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Biphasic effects of haloperidol on sodium currents in guinea pig ventricular myocytes

Lu-feng CHENG, Dong YAN, Subat TURDI, Parhat KERRAM[1]

Department of Pharmacology, School of Pharmacy, Xinjiang Medical University, Urumqi 830054, China

Key words

haloperidol; sodium current; ventricular myocytes

[1] Correspondence to Prof Parhat KERRAM.
Phn 86-991-436-2413.
Fax 86-991-431-4513.
E-mail parhatke@vip.sina.com

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Abstract

Aim: To study the effects of haloperidol on sodium currents (I_{Na}) in guinea pig ventricular myocytes. **Method**: Whole-cell patch clamp technique was employed to evaluate the effects of haloperidol on I_{Na} in individual ventricular myocytes. **Results**: Haloperidol (0.1–3 μmol/L) inhibited I_{Na} in a concentration-dependent manner with an IC_{50} of 0.253±0.015 μmol/L. The inhibition rate of haloperidol (0.3 μmol/L) on I_{Na} was 22.14%± 0.02%, and the maximum conductance was reduced. Haloperidol significantly reduced the midpoints for the activation and inactivation of I_{Na} by 2.09 and 4.09 mV, respectively. The time constant of recovery was increased. The increase in time intervals could only recover by 90.14%±1.4% (n=6); however, haloperidol at 0.03 μmol/L enhanced I_{Na} conductance. The midpoints for the activation and inactivation of I_{Na} were shifted by 1.38 and 5.69 mV, respectively, at this concentration of haloperidol. **Conclusion**: Haloperidol displayed a biphasic effect on I_{Na} in guinea pig cardiac myocytes. High concentrations of haloperidol inhibited I_{Na}, while lower concentrations of haloperidol shifted the activation and inactivation curve to the left. Full recovery of recovery curve was not achieved after 0.3 μmol/L haloperidol administration, indicating that the drug affects the inactivated state of sodium channels.

Introduction

Haloperidol is used to treat certain mental/mood disorders (eg schizophrenia and schizoaffective disorders). In 2004, it was one of the 6 most frequently prescribed antipsychotic drugs[1]. However, changes in the electrokardiogram (EKG) pattern (Q-T prolongation[2–4], T wave inversion, and ST segment depression) after haloperidol treatment are common. Excessive prolongation in the Q-T interval may lead to after-depolarization which may result in torsades de points (Tdp)[5]. It was demonstrated that haloperidol decreased inward sodium currents (I_{Na}) in a frequency-dependent manner in cardiac myocytes[6]. Yet another recent investigation revealed that haloperidol had biphasic frequency-dependency on cardiac myocytes, frequency-dependent decrease in intraventricular conduction, and reverse frequency-dependent prolongation of repolarization[7]. The aim of the present study was to examine the effect of haloperidol on I_{Na} in isolated guinea pig ventricular myocytes in order to explore the possible mechanism(s) of drug-induced arrhythmias.

Materials and methods

Myocyte preparation and solutions Guinea pig ventricular myocytes were enzymatically dissociated as described previously[8]. In brief, guinea pigs of either gender (300±20 g) were anesthetized, and the hearts were quickly removed and placed in oxygenated Tyrode's solution. The hearts were mounted on a Langendorff apparatus and perfused with 37 °C oxygenated Tyrode's solution containing (in mmol/L): 140 NaCl, 5.4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 0.33 NaH_2PO_4, 10 glucose, and 10 HEPES (pH adjusted to 7.4 with NaOH). Then after perfusion with Ca^{2+}-free Tyrode's solution for 5–10 min, the hearts were digested with solution containing 0.5 mg/mL collagenase (type II, Worthington, Lakewood, New Jersey, USA) and 1 mg/mL bovine serum albumin (BSA) (Sigma, Saint Louis, Missouri, USA). The isolated myocytes were

stored in high-K^+ media containing (in mmol/L): 10 KCl, 10 KH_2PO_4, 120 potassium glutamate, 10 taurine, 1.0 $MgSO_4$, 20 glucose, and 0.5 EGTA (pH adjusted to 7.2 with KOH) and were used within 8 h of isolation.

