Biomedicine & Pharmacotherapy 109 (2019) 2348–2356

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

The cardiac electrophysiology effects of higenamine in guinea pig heart

Yuhong Wang[a,1], Jing Geng[b,1], Min Jiang[b], Cong Li[a], Yanxing Han[a], Jiandong Jiang[a,*]

[a] State Key Laboratory of Bioactive Substances and Function of Natural Medicine, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, 100050, China

[b] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, 100050, China

ARTICLE INFO

Keywords:
Higenamine
Cardiac electrophysiology
Action potential duration (APD)
Ion channels
β-Adrenergic receptor

ABSTRACT

Background: Higenamine (HG) is an active compound derived from Aconiti root with a cardiotonic effect. It has been approved by the Chinese SFDA for clinical trials due to its effect as a potent inotropic and chronotropic agent in the heart. However, the direct mode of action of HG on cardiac electrophysiology is unclear.

Methods: The experiments were performed at both cell levels and the isolated organ. The major cardiac ion currents and the action potential duration (APD) were measured using patch-clamps in single guinea-pig left ventricular myocytes. ECG was recorded in isolated guinea pig hearts.

Results: In the left ventricular myocytes, HG increased $I_{Ca\text{-}L}$ and I_{Ks} in concentration- and voltage-dependent manners in the left ventricular myocytes. It potentiated the $I_{Ca\text{-}L}$ and I_{Ks} simultaneously for synchronization. The EC_{50} values were 0.27 μM and 0.64 μM for the $I_{Ca\text{-}L}$ and I_{Ks}, respectively. HG (0.1 μM, 0.5 μM and 1 μM) had no effect on the I_{Kr} and I_{Na}. HG slightly prolonged APD at lower concentrations, and shortened the APD at higher concentrations. HG can induce the delayed after depolarization (DAD), which showed some pro-arrhythmic effect. In the isolated perfused heart, HG increased the heart rate *via* an action on the sinoatrial node cells, but did not induce cardiac arrhythmias, even at high concentrations. The EC_{50} value for the sinoatrial node that controls the heart rate was 0.13 μM. The sinoatrial node cells appeared to be more sensitive than ventricular myocytes to HG. The effects of HG on ventricular cells and sinoatrial node cells were both mediated through stimulation of β1-AR.

Conclusion: We show for the first time that HG produced a predominant action on the sinoatrial node. HG appears to control the cardiac electrophysiology through its predominant effect on the sinoarial node cells, without induction of the ectopic activity that causes cardiac arrhythmias. Thus, HG might be useful for the treatment of bradycardia.

1. Introduction

Aconite has been used as a medicinal herb in Asia for thousands of years, and is prescribed as a principal herb, in combination with other components, to treat patients with heart failure symptoms Higenamine (HG), (1-[(4-hydroxyphenyl) methyl]-1,2,3,4-terahydroisoquinoline-6,7-diol), is an active compound of the Aconiti root [1]. HG can also be found in other plants, such as lotus (Nelumbo nucifera), Gnetum parvifolium, Tinospora crispa and Tinospora cordifolia. Current studies have shown multiple pharmacological effects of HG, including positive inotropic and chronotropic effects, vascular and tracheal relaxation effect, anti-thrombotic effects, anti-oxidative effects, anti-apoptotic effects and anti-inflammatory, or immunomodulatory effects, for which detailed mechanisms have been reported [2]. These beneficial bioactivities have made HG an attractive botanic drug for further study. Because of its positive inotropic and chronotropic effects, HG was approved by the Chinese SFDA for clinical study and its Phase III clinical trial has been completed in China with promising results. Therefore, it has drawn attention as a potent inotropic and chronotropic agent.

The chemical structure of HG shows some similarity to that of catecholamine, and it activates β adrenergic receptors (β-AR) [3]. HG can stimulate β1-AR, mediating the inotropic and chronotropic effects on the heart [4,5]. HG has also been reported to have β2-AR agonist activity [6], reducing cardiac injury and myocytes apoptosis [7]. HG is

Abbreviations: HG, higenamine; APD, action potential duration; DAD, delayed after depolarization; Iso, isoproterenol; CGP20712A, (2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluorometyl)-1H-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide); ICI118551, (1-[2,3-dihydro-7-methyl-1H-inden-4-yl]oxy-3-[(1-methylethyl)amino]-2-butanol)

* Corresponding author at: 1 Xian Nong Tan Street, 100050, Beijing, China.
E-mail address: jiand.jiang@163.com (J. Jiang).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biopha.2018.10.022
Received 11 August 2018; Received in revised form 30 September 2018; Accepted 4 October 2018

