of −40 mV. Reduction of I_{Ca} with time ('rundown') was observed initially after rupture of the membrane patch. The rundown phenomenon was more prominent during the initial 5–10 min access of the patch pipette to the interior of the cell. In our predrug control study, I_{Ca} measured at 5, 7, 15 and 20 min after disruption of the cell membrane were 91.2±7.5, 80.5±5.4, 75.8±3.2 and 73.4±4.9% ($n=11$) of that measured at 1 min after disruption of membrane patch, respectively. For this reason, the concentration-related effects were measured by taking the values at the end of the 8 min control period as unity. The increasing concentrations of HA-7 on I_{Ca} are shown in Figure 6a and b. Average control peak current amplitude at 0 mV is 11.4±1.2 pA/pF ($n=8$). From these data an IC_{50} value of 5.3±1.4 μM and a maximal inhibition of 31.8±2.3% were obtained with a Hill coefficient (n_H) of 1.30±0.24 ($n=8$).

Effects of HA-7 on K^+ currents

Depolarization or hyperpolarization of rat ventricular myocytes from a holding potential of −80 mV resulted in the activation of K^+ currents. In the presence of 30 μM TTX and 1 mM Co^{2+} (to block Na^+ and Ca^{2+} current), step depolarizations to potentials more positive than −30 mV produced a rapidly activating and inactivating transient outward current (I_{to}) and a slowly inactivating steady-state K^+ outward current

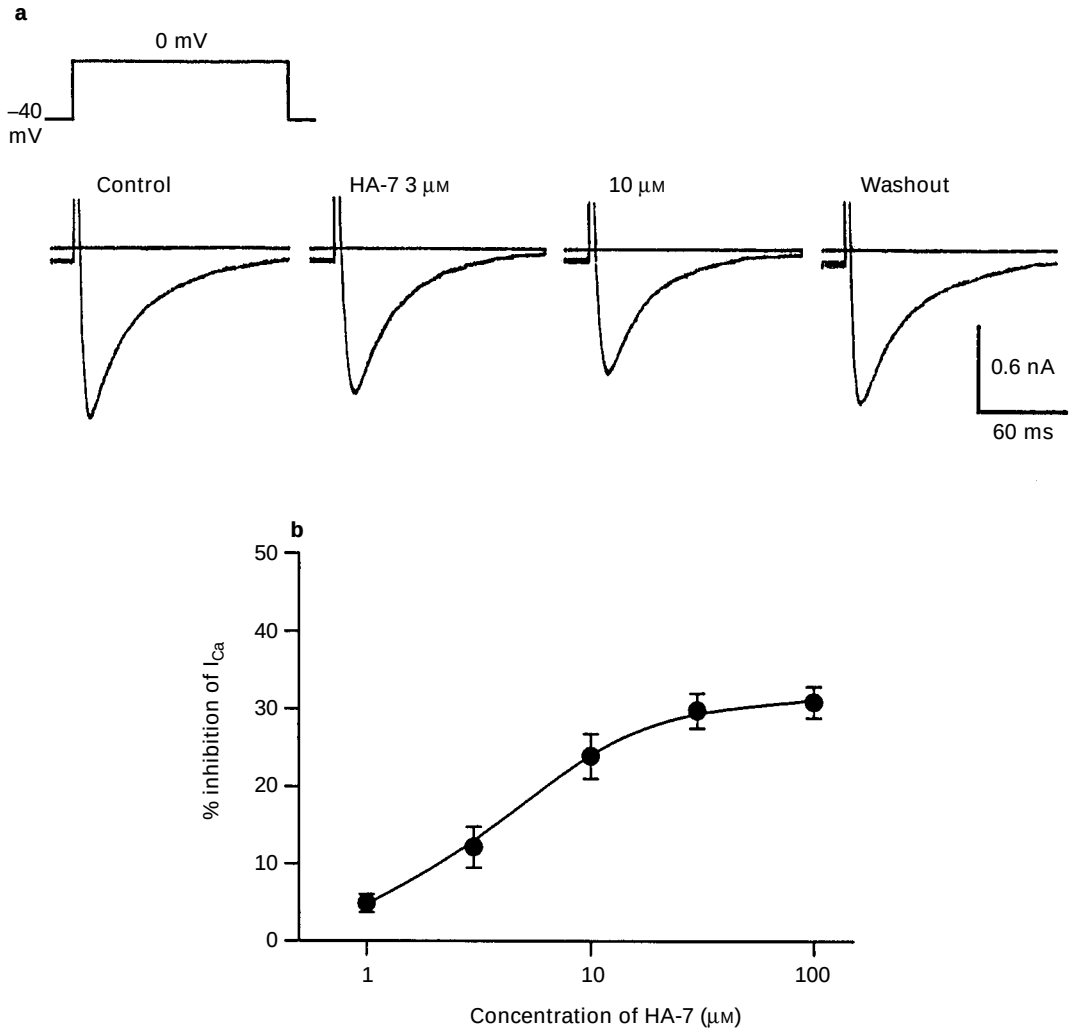


Figure 6 Effects of HA-7 on I_{Ca}. (a) Effect of cumulative application of 3 and 10 μM HA-7 on the peak I_{Ca}, activated by a depolarizing pulse to 0 mV from a holding potential of −40 mV. Horizontal line in each panel indicates zero current level. (b) Concentration-dependent effect of HA-7 on I_{Ca}. Percentage inhibition of the amplitude of I_{Ca} by HA-7 was plotted against drug concentration. Each symbol is means±s.e.means for eight cells. The curve for I_{Ca} was fitted to the mean data according to: inhibition of current (%) $= E_{max}/[1+(IC_{50}/C)^{n_H}]$, where E_{max} is the maximal attainable percent decrease, IC_{50} is the concentration produced half-maximal block and C is the drug concentration.