The myocytes were placed in a chamber (about 0.3 mL) on an inverted microscope and superfused with solution containing (in mmol/L): 5 NaCl, 135 CsCl, 1 $MgCl_2$, 10 glucose, 0.5 $CaCl_2$, 0.5 $CoCl_2$, and 5 HEPES (pH adjusted to 7.4 with CsOH) at 21–22 °C. Only quiescent, rod-shaped cells showing clear striations were selected for the experiments.

Electrophysiological recording The I_{Na} was recorded by whole cell patch-clamp technique (HEAK EPC-10 plus, HEAK Instruments, D-67466 Lambrecht/Pfalz, Germany). Data were acquired at 50 kHz, and command pulses were generated by PULSE+PULSEFIT (HEAK, D-67466 Lambrecht/Pfalz, Germany). Recordings were low-pass filtered at 5 kHz with an 8-pole Bessel filter (HEAK Instruments, D-67466 Lambrecht/Pfalz, Germany) and stored on hard disk.

Borosilicate glass [1.5 mm outside diameter (OD)] patch pipettes were filled with (in mmol/L) 5 NaCl, 20 CsCl, 110 CsF, 1 $MgCl_2$, 5 HEPES, 5 EGTA, and 5 Mg_2ATP (pH adjusted to 7.2 with CsOH). Junction potentials were compensated before the pipette touched the cell. Series resistance (Rs) and capacitance were electronically compensated; Rs=1.1±0.2 mV (n=30) after compensation. Caution was taken to ensure that the voltage drop across the Rs was <5 mV. Data were discarded if there was evidence of inadequate voltage control.

Statistical analysis A nonlinear curve fitting was done in Clampfit 9.0 (Axon, Sunnyvale, California, USA) and Sigmaplot 8.0 (SPSS, Chicago, Illinois, USA). Paired and unpaired Student's t-tests were used to evaluate differences between 2 means. P<0.05 was considered to indicate significance. Group data are expressed as mean±SEM.

Results

Inhibitory effect of 0.3 μmol/L haloperidol on I_{Na}

Concentration-dependent effect of haloperidol (0.1-3.0 μmol/L) on I_{Na} Under our current experimental condition, the maximum activation voltage for I_{Na} was between -30 and -40 mV. Haloperidol (0.1–3.0 μmol/L) elicited a concentration-dependent inhibition on I_{Na} (Figure 1). I_{Na} elicited by depolarization to -35 mV in haloperidol was normalized by control I_{Na} in the same cell. The inhibition ratios of 0.1, 0.3, 1, and 3 μmol/L haloperidol were 3.94%±0.014% (n=6, P<0.05), 22.14%±0.02% (n=7, P<0.001), 34.97%±0.02% (n=6, P<0.001), and 37.21%±0.10% (n=6, P<0.001), respectively. If administered higher concentrations (10 μmol/L), there was no current appearing even under about -35 mV, which supposed the cell would be dead. So it was supposed that 3 μmol/L haloperidol was the concentration approached the maximum effect. Using the inhibition ratio to plot against haloperidol concentration, the concentration-response curve was fitted by the Hill formulation $\{y=1/[1+(C/IC_{50})^b]\}$. On the basis of cell-by-cell fits, the haloperidol concentration giving a 50% block (IC_{50}) at -35 mV was 0.253±0.015 μmol/L with a Hill coefficient of 2.24 (n=25 cells).

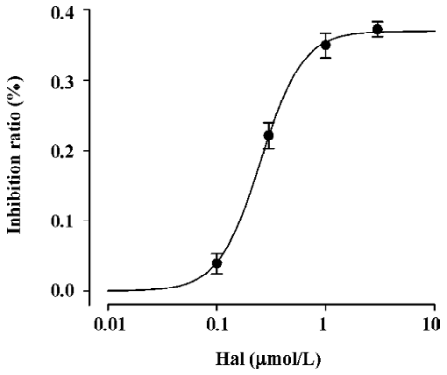

Figure 1. Dose dependence of block of I_{Na}. Inhibition ratio (IR)=$(I_{Na\text{-}Cont}-I_{Na\text{-}Hal})/I_{Na\text{-}Cont}\times100\%$, test potential (TP)=-35 mV, holding potential=-130 mV. Data fitted cell-by-cell to the following equation: $IR/IR_{max}=1/(1+[C/IC_{50}]^b)$, where IC_{50} is the concentration giving 50% inhibition, C is the haloperidol concentration, and b is the Hill coefficient. IC_{50} was 0.253±0.015 μmol/L, and b was 2.24 (n=25 cells, 4 concentrations each).