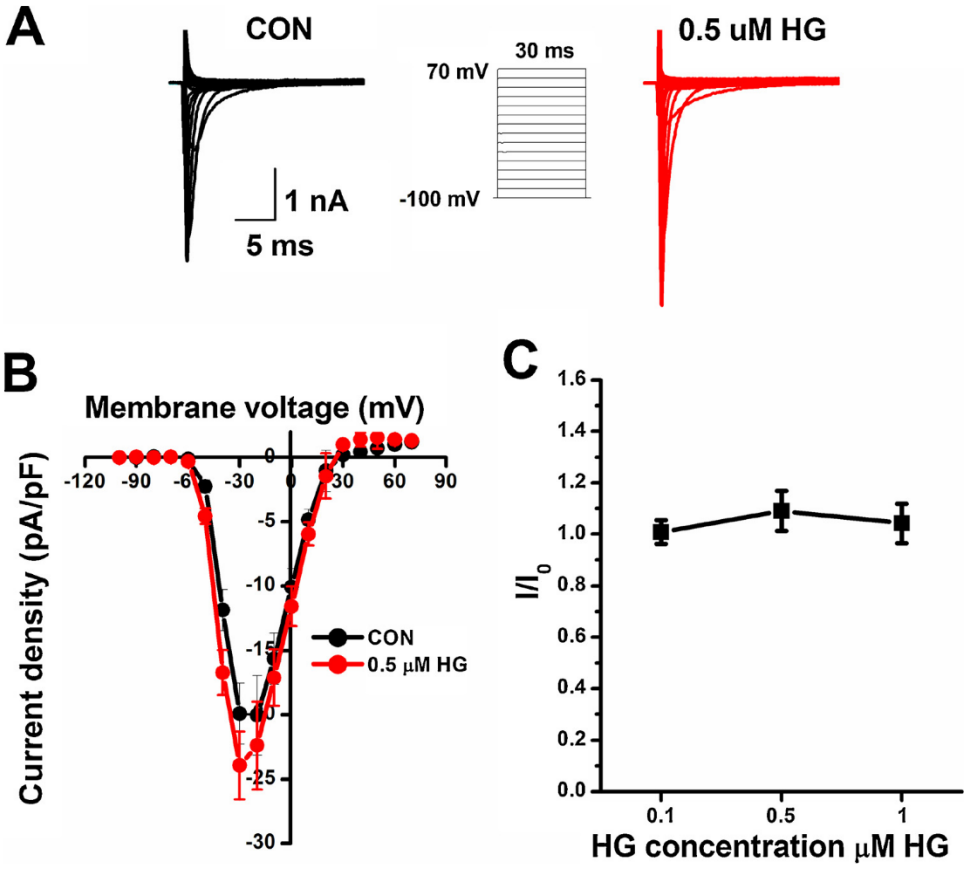

Fig. 1. The effect of HG on I_{Na} in isolated guinea-pig left ventricular myocytes. (A) The representative current trace of I_{Na} activated by a 25 ms voltage step to between -100 mV and 70 mV from a holding potential of -100 mV in a representative ventricular myocytes in the absence and presence of 0.5 μM HG; (B) Voltage-current relationship (IV-curve) of I_{Na} density in the absence and presence of 0.5 μM HG. (C) Summarized data of normalized I_{Na} in the presence of HG (0.1 μM, 0.5 μM and 1 μM) at -20 mV. n = 5 to 8.