(I_{ss}) (Apkon & Nerbonne, 1991). When the cell was hyperpolarized to potentials more negative than −80 mV an inward current through the inward rectifier K^+ channel (I_{K1}) was observed. As shown in Figure 7a, 3 μM HA-7 significantly decreased the peak amplitude and accelerated the inactivation of I_{to} and this effect was reversible after washout of the drug. The I-V relation for peak outward and inward K^+ current amplitude is shown in Figure 7b. HA-7 also reduced the magnitude of the slowly inactivating outward current (I_{ss}) (Figure 7a). The I-V relation for I_{ss} is shown in Figure 7c. Figure 7d shows dependence of the decay time constant on membrane potential in the absence and presence of 3 μM HA-7. In control condition the time constant was 20–30 ms and was not significantly dependent on membrane potential in the

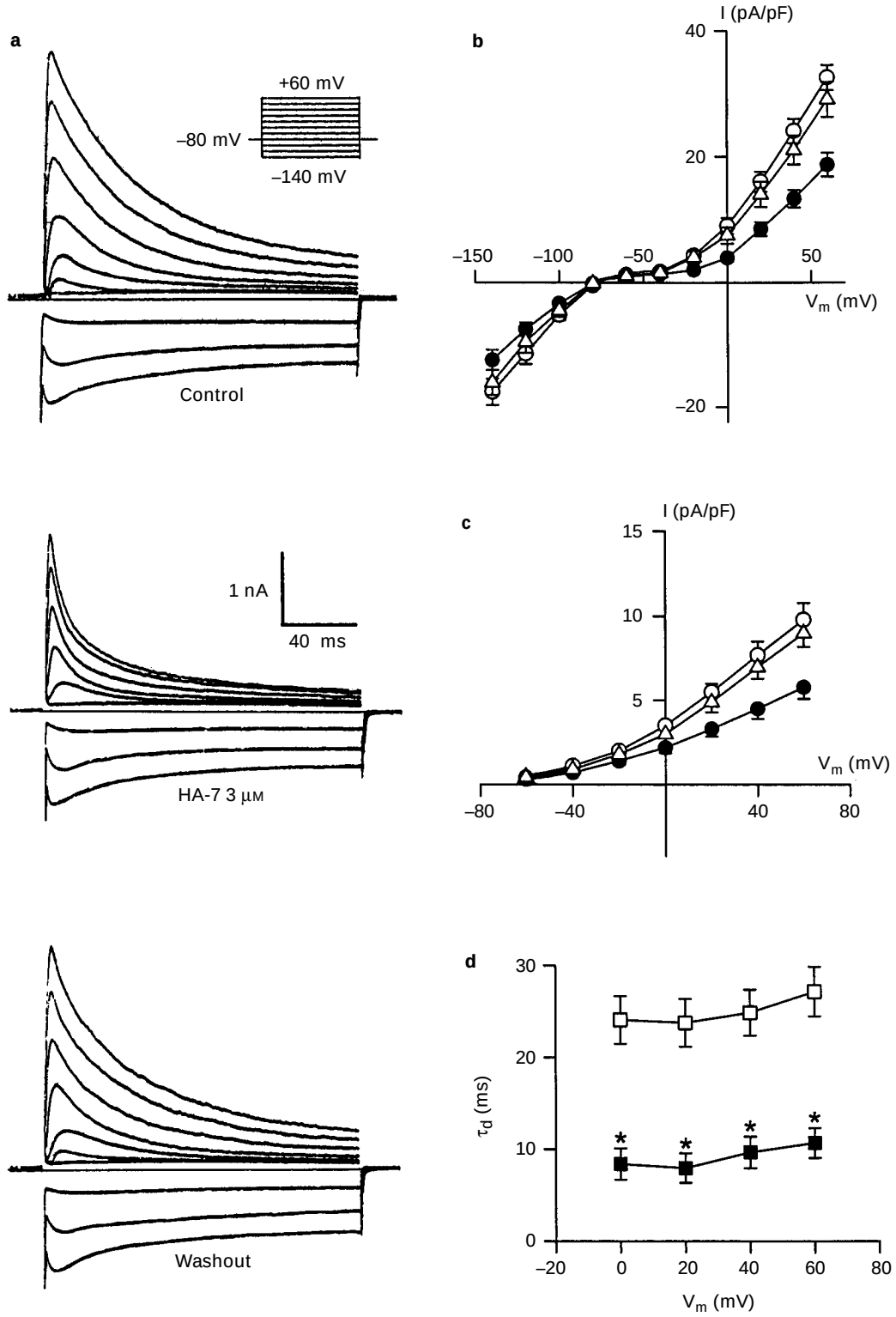


Figure 7 Effects of HA-7 on K^+ currents recorded in rat isolated ventricular myocyte. (a) Family of current traces elicited by a series of 160 ms long depolarizing and hyperpolarizing pulses from a holding potential of −80 mV in the absence and presence of 3 μM HA-7 and following washout of the drug. Horizontal line in each panel indicates zero current level. (b) and (c) Averaged I-V relation for I_{to} and I_{K1} (panel b) and I_{ss} (panel c) in the absence (○), and presence of 3 μM HA-7 (●) and after washout (△). Data points are means ± s.e.mean (n = 10 both in panel b and c). (d) Time constant of decay (τ_d) of the I_{to} vs membrane potential, before (□) and after (■) 3 μM HA-7. Data points are means ± s.e.mean (n = 10).