Effect of 0.3 μmol/L haloperidol on I_{Na} As IC_{50} (0.253 μmol/L) was near 0.3 μmol/L, we made 0.3 μmol/L as representative concentration to investigate the changes of various parameters of inhibition effect on I_{Na} by the drug. Families of capacity- and leak-corrected I_{Na} elicited with 40 ms time courses from -130 mV to between -80 and +15 mV of the control and 0.3 μmol/L haloperidol are shown in Figure 2A. Inward currents were substantially reduced by 0.3 μmol/L haloperidol. Taking the test voltage as the X axis and current density (pA/pF) as the Y-axis, current-voltage (I–V) relationship curves are plotted in Figure 2B. The time course of block of I_{Na} by haloperidol at -35 mV and recovery on washout are illustrated in Figure 2C. The block reached a steady state after about 10 min, but recovery was biphasic and incomplete during the 10 min washout period. The block of peak I_{Na} was associated with the slowing of activation and inactivation kinetics. In superimposed traces (Figure 2D), peak times had nearly no changes which reflected no changes of activation kinetics, but inactivation courses were earlier than the control.

Voltage dependence of conductance and availability of I_{Na} before and after 0.3 μmol/L haloperidol treatment

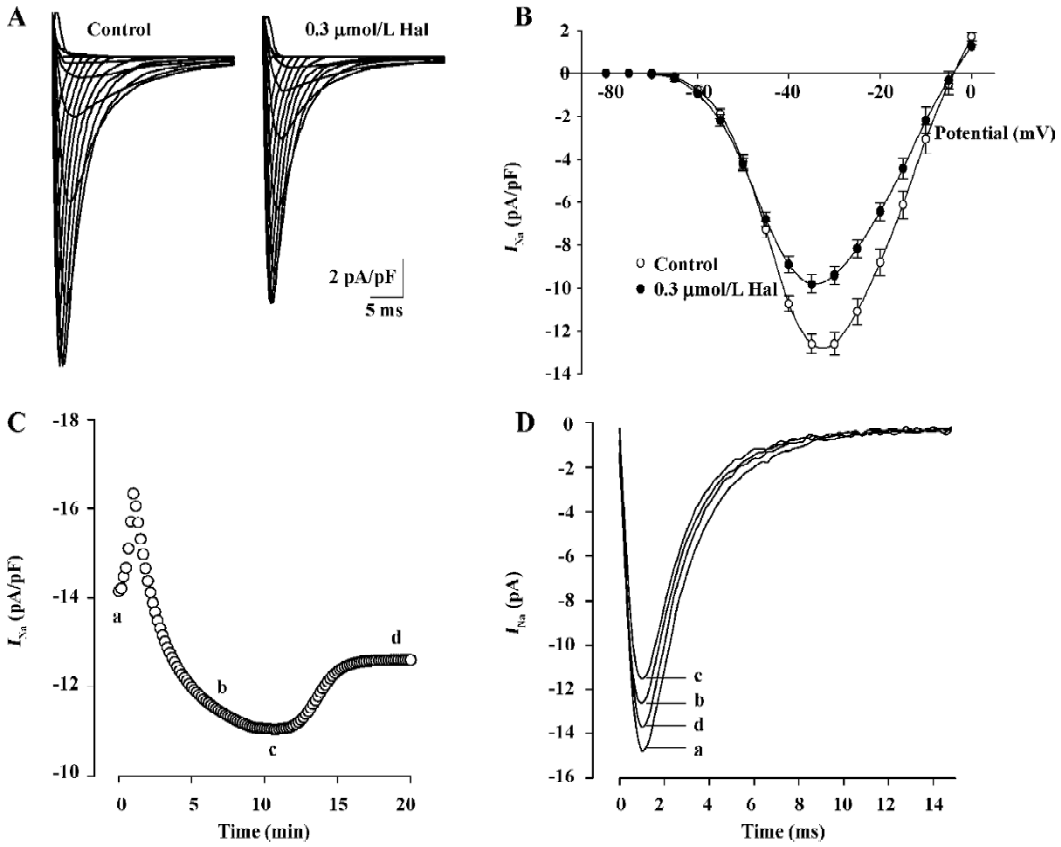

Figure 2. Effect of haloperidol (0.3 μmol/L) on Na^+ current (I_{Na}) in ventricular myocytes. (A) initial current-voltage superimposed traces of control and 0.3 μmol/L haloperidol; 40 ms steps from -130 mV to between -80 and +15 mV at 0.2 Hz. (B) current-voltage relationship curves before and after 0.3 μmol/L haloperidol. (C) time course of block at -35 mV; washout was incomplete. (D) I_{Na} at times (*a*–*d*) indicated in C.