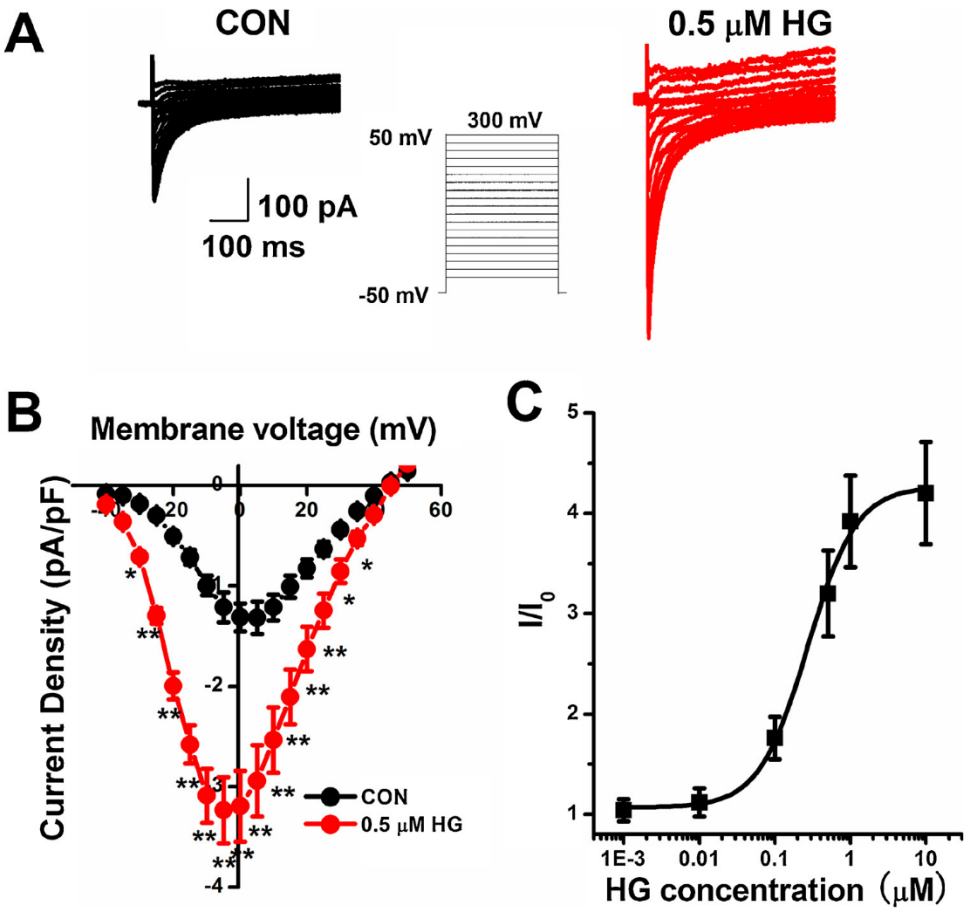

Fig. 2. The effect of HG on $I_{Ca\text{-}L}$ in isolated guinea-pig left ventricular myocytes. (A) The representative current trace of $I_{Ca\text{-}L}$ activated by a 300 ms voltage step to between -50 mV and 50 mV from a holding potential of -50 mV in a representative ventricular myocytes in the absence and presence of 0.5 μM HG; (B) Voltage-current relationship (IV-curve) of $I_{Ca\text{-}L}$ density in the absence and presence of 0.5 μM HG; (C) Concentration-enhancement ratio of $I_{Ca\text{-}L}$ at 0 mV was fitted to Hill equation. n = 5 to 7, *P < 0.05 and **P < 0.01 *vs* CON.

expected to exert positive inotropic and chronotropic effects on the heart, similar to that of isoproterenol (Iso). It is also widely believed that HG has effects on the cardiac electrophysiology similar to that of Iso. However, the effect of HG on cardiac electrophysiology has not been well studied. HG prolonged the action potential duration (APD) of porcine myocardial cells, probably due to an increase in the L-type Ca^{2+} current [8]. Yu et al. [9] observed that HG could heighten Purkinje fibre self-discipline and could shorten the APD of the right ventricular papillary muscle of dogs in an *in vitro* test. It is not clear whether, or not, the effects of HG on cardiac electrophysiology are similar to those of Iso.

In the present study, we investigated the direct effects of HG on cardiac electrophysiology. The effect of HG on the major cardiac ion currents and the APD was examined in single guinea-pig left ventricular myocyte using the patch-clamp technique. The effect of HG on ECG was examined in isolated heart. In addition, the effect of HG was compared with the effect of Iso that was previously reported, from which we found the advantage of HG over that of Iso.

2. Material and methods

2.1. Animals

Animal protocols used in this study were approved by the Laboratories Institutional Animal Care and Use Committee of the Chinese Academy of Medical Sciences and Peking Union Medical College. Hartley guinea-pigs (weight 350–400 g, male) were supplied by Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Animals were housed in SPF-grade rooms and had free access to food and water, with a 12 h light/dark cycle (light on from 8:00 AM to 8:00 PM) at ambient temperature (22–24 °C) and 45% relative humidity. Guinea-pigs were anaesthetized by intraperitoneal injection of 2% pentobarbital sodium (30 mg/kg) and **heparin** (1000 u/ml). When deep anesthesia was achieved, the heart and sufficient length of aorta were excised gently. The heart was used for the following experiments.

2.2. Isolation of single ventricular myocytes

Single ventricular myocytes were enzymatically dissociated from the heart of adult guinea pigs as described in our previous report [10]. In brief, hearts were retrogradely perfused with Ca^{2+}-free modified Tyrode's solution composed of (in mM) NaCl 140; KCl 5.4; $MgCl_2$ 1; HEPES 10, and glucose 10 (pH 7.4 with NaOH). After 5 min of perfusion, the solution was switched to one containing Type II collagenase (0.4 mg/ml, Worthington Biochemical Corporation) and the hearts were removed from the perfusion apparatus after 10–15 min of perfusion. Myocytes were obtained from most of the transmural left ventricular free wall. The myocytes were kept in a high K^+ solution, which contained (in mM) KOH 80, KCl 40, KH_2PO_4 25, $MgSO_4$ 3, Glutamic acid 50, Taurine 20, EGTA 0.5, HEPES 10 and glucose 10 (pH 7.3 with KOH). Cells were then harvested and were used for electrophysiological recording within 6–8 h after isolation.