Table 3 Kinetic parameters of steady-state inactivation and recovery from inactivated state of I_{to} channel under the influence of HA-7

	n	*Control*	*HA-7 (μM)* *0.3*	*1*	*3*
τ_d (ms)	10	27.2±2.6	22.4±3.6	16.3±1.7**	10.7±1.6***
V_h (−mV)	9	25.7±2.5	37.8±2.8**	45.7±2.1***	51.9±1.5***
s (mV)	9	7.1±0.4	7.2±0.5	7.0±0.2	7.4±0.6
τ_r (ms)	8	38.1±2.0	39.5±2.1	42.0±2.0	42.5±1.3

τ_d indicates the time constant for decay of I_{to}; V_h and s indicate half-maximal inactivation voltage and slope factor; τ_r indicates the time constant for I_{to} channel recovery from inactivation state. Values are means±s.e. *n* is number of experiments. $^{**}P<0.01$, $^{***}P<0.001$ compared to respective control.

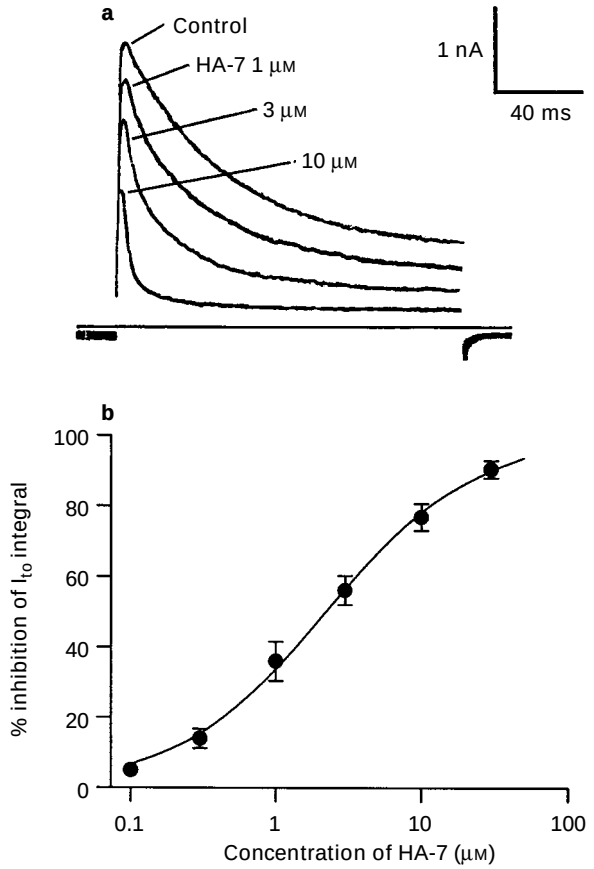


Figure 8 Concentration-dependent inhibition of K^+ currents by HA-7. (a) Superimposed family of current traces produced by 160 ms depolarizing pulses from −80 to +60 mV in the absence and presence of HA-7. Horizontal line indicates zero current level. (b) Concentration–response curve for the inhibition of the I_{to} induced by HA-7 measured as the integral of the first 160 ms of the current elicited by a depolarizing pulse from −80 mV to +60 mV applied once every 30 s. Each symbol is means±s.e.mean for 12 cells at each concentration. The continuous line is drawn according to the fitting of the Hill equation.

range from 0 to +60 mV. This time constant was significantly reduced in the presence of HA-7, but was still independent of membrane potential. The averaged decay time constants of I_{to} at +60 mV from 10 cells in the absence and presence of HA-7 are depicted in Table 3.

The concentration-dependent inhibition of outward K^+ currents by HA-7 is shown in Figure 8. Inhibition of I_{to} was quantified by measuring the integral of the inactivating component of the outward current evoked by a 160 ms depolarization to +60 mV whereas block of I_{ss} was determined from the reduction on the sustained component measured at the end of the depolarizing pulse. The concentration–response curve derived for the inhibition of I_{to} is shown in Figure 8b. The data points were fitted according to the Hill equation with an IC_{50} of 2.9±0.7 μM, and n_H, 0.96±0.05 ($n=12$). Peak I_{ss} decreased by 33±3, 57±3, 80±4 and 91±2% ($n=12$) after 1, 3, 10 and 30 μM HA-7. The calculated IC_{50} value for HA-7 on I_{ss} was 2.5±0.5 μM, $n_H=0.98\pm0.10$ ($n=12$).

In addition to the inhibition of I_{to} and I_{ss}, inward currents through the $I4_{K1}$ channels were also decreased somewhat by HA-7 (Figure 7a and b). The slope conductance measured between the membrane potential −60 and −140 mV after 3, 10, 30 and 100 μM HA-7 was decreased by 24±4, 41±5, 54±8 and 76±1% ($n=13$) respectively. The calculated IC_{50} was 14.5±2.6 μM and n_H, 0.92±0.05 ($n=13$).