The voltage dependence of the conductance activation variable (g/g_{max}) of I_{Na} was determined from *I–V* relationships for each cell (Figure 3B) and conductance equation: $g=I_{Na}/(V_m-V_r)$ (where I_{Na} is current density, V_m and V_r are membrane potential and reverse potential, respectively) and was fitted to the Boltzmann equation: $y=1/\{1+\exp[(V_m-V_{0.5})/S]\}$ to obtain the voltage for half-activation ($V_{0.5}$) and slope factor (S). The voltage dependence of availability (I/I_{max}) was determined as illustrated in Figure 3A and was also fitted to the Boltzmann equation. Figure 3B shows that 0.3 μmol/L haloperidol shifted the midpoint for conductance and availability of I_{Na} to more negative potentials. The $V_{0.5}$ for activation shifted 2.09 mV, from -41.21±0.50 mV in the control to -43.86±0.47 mV in haloperidol (n=7, P<0.01). The shift of the availability curve was slightly more, 4.09 mV, from -86.11±0.98 mV in the control to -90.20±1.04 mV in haloperidol (n=7, P<0.001). The S values for conductance, which were not significantly altered, were -5.30±0.14 and -5.06±0.18 mV (n=7, not significant) and in contrast, the S values for availability were 5.00±0.11 and 4.67±0.18 mV (n=7, P<0.05) in the control and haloperidol, respectively.

Kinetics of recovery from inactivation The recovery of I_{Na} from inactivation was studied with a paired-pulse protocol. Superimposed currents and the time course of recovery are illustrated in Figure 4. The I_{Na} recovery was not complete (recovery was about 90.14%±1.4%, n=6) and well fitted by monoexponential functions with time constants of 7.84±0.74 ms in the control and 9.20±0.71 ms in 0.3 μmol/L haloperidol (n=6, P<0.01). This indicates that 0.3 μmol/L haloperidol did affect the recovery of I_{Na} from inactivation at hyperpolarized potentials.

Biphasic effect of 0.03 μmol/L haloperidol on I_{Na}

Enhanced effect of 0.03 μmol/L haloperidol on I_{Na} Under lower concentrations of haloperidol (0.01 and 0.03 μmol/L), I_{Na} appeared enhanced effect. The effect was enhanced more obviously in 0.03 μmol/L (by 57.01%±6.35%, n=6, P<0.01). Initial current-voltage superimposed curves before and after 0.03 μmol/L haloperidol treatment are shown