2.3. Electrophysiological recordings

I_{Na} and I_{Kr} were recorded using the whole cell patch-clamp technique. $I_{Ca\text{-}L}$, I_{Ks} and AP were recorded using the perforated patch-clamp technique, and the patch pipettes were backfilled with amphotericin (500 μg). Borosilicate glass electrodes had tip resistances of 1–3 MΩ when filled with the pipette solution. Uncompensated capacitance currents in response to small hyperpolarizing voltage steps were recorded for offline integration as a means of measuring cell capacitance. All experiments were performed at room temperature (22–23 °C) using an Axopatch 200B amplifier (Axon Instrument, Foster City, CA, USA). The electrical signals were sampled at 2.5–10 kHz and filtered at 1 kHz using a low-pass filter, and were digitized with an A/D converter (Digidata 1322; Axon Instruments). pCLAMP software (Version 8.1; Axon Instrument) was used to generate voltage-pulse protocols, and to acquire and analyse the data.

For I_{Na} recordings in the ventricular myocytes, the external solution contained (in mM) NaCl 20, $MgCl_2$ 1, $CaCl_2$ 1, CsCl 55, CsOH 10, Glucose 10, glucose 10, HEPES 20, TEA-Cl 5 and $CdCl_2$ 0.5 (pH 7.4 with CsOH). The pipette solution contained (in mM) NaCl 5, CsF 135, Mg-ATP 5, EGTA 10, and HEPES 5 (pH 7.2 with CsOH). TEA-Cl (5 mM) was added to the external solution to block the potassium current. $CdCl_2$ (0.5 mM) was added to the external solution to block the L-type Ca^{2+} current.

For $I_{Ca\text{-}L}$ recordings in ventricular myocytes, the external solution contained (in mM) N-methyl-D-glucamine 140, CsCl 5, $CaCl_2$ 2, $MgCl_2$ 0.5, glucose 10 and HEPES 20 (pH 7.4 with HCl). The pipette solution contained (in mM) CsCl 120, TEA-Cl 20, Mg-ATP 4, EGTA 10, and HEPES 10 (pH 7.2 with CsOH).

For potassium channel recordings in ventricular myocytes, the external solution contained (in mM) NaCl 132, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1.2, glucose 5 and HEPES 10 (pH 7.4 with NaOH). The pipette solution contained (in mM) KCl 140, Mg-ATP 4, $MgCl_2$ 1, EGTA 5, and HEPES 10 (pH 7.2 with KOH). Nimodipine (Nim, 1 μM) was added to the external solution to block the L-type Ca^{2+} current. Na^+ and T-type Ca^{2+} currents were inactivated by a holding potential of -40 mV. To record I_{Ks}, I_{Kr} was blocked by the addition of 1 μM Dofetilide. To record I_{Kr}, I_{Ks} was blocked by the addition of 20 μM chromanol 293B.

For the action potential (AP) recordings, the pipette solution contained (in mM) potassium glutamate 120, KCl 25, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10 (pH 7.4 with KOH). The external solution contained (in mM) NaCl 138, KCl 4, $MgCl_2$ 1, $CaCl_2$ 2, NaH_2PO_4 0.33, glucose 10 and HEPES 10 (pH 7.4 with NaOH). Action potentials were evoked at a rate of 0.5 Hz or 1 Hz with supra threshold current pulse of 4 − 6 ms duration applied *via* patch electrodes in the current-clamp mode. The APD was measured at 50% and 90% repolarization (APD_{50} and APD_{90}).

2.4. Langendorff-perfused isolated heart preparations and pseudo-ECG recording

Brief, the hearts of guinea-pigs were quickly removed, and the ascending aorta was then immediately cannulated to a Langendorff perfusion system (Radnoti Working Heart System, Monrovia, CA, USA) with warm normal Tyrode's solution equilibrated with 100% O_2 at a constant flow of 15 ml/min that maintained an initial perfusion pressure of 60–80 mmHg. The composition of Tyrode's solution (in mmol/L) was: NaCl 125, KCl 4.5, NaH_2PO_4 1.8, $NaHCO_3$ 24, $CaCl_2$ 1.8, $MgCl_2$ 0.5, dextrose 5.5, and albumin 50 mg/l in deionized water. A pseudo-electrocardiogram (pseudo-ECG) was recorded by three electrodes placed widely apart with one pole on the aortic root and the other on the ventricular apex; the last one was used for grounding. The hearts were allowed to equilibrate for a minimum of 30 min to ensure their stability over the course of an experiment (up to 4 h of perfusion) when no pharmacological intervention was applied in a pilot study. The pseudo-ECG was filtered using a low pass filter at 100 Hz and a high pass filter at 1–10 Hz, and digitized with the BIOPAC/MP100 system (BIOPAC Systems, Inc., Goleta, CA, USA). ECG parameters were measured from at least 10 consecutive complexes at every experimental time point and averaged valves were analysed. Rate correction of the QT interval was achieved using Bazetts's formula [11].