Time-dependent inhibition of I_{to}

To test whether the increase in the rate of decay of I_{to} in the presence of HA-7 could be associated with time-dependent block of the open channel, the magnitude of current inhibition at various times after the initiation of the depolarizing pulse was determined. Figure 9a, which was derived from Figure 8a, shows the development of I_{to} inhibition by HA-7 during depolarization. The reduction of I_{to} in the presence of 1, 3 and 10 μM HA-7, expressed as a proportion of the control current, was plotted as a function of time after the start of depolarization. The data clearly show time-dependent increases in inhibition which are particularly evident with the highest concentration of HA-7 which was used (10 μM, top trace of Figure 9a). The rate of block of I_{to} at several different concentrations of HA-7 was described semi-quantitatively by using the following monoexponential equation:

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

where B_{max} equals maximum block and B equals the amount of block at time t. At 1, 3 and 10 μM HA-7, the maximal fraction of steady-state inhibition at +60 mV were 50.4±2.8, 68.9±2.2 and 97.7±0.6% ($n=9$) and the time constants for the developing block (τ_b) were 11.2±1.6, 8.1±1.2 and 3.8±0.5 ms ($n=9$), respectively. Figure 9b shows that the relation between $1/\tau_b$ and the concentration of HA-7 was well described by the following linear function:

$$1/\tau_b = k_{+1}[D] + k_{-1}$$

where k_{+1} and k_{-1} are the association and dissociation rate constants for the drug. The fitted parameters: k_{+1} and k_{-1}, as estimated by least-square analysis, were $24.0\pm2.9\ \mu M^{-1}\ s^{-1}$ and $71.2\pm12.5\ s^{-1}$, respectively; the equilibrium dissociation constant ($K_d=k_{-1}/k_{+1}$) for the inhibition of I_{to} by HA-7 was about 3.0 μM.

Effects of HA-7 on steady-state inactivation and recovery from inactivation of I_{to}

The voltage-dependence of steady-state inactivation of I_{to} was evaluated by using a conventional two-pulse protocol (200 ms conditioning pulses to varying potentials followed by a 200 ms test pulse to +50 mV, see Figure 10a(ii) inset). The resulting steady-state inactivation curves were fitted to the Boltzmann equation. As shown by the graph in Figure 10a(ii) and Table 3,

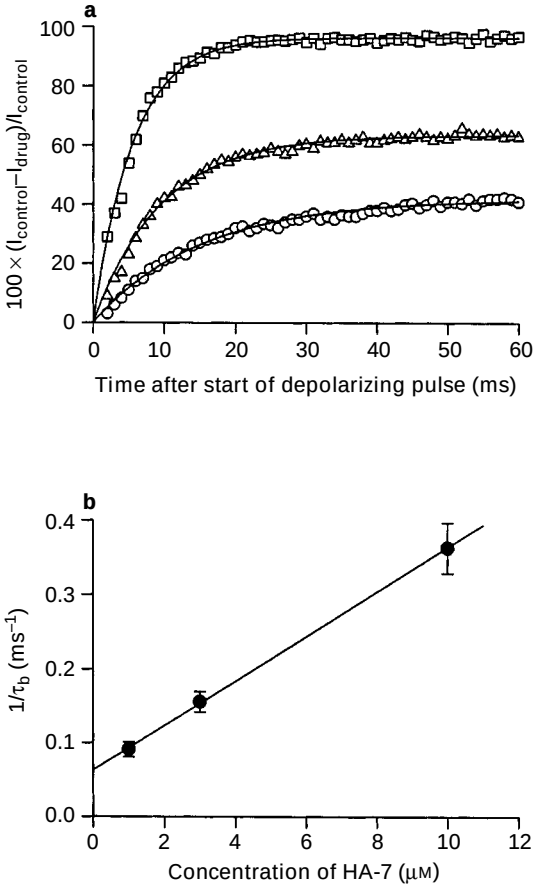


Figure 9 Time-dependent inhibition of I_{to} by HA-7. (a) Time course of the development of inhibition of I_{to} by 1 (○), 3 (△) and 10 μM (□) HA-7 after a depolarizing pulse to +60 mV from a holding potential of −80 mV (derived from the original traces of Figure 8a under the conditions where the noninactivating component of the outward current has been subtracted). The onset of block was well fitted by a one-exponential equation and is indicated in the figure by a smooth line through the points. The time constant for the onset of block (τ_b) was 15.2 for 1 μM, 10.1 for 3 μM and 5.6 ms for 10 μM HA-7. (b) Rate constant for the block of I_{to} by HA-7. The reciprocal of τ_b is plotted against the drug concentration. The line is regression fit of the equation, $1/\tau_b = k_{+1}[D] + k_{-1}$ to the data (see text for details). Each point is means ± s.e.mean for nine cells at each concentration.

exposure to HA-7 produced a marked leftward shift in the voltage dependence of the steady-state I_{to} inactivation. The time dependence of I_{to} recovery from inactivation was measured using a paired pulse protocol applied every 10 s (Figure 10b). Identical clamp steps of 200 ms duration were applied from a holding potential of −80 mV to +50 mV, and were separated by a variable time interval from 10 to 600 ms (see inset of Figure 10b(ii)). The time course of recovery from inactivation could be well fitted by a single exponential process. The recovery of I_{to} from steady-state inactivation was not changed significantly by the exposure of the myocytes to HA-7 (Figure 10b(ii) and Table 3).