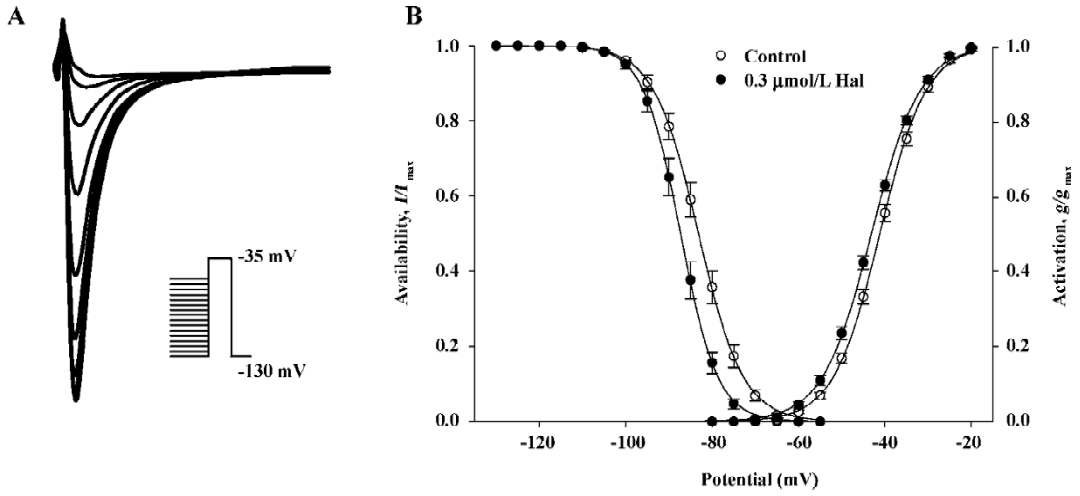

Figure 3. Negative shift of activation and availability. (A) protocol and representative recordings used to assess availability (I/I_{max}). Currents (I) at -35 mV after 1 s conditioning pulses to between -130 and -50 mV with 30 ms square wave were normalized by maximum current (I_{max}). (B) voltage dependence of activation and availability in the control and with 0.3 μmol/L haloperidol (n=7). I/I_{max} and g/g_{max} were fitted to Boltzmann distribution: $y=1/(1+\exp[\{V_m-V_{0.5}\}/S])$, where V_m is the membrane potential, $V_{0.5}$ is the midpoint, and S is the slope. For the activation, $V_{0.5}$ and S were -41.21±0.50 mV and -5.30±0.14 mV in the control, and -43.86±0.47 mV and -5.06±0.18 mV in haloperidol. For the availability, $V_{0.5}$ and S were -86.11±0.98 mV and 5.00±0.11 mV in the control and -90.20±1.04 and 4.67±0.18 mV for haloperidol.

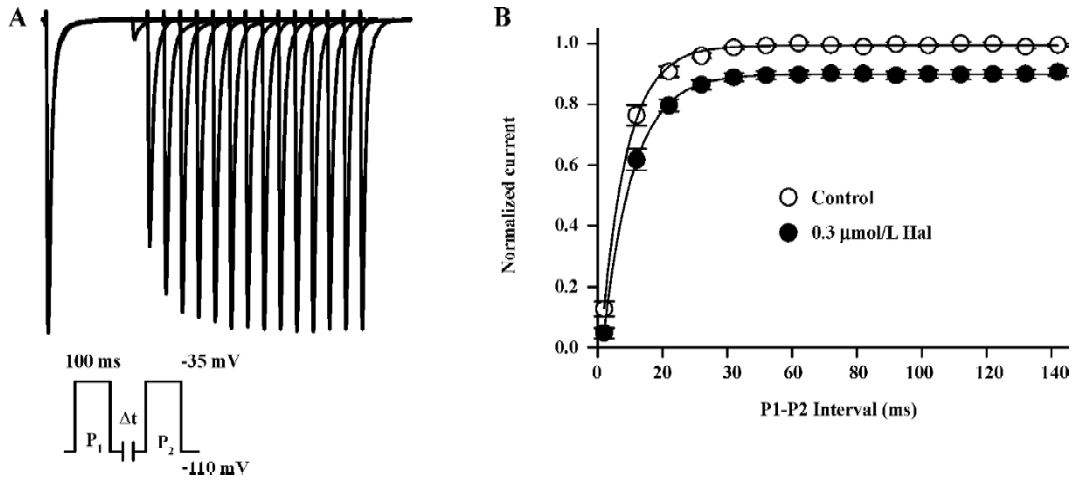

Figure 4. Haloperidol did affect recovery from inactivation. Kinetics of recovery assessed with paired 100 ms pulses (P_1 and P_2) from -110 mV to -35 mV at 0.1 Hz with varying P_1–P_2 intervals (inset). (A) typical currents are superimposed. (B) P_2 current normalized by P_1 current and plotted as the P_1–P_2 interval. I_{Na} recovery was only 90.14%±1.4% (n=6). Recovery was fitted with mono-exponential functions; time constants were 7.84±0.74 ms for the control and 9.20±0.71 ms for 0.3 μmol/L haloperidol (n=6).

in Figure 5A, and the *I–V* curves are indicated in Figure 5B.