2.5. Drugs

Higenamine (HG) was from the Institute of Materia Medica Chinese Academy of Medical Sciences (Beijing, China), with a purity over 98%. The other chemicals were bought from the Sigma or Tocris. In some of experiments, the cardiac electrophysiology effects of HG (0.5 μM) were tested in isolated cardiomyocyte and isolated heart in the presence of β-AR blocker propranolol (Pro, 10 μM), or a selective β1-AR antagonist

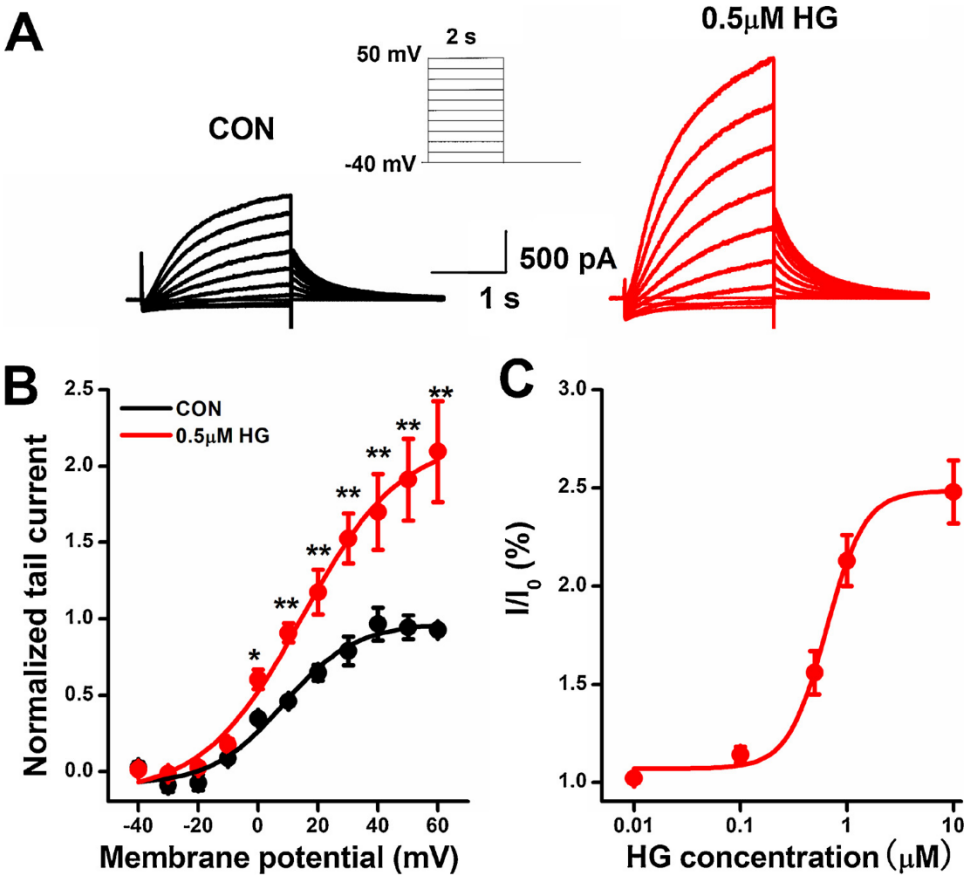

Fig. 3. The effect of HG on I_{Ks} in isolated guinea-pig left ventricular myocytes. (A) The representative current trace of I_{Ks} in a representative ventricular myocytes in the absence and presence of 0.5 μM HG. I_{Ks} tail currents is elicited on repolarization to a test potential of -40 mV after 2 s pre-pulse potentials between -40 mV and 50 mV from a holding potential of -40; (B) Voltage-current relationship (IV-curve) of I_{Ks} in the absence and presence of 0.5 μM HG. Normalized I_{Ks} tail currents were fitted Boltzmann equation; (C) Concentration-enhancement ratio of I_{Ks} for increasing the current at 40 mV was fitted to Hill equation. n = 5 to 7, *P < 0.05 and **P < 0.01 *vs* CON.