Antiarrhythmic efficiency on reperfusion arrhythmias

At a concentration of 1–30 μM, HA-7 consistently converted a polymorphic ventricular tachyrhythmia induced by ischaemia-reperfusion experiment model (Figure 11a and b). In the control heart preparation, following 20 min occlusion of left coronary artery, reperfusion elicits arrhythmias within 5–10 s which was maintained for about 92.4 ± 6.6 min ($n = 12$). Ventricular tachycardia (VT) (defined as continuous run of ventricular extrasystoles) is usually observed and is often followed by ventricular fibrillation (VF) (defined as a total irregularity of morphology of the repetitive rapid ventricular beats). Both of these two type arrhythmias are described as polymorphic ventricular tachyrhythmia. Figure 11a shows a typical trace for the conversion of ventricular tachyrhythmia induced by reperfusion after a 20 min occlusion period to normal sinus rhythm by 3 μM HA-7. Out of 15 episodes of ventricular tachyrhythmia induced by ischaemia-reperfusion, HA-7 1 μM converted the tachyrhythmia to normal sinus rhythm in one instance, 3 μM converted seven of the remaining 14 episodes, 10 μM converted four of the remaining seven episodes and 30 μM converted two of the other three episodes. However, for the one episode of refractory tachyrhythmia, 50 μM of HA-7 failed to convert the arrhythmia. The concentration–response curve for arrhythmia conversion showed an EC_{50} of 2.8 μM (Figure 11b). No new tachyrhythmias upon the perfusion of HA-7 for about 1.5–2 h were observed during the experiment.

Discussion

In the present study we have demonstrated positive inotropy as well as antiarrhythmic efficacy of HA-7, a new synthetic furoquinoline derivative. In rat heart this antiarrhythmic action is mediated mainly through blockade of the I_{to}, I_{ss} and Na^+ channels, although currents through the I_{K1} and L-type Ca^{2+} channels are also altered somewhat. These actions are similar to those found for other class I and class III antiarrhythmic agents (Castle, 1990; Dukes *et al*., 1990; Beatch *et al*., 1991; Clark *et al*., 1995), but are unique in the relatively potent I_{to} suppression, safety margin of I_{K1} suppression and minimal inhibition on I_{Ca}.

Our results show that the extent of Na^+ channel blockade by HA-7 is strongly correlated with the observed decrease in the action potential upstroke velocity. Most class I antiarrhythmic agents cause a use-dependent inhibition of I_{Na}, a negative shift of the voltage-dependent inactivation curve of I_{Na} and a slower recovery of Na^+ channels from their inactivation state (Carmeliet & Saikawa, 1982; Sanchez-Chapula *et al*., 1983; Vaughan Williams, 1984; Gilliam *et al*., 1989; Grant & Wendt, 1992; Nattel, 1993). HA-7 exhibits somewhat similar block of the Na^+ channels in rat ventricle. The HA-7 block is voltage-dependent, as evidenced by a shift of the steady-state Na^+ channel inactivation curve to more negative potentials. The shift of the inactivation curve could result from a higher affinity of HA-7 for the inactivated state of Na^+ channels rather than the rested state (Hondeghem & Katzung, 1977). Under the modulated receptor hypothesis (Hille, 1977; Hondeghem & Katzung, 1977), the affinity of the receptor of the drug is modulated by the channel state, rested, activated and inactivated states. In this scheme it is possible to estimate the relative affinities of drugs for the rested and inactivated states from the concentration dependence of the shift in the midpoint of the inactivation curves (Bean *et al*., 1983). The shift in the midpoint, $\triangle V_h$, is related to the relative affinities of the rested and inactivated states according to the following equation:

$$\Delta V_h = s \times \ln[(1 + C/K_r)/(1 + C/K_i)]$$

where s is the slope factor, C is the drug concentration, and K_r and K_i are the apparent dissociation constants for the rested and inactivated states, respectively. From the negative shift of V_h obtained with two concentrations of HA-7 (3 and 10 μM, Figure 5a and Table 2), the values of K_r and K_i were calculated to be 20.3 and 1.2 μM, respectively. The difference between the dissociation constant for the rested and inactivated state suggests that HA-7 binds preferentially to the inactivated Na^+ channel, which results in a decrease of the available Na^+ channels in steady-state condition. In view of such a high affinity of HA-7 for inactivated Na^+ channels, the drug may depress excitability and conduction in cardiac tissues, especially under pathological conditions associated with rapid heart beats and partially depolarized tissues.

The slowed rate of recovery of Na^+ channel from inactivation provides additional evidence that HA-7 can interact with inactivated Na^+ channels. The insignificant change in the fractional amounts of the fast and slow recovery phases of I_{Na}, and the prolongation of both τ_f and τ_s by HA-7 suggests that the drug may have similar affinity for inactivated Na^+ channels during both in fast and slow recovery state (Brown *et al.*, 1981; Follmer *et al.*, 1987). The slow repriming of I_{Na} by HA-7 leads to incomplete recovery of Na^+ channels from inactivation during short diastolic intervals and could contribute to use or frequency-dependent block of this agent. As the time course of I_{Na} decay at -20 mV was not accelerated by HA-7, the rate of transformation of channels from open to inactivated state may be unaffected by HA-7 and the binding of HA-7 to channels in open state may be insignificant or not fast enough to affect the time course of I_{Na} inactivation.