Voltage dependence of conductance and availability of I_{Na} by 0.03 μmol/L haloperidol The midpoint for conductance (g/g_{max}) and availability (I/I_{max}) of I_{Na} was shifted to positive potentials by 0.03 μmol/L haloperidol (Figure 5C), in which $V_{0.5}$ for activation increased by 1.38 mV from -44.52±1.56 mV in the control to -43.15±1.48 mV in 0.03 μmol/L haloperidol (n=6, P<0.01); S values were -4.86±0.20 mV in the control and -4.09±0.16 mV in haloperidol (n=6, P<0.01), respectively. The $V_{0.5}$ for inactivation increased by 5.69 mV from -90.02±1.15 to -84.33±1.01 mV (n=6, P<0.01); the S value for inactivation was enhanced from 4.87±0.19 to 6.01±0.21

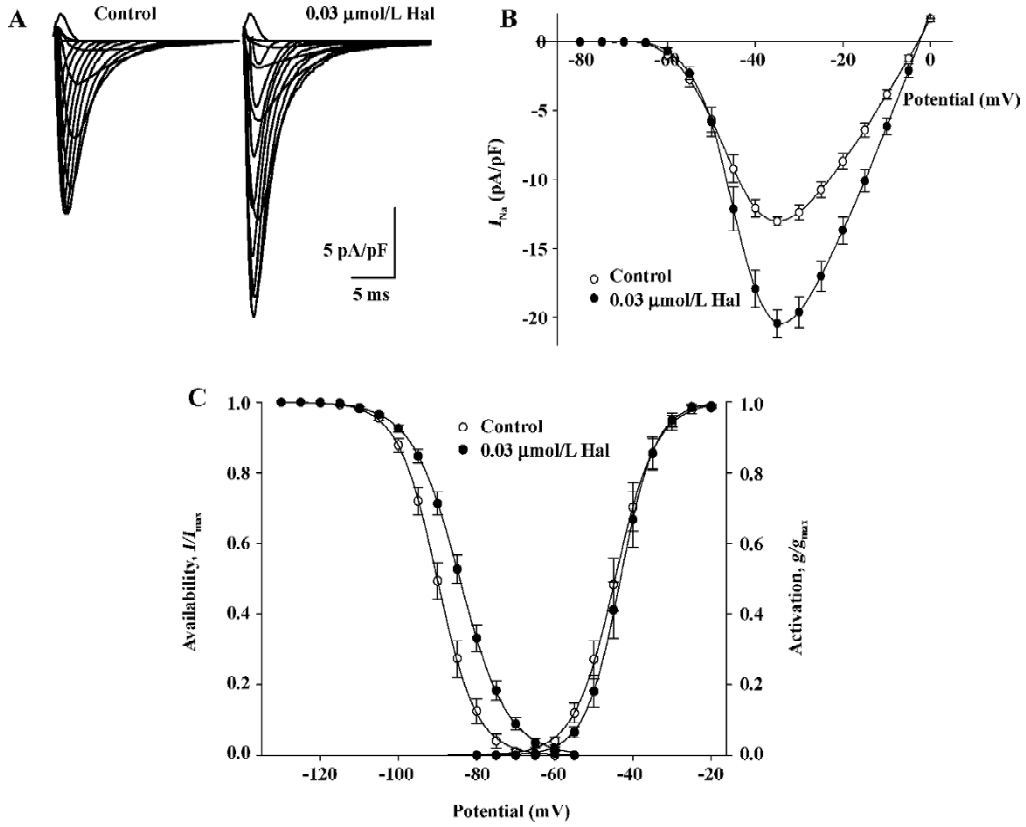

Figure 5. Biphasic effect of 0.03 μmol/L haloperidol on I_{Na}. (A) initial current-voltage superimposed traces of control and 0.03 μmol/L haloperidol; (B) current-voltage curves before and after 0.03 μmol/L haloperidol treatment; (C) voltage dependence of activation and availability in the control and with 0.03 μmol/L haloperidol (n=6). I/I_{max} and g/g_{max} were fitted to the Boltzmann distribution. For the activation, $V_{0.5}$ and S were -44.52±1.56 mV and -4.86±0.20 mV in the control and -43.15±1.48 mV and -4.09±0.16 mV in haloperidol (P<0.01). For the availability, $V_{0.5}$ and S were -90.02±1.15 mV and 4.87±0.19 mV in the control and -84.33±1.01 mV and 6.01±0.21 mV in haloperidol (P<0.01).

mV before and after 0.03 μmol/L haloperidol (n=6, P<0.01).

Discussion

I_{Na} is the major depolarizing current of the working cardiac myocytes. Class I anti-arrhythmic drugs exert their actions by inhibiting I_{Na} in cardiac myocytes. Animal studies have found that haloperidol depresses action potential amplitude and phase 0 maximum upstroke velocity (V_{max}), in which the effects are similar to that of quinidine.

Our study showed that haloperidol (0.1−3 μmol/L) depressed I_{Na} in a concentration-dependant manner (Figure 1), and the results are in accordance with previous studies that show that haloperidol has the same effects on the action potentials of isolated Purkinje fiber and papillary muscles.

A negative shift in the activation/inactivation curves were observed after 0.3 μmol/L haloperidol, where the voltage at both half activation and half inactivation was decreased. We assumed haloperidol caused an early activation and inactivation of sodium channels. According to the modulated receptor theory, the drug mainly affected the inactivated state of sodium channels. A slow recovery of the inactivation curve