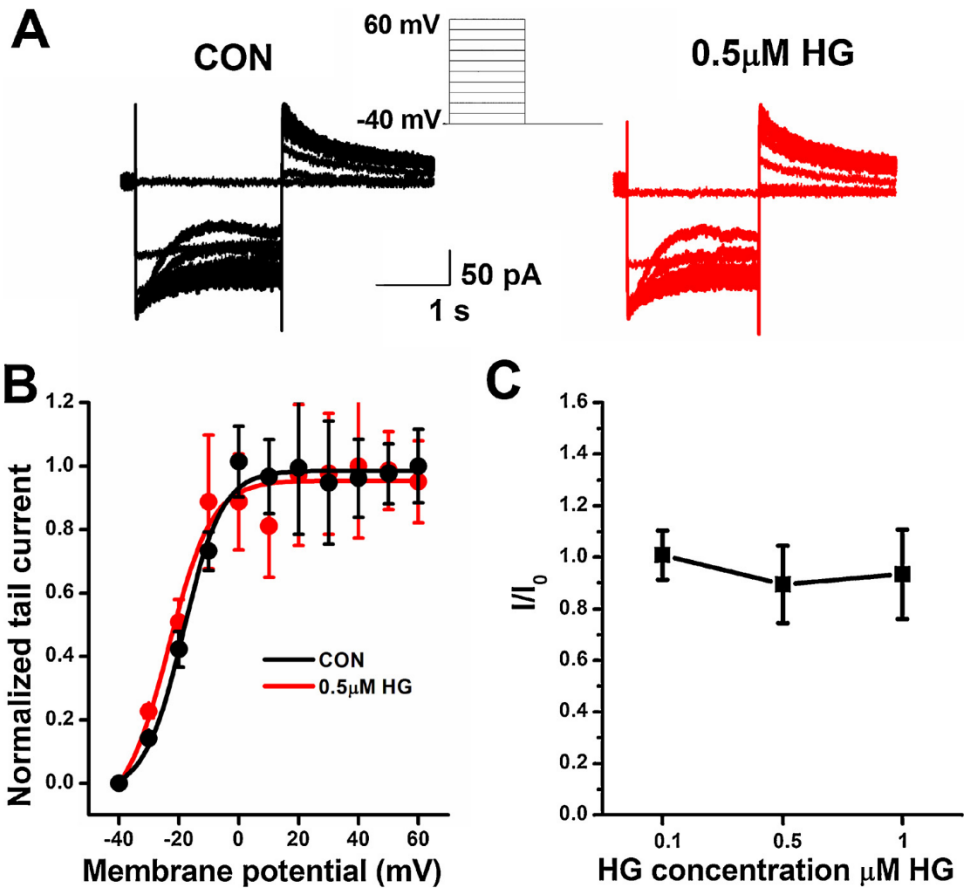

Fig. 4. The effect of HG on I_{Kr} in isolated guinea-pig left ventricular myocytes. (A) The representative current trace of I_{Kr} in a representative ventricular myocytes in the absence and presence of 0.5 μM HG. I_{Kr} tail currents is elicited on repolarization to a test potential of -40 mV after 2 s pre-pulse potentials between -40 mV and 60 mV from a holding potential of -40; (B) Voltage-current relationship (IV-curve) of I_{Kr} in the absence and presence of 0.5 μM HG. Normalized I_{Kr} tail currents were fitted Boltzmann equation. n = 5 cells. (C) Summarized data of normalized I_{Kr} in the presence of HG (0.1 μM, 0.5 μM and 1 μM) at 0 mV. n = 5 to 6.