The present study has shown that HA-7 can prolong the action potential duration in rat ventricular myocytes. This

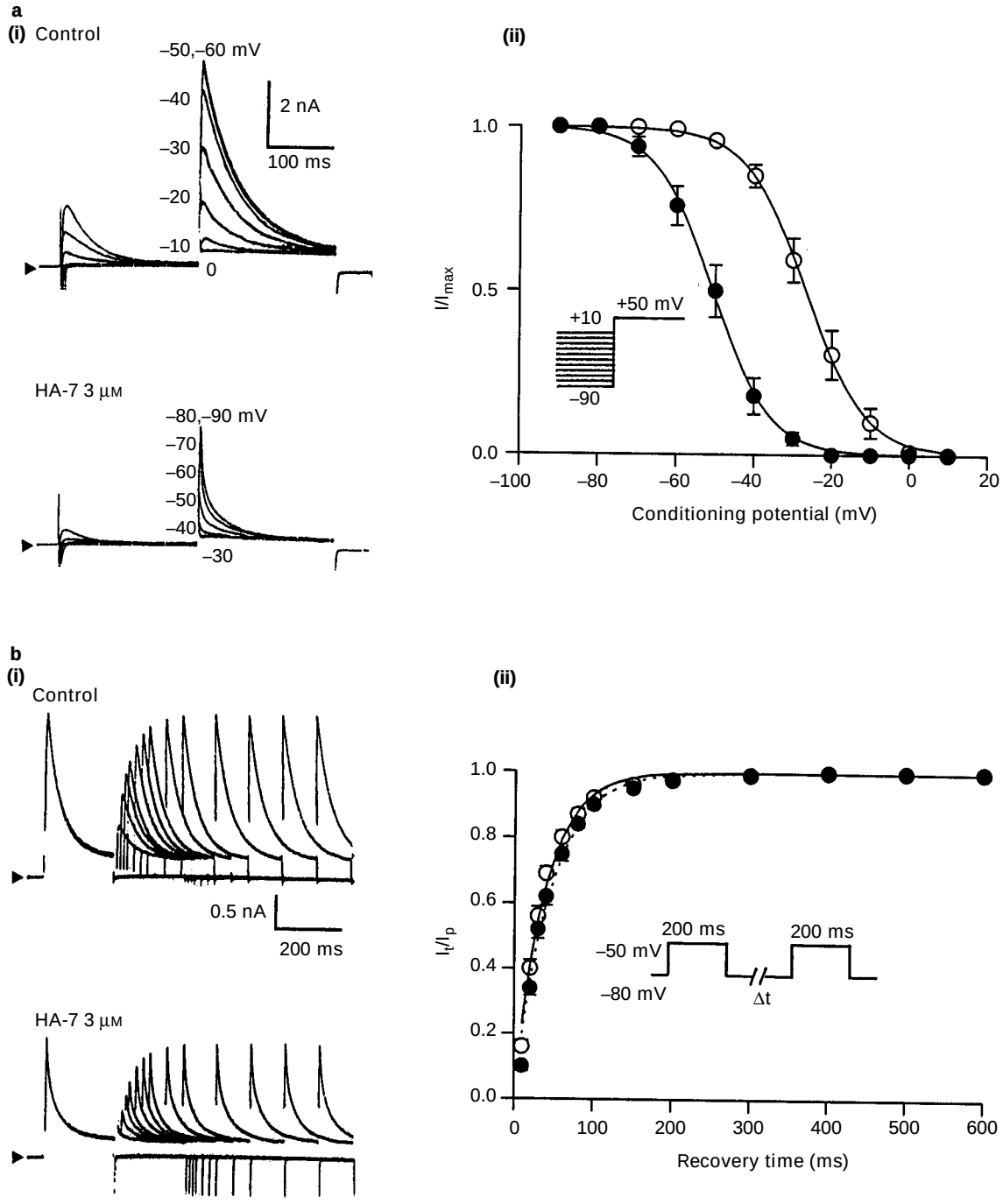


Figure 10 (a) Effects of HA-7 on the voltage-dependence of steady-state inactivation of I_{to}. In panel (i), outward currents were evoked during 200 ms depolarizations to +50 mV after 200 ms conditioning pulses to potentials between -90 and +10 mV from a holding potential of -80 mV in the absence (upper panel) and presence (lower panel) of 3 μM HA-7. In panel (ii), steady-state inactivation curves in the absence (○) and presence (●) of 3 μM HA-7. Peak currents were normalized to the test pulse current amplitude after a prepulse to -90 mV minus the current amplitude after a prepulse to +10 mV. The data were fitted using the Boltzmann equation. Data points are means ± s.e.means from nine cells. (b) Effects of HA-7 on reactivation of I_{to}. In panel (i), tracings show examples of HA-7 on the reactivation of I_{to}. The reactivation curve was generated by the protocol shown in the inset of panel (ii) and consisted of two identical depolarizing pulses to +50 mV for 200 ms from a holding potential -80 mV. In panel (ii), the peak of I_{to} induced by the second pulse was measured and normalized to the first pulse I_{to} and plotted as a function on interpulse interval. The continuous line and dashed line through the data points resulted as the best fits from a single exponential equation in the absence (○) and presence of 3 μM HA-7 (●), respectively. Data points are means ± s.e.means from eight cells.