indicated recovery of sodium channels after inactivation was slow, which is a reflection on the slow unbinding rate of the dissociation of the drug from the channel receptor. After administering 0.3 μmol/L haloperidol, the numbers of sodium channels recovered by 90.14%±1.4% (n=6) and did not completely recover with longer time intervals. This might be because only about 90% of the channels were dissociated after giving 0.3 μmol/L haloperidol and this dissociation rate remained constant as the time for haloperidol exposure was prolonged. Haloperidol delayed the recovery of I_{Na} from inactivation and this explains the negative shift in the inactivation curve[9]. We conclude that haloperidol may have some properties of class I anti-arrhythmic drugs[10].

Paradoxically, haloperidol at lower concentrations (0.01 and 0.03 μmol/L) stimulated I_{Na}. Haloperidol (0.03 μmol/L) caused a positive shift in the inactivation curve and enhanced the kinetic parameters of the sodium channel (Figure 5). We found no similar results in the literature and to our knowledge, report on this biphasic effect of haloperidol for the first time.

The inhibition of sodium channels by haloperidol decreases conductivity and makes the heart more susceptible to the development of unidirectional conduction block. A depressed membrane potential not only decreases V_{max}, but also inhibits further propagation of the impulse and results in re-entrant excitation. However, pathogenesis of fatal arrhythmia is complex. Experimental models of Tdp are usually associated with the induction of early after-depolarization (EAD) and there is a participation of a re-entry mechanism. The ionic basis of EAD is related to activated L-type Ca^{2+} currents during plateau, increased sodium influx, and the inhibition of K^+ efflux via delayed rectified potassium channels. Therefore, we deduce that the stimulation of I_{Na} by low concentrations of haloperidol contributes to the formation of EAD and this may be one of the mechanisms of haloperidol-induced Tdp.

Arrhythmia is associated with both abnormalities in depolarization and repolarization processes. The ionic currents which participate in the repolarization are even more complicated. As part of the investigation, we will conduct further research on haloperidol's effect on other ionic channels as well as their subtypes to elucidate the electrophysiological mechanisms of haloperidol-induced arrhythmia; this may add weight for understanding drug-induced arrhythmias.

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