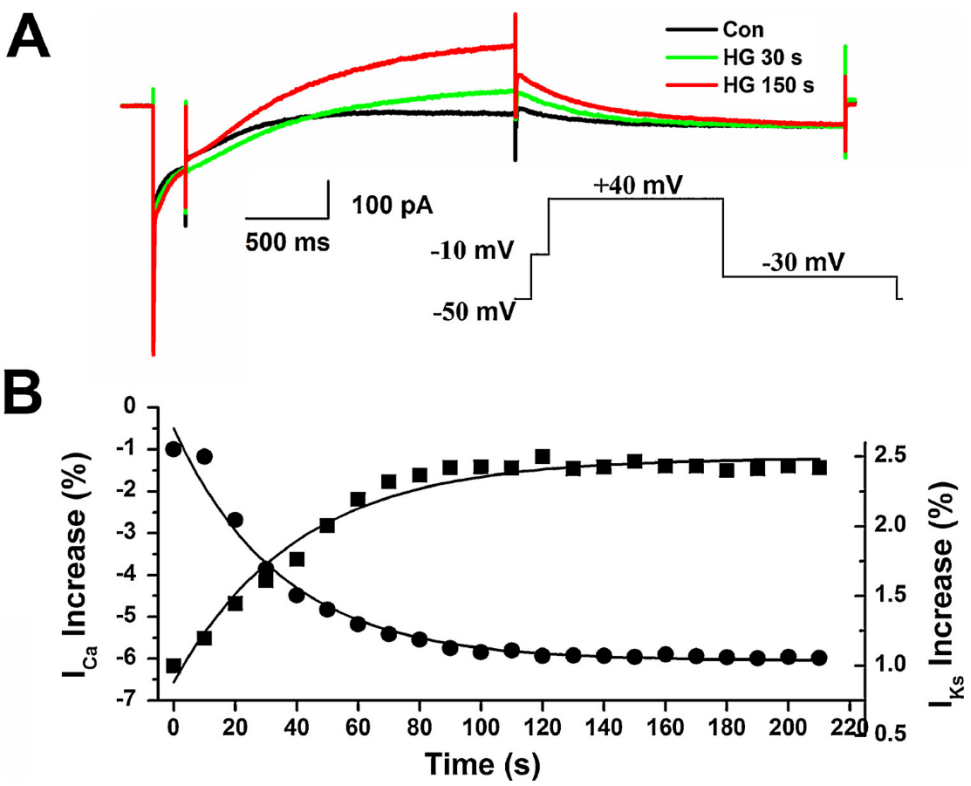

Fig. 5. HG increased both I_{Cal} and I_{Ks} in a time-dependent manner. (A) The representative current trace of I_{Ca} and I_{Ks}that recorded at the same time in a representative ventricular myocytes without and with 0.5 μM HG. Holding potential is −50 mV, voltage first jump to −10 mV for 200 ms to record peak Ca^{2+} current, then further jump to + 40 mV for 2 s to record slow activated I_{Ks}. Tail current of I_{Ks} could be best observed at −30 mV. The pulse was repeated every 10 s following the HG perfusion. Recordings were done in the presence of 5 μM E-4031 to eliminate I_{Kr}. (B) The current is shown as a function of time. The I_{CaL} was measured as peak current at −10 mV. Steady-state I_{Ks} was measured at the beginning of the -30 mV repolarization potential. They could best be fitted with one exponential function. (n = 6).

CGP 20712A (300 nM), or a selective β2-AR antagonis ICI-118,551 (ICI, 100 nM).

2.6. Statistical analysis

The results are presented as the mean ± standard error (S.E.M.). Statistical comparisons were done using the unpaired or paired Student's t-tests. All data were analysed using two-tailed tests and $p < 0.05$ was set as the cut-off for significance level. The EC_{50} value was calculated using the Hill equation to fit the experimental points. The software SPASS (19.0) was used for the calculations.

3. Results

3.1. Effects of HG on the major cardiac currents in left ventricular myocytes

In isolated guinea pig ventricular myocytes, we examined the effect of HG on the major cardiac ion currents, including the sodium channel (I_{Na}), the L-type Ca^{2+} current (I_{Ca-L}), the slowly activating delayed-rectifier potassium current (I_{Ks}), and the fast delayed rectifier potassium channel (I_{Kr}).

We first investigated the effects of HG on inward currents, including I_{Na} and I_{Ca-L}. Fig. 1A shows the representative I_{Na} activated by the voltage protocol as shown in the insert in a representative ventricular myocyte, in the absence or presence of 0.5 μM HG. Ventricular myocyte was depolarized from a holding potential of −100 mV to various test potentials of −100 to 70 mV for 25 ms to evoke inward currents. The relationship between voltage and current was calculated based on the inward peak current density (Fig. 1B). Fig. 1C shows that there was no significant different in the amplitude of I_{Na} recorded in the presence of HG (0.1 μM, 0.5 μM and 1 μM) that compared to the control. Fig. 2A shows the representative I_{Ca-L} activated by the voltage protocol as shown in the insert in a representative ventricular myocyte, with or without 0.5 μM HG. Ventricular myocytes was depolarized from a holding potential of −50 mV to various test potentials of −40 to 50 mV for 300 ms to evoke inward currents. A holding potential of -50 mV inactivated I_{Na}. The I_{Ca-L} current was remarkably increased by 0.5 μM HG. The enhancement of I_{Ca-L} current reached to a stable state within 5 min after addition of HG into the bath, and the effect was completely reversible (data not shown). The IV curves showed that HG markedly increased the inward currents during different depolarization potential (Fig. 2B). The current density of I_{Ca-L} was increased from 1.21 ± 0.15 to 3.24 ± 0.33 pA pF^{-1} at 0 mV ($n = 6$, $P < 0.01$). The percentage of increase in the amplitude of the I_{Ca-L} current elicited on test potentials (0 mV) was calculated and plotted against concentrations of HG (Fig. 2C). The mean data are well described by the Hill equation with an IC_{50} value of 0.27 μM.

The action of HG on outward current including I_{Ks} and I_{Kr} was also examined in the guinea pig ventricular myocytes. Fig. 3A shows the representative voltage-dependent I_{Ks} traces recorded by the voltage protocol as shown in the inset in a representative ventricular myocyte in the absence or presence of 0.5 μM HG. Ventricular myocytes were depolarized from a holding potential of −40 mV to various pre-pulse potentials of −40 to 60 mV for 2 s and were repolarized to −40 mV to evoke outward tail currents in the presence of the I_{Kr} blocker, dofetilide (1 μM). The I_{Ks} tail current was remarkably increased by 0.5 μM HG. The enhancement of I_{Ks} by HG was completely reversible (data not shown). The voltage dependence of the activation was calculated based on the normalized I_{Ks} tail current (Fig. 3B). HG was more potent when increasing the tail current at more positive potentials. The activation curve was fit using the Boltzmann equation. There was no significant shift of $V_{1/2}$ between before and after HG application. The percentage of increase in the amplitude of the I_{Ks} tail current elicited on repolarization to a test potential of −40 mV after a 2-s prepulse potential of 40 mV was calculated and plotted against concentrations of HG (Fig. 3C). The mean data are well expressed by the Hill equation with an EC_{50} value of 0.64 μM. We recorded I_{Kr} tail current like before our previous report [10]. There was no effect of HG (0.1 μM, 0.5 μM and 1 μM) on I_{Kr} in guinea pig ventricular myocytes (Fig. 4).