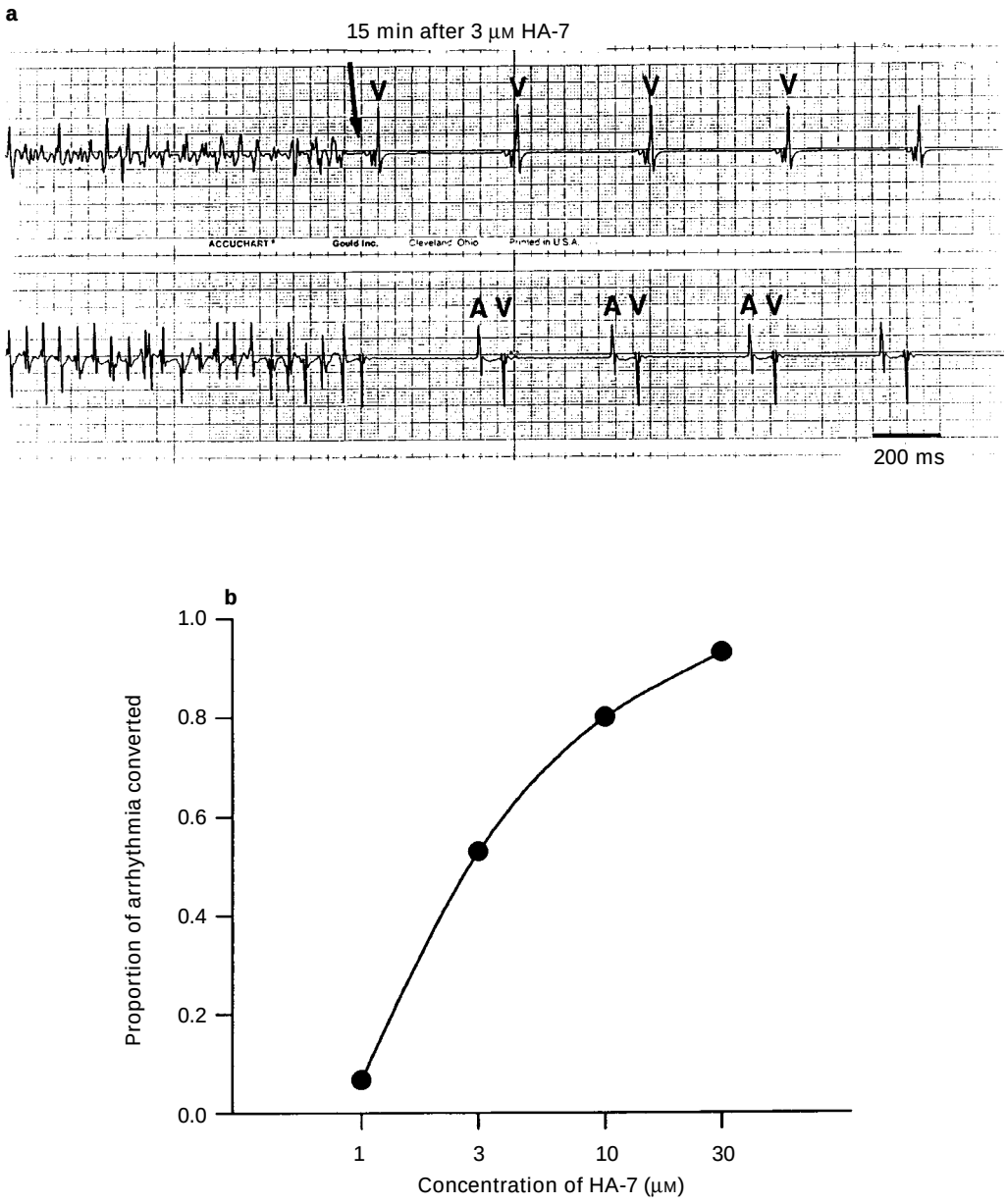


Figure 11 (a) Conversion of polymorphic ventricular tachyrhythmia induced by ischaemia-reperfusion to normal sinus rhythm by 3 μM HA-7. Upper panel shows the ventricular electrogram. The electrogram of the lower panel was recorded at lower right atrium and shows the atrial (A) and ventricular depolarization (V). The paper speed was 100 mm s^{-1}. (b) Antiarrhythmic efficiency of HA-7. The proportion of arrhythmias converted by HA-7 at various concentrations is plotted against the drug concentration.

action of HA-7 is associated with an inhibition of three distinct classes of K^+ channels. Apkon & Nerbonne (1991) have provided evidence that I_{to} is responsible for the initial fast phase of repolarization in rat ventricle, where I_{ss} is a major contributor to the late slower phase of repolarization. The concentration of HA-7 needed to prolong the action potential is pretty close to that required to block I_{to} and I_{ss}, suggesting that inhibition of both of these two currents may contribute to action potential prolongation. The apparent increase in the rate of I_{to} inactivation induced by HA-7 most probably reflects block of open channels. Such I_{to} inhibition is similar to that induced by quinidine (Clark *et al.*, 1995), bupivacaine (Castle, 1990) and SL-1 (Chang *et al.*, 1995). However, a drug-induced acceleration in the conversion of open channels to the inactivated state cannot be completely ruled out. As compared with agents such as quinidine which was known to accelerate I_{to} decay in a potential-dependent manner (Clark *et al.*, 1995), we found that HA-7 accelerates I_{to} decay in a potential-independent manner over the potential range from 0 to +60 mV. In view of the rate of developing block of I_{to} at several different concentrations of HA-7, the apparent equilibrium dissociation constant (K_d) to open channels was calculated to be 3.0 μM, which was similar to the IC_{50} value derived from the concentration–response curve for inhibition of I_{to}, measured as the integral of the current change at +60 mV. In addition, the extrapolation to zero time shows that HA-7 did not significantly block I_{to} channel before its activation by the depolarization. This suggests that HA-7 does not interact with the resting state of the I_{to} channel. Furthermore, HA-7 produced a marked hyperpolarizing shift in the voltage-dependent steady-state inactivation curve of I_{to} concentration-dependently. Hence, HA-7 may also bind to the inactivated channels which could then result in a decrease of number of resting I_{to} channels available for activation. The finding that HA-7 did not change the recovery time constant of I_{to} suggests that the rate of recovery from I_{to} block by HA-7 upon repolarization will not be slower than the rate of recovery from inactivation.

It is well known that transient coronary artery occlusion can lead to malignant ventricular arrhythmia during ischaemia and

reperfusion (Penkoske *et al*., 1978; Janse & Kléber, 1981; Ferrier *et al*., 1985). The mechanism(s) underlying the genesis of these lethal arrhythmias (ventricular tachycardia and ventricular fibrillation) are complex but may include the reentry and the oscillatory afterpotentials (Manning & Hearse, 1984; Ferrier *et al*., 1985; Pogwizd & Corr, 1987). In this study, the concentration range studied for the antiarrhythmic efficiency of HA-7 was about that of $\dot{V}_{max}$ suppression and the Na^+ channel blockade. These results suggest that HA-7 may exert antiarrhythmic activity mainly by suppressing oscillatory afterpotentials or extrasystole by blocking the Na^+ channels, as do the class I antiarrhythmic agents (Sanchez-Chapula *et al*., 1983; Li & Ferrier, 1991).

Some class I and class III antiarrhythmic agents are well known to exert part of their antiarrhythmic effects by suppressing K^+ outward currents, which prolongs the APD and refractoriness of conduction system (Gibson & Kersten, 1990; Lynch *et al*., 1992; Clark *et al*., 1995). I_{to} is one of the predominant repolarizing currents in the action potential of several mammalian cardiac tissues, including human atrium and ventricle (Escande *et al*., 1987; Näbauer *et al*., 1993) and in rat and rabbit ventricular myocytes (Josephson *et al*., 1984; Giles & Imaizumi, 1988). The potential importance of the I_{to} in the origin of ventricular arrhythmias 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 also been shown to be 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 phase 2 re-entry (Litovsky & Antzelevitch, 1988; Clark *et al*., 1993; Di Diego & Antzelevitch, 1994). The finding that blockers of this current (such as tedisamil) can reduce the incidence of ischaemia-induced extrasystolic activity suggests that compounds which block the I_{to} could exert an antiarrhythmic effect (Beatch *et al*., 1991). In the present study, the fact that the extent of HA-7 induced prolongation of APD was attenuated by an I_{to} channel blocker 4-AP together with the voltage clamp experiments which have demonstrated that HA-7 produced a significant inhibition of I_{to}, suggesting that the prolongation of APD by HA-7 could be attributed to the I_{to} inhibition. Therefore, I_{to} blockade of HA-7 may contribute to the observed antiarrhythmic effects of this drug in the ischaemia-reperfusion model. In addition, we found that HA-7 also blocks I_{ss} significantly with an IC_{50} in the range of 2.5 μM which is very similar to the EC_{50} for the antiarrhythmic effect, indicating that part of the antiarrhythmic effect of HA-7 could be attributed to the blockade of I_{ss}.

The physiological role of I_{K1} involves the stabilization and maintenance of resting membrane potential and repolarization during the last part of the action potential, which is an important prerequisite for complete recovery of the fast I_{Na} (Carmeliet, 1993). As I_{K1} is responsible for a considerable fraction of the outward K^+ current between -40 mV and the resting membrane potential, drugs that inhibit the I_{K1} are more likely to slow the phase 3 repolarization and to increase the diastolic membrane resistance (Arnsdorff, 1989; Colatsky *et al*., 1990). These electrophysiological changes may favour the appearance of new arrhythmias by slowing conduction through inactivation of the Na^+ channels and causing abnormal automaticity (Arnsdorff, 1989; Colatsky *et al*., 1990). However, if the I_{K1} was only modestly decreased, the action potential upstroke could be spread to a greater distance, thereby facilitating the conduction. Additionally, it has been shown that during acute ischaemia and reperfusion selected concentrations of a drug sufficient to reduce but not abolish I_{K1} may delay repolarization, preventing ventricular fibrillation, without producing proarrhythmia (Rees & Curtis, 1993). In this study, HA-7 only modestly decreased the I_{K1} at the concentration range possessing the antiarrhythmic activity and may be an additive mechanism of HA-7 effects.

The HA-7-induced positive inotropy, is not mediated by the release of catecholamines or stimulation of α- or β-adrenoceptors, or inhibition of phosphodiesterase (unpublished results). It could be related to the action potential duration prolongation along with a minimal I_{Ca} suppression. In general, a prolonged APD is known to increase contractile force and this effect is caused by increases SR Ca^{2+} loading and release (Clark *et al*., 1993; Bouchard *et al*., 1995). The increase in SR Ca^{2+} loading is due to enhanced Ca^{2+} influx via 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; Negretti *et al*., 1995). Most antiarrhythmic drugs, especially the Na^+ and Ca^{2+} channel blockers, may exert negative inotropic effects (Schlepper, 1989) which frequently limit their clinical use (Jewitt, 1980). Therefore, such positive inotropy of HA-7 is advantageous especially in dealing with patients with heart failure.

In summary, we have demonstrated an antiarrhythmic efficacy for the newly synthesized furoquinoline derivative, HA-7. This drug exerts mixed class I and class III antiarrhythmic properties. The antiarrhythmic efficacy is linked with positive inotropy. We suggest that HA-7 may be a promising drug for the treatment of cardiac arrhythmias associated with heart failure.

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