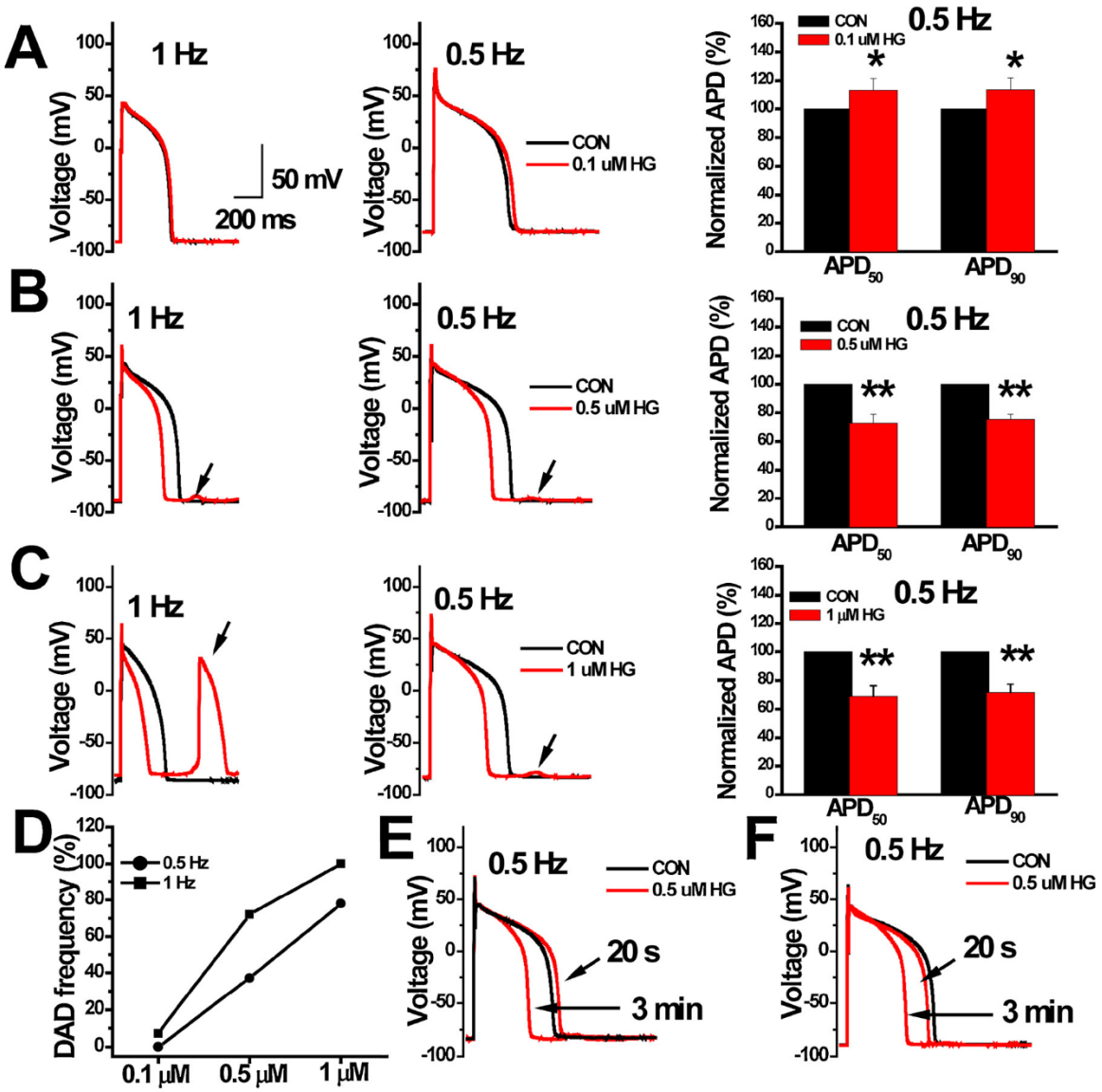

Fig. 6. The effect of HG on the action potential duration in isolated guinea-pig left ventricular myocytes. (A–C) The representative action potential recorded in different condition and the summary of changes of action potential duration; (D) Summary the incidence of DAD in the presence of HG; (E) and (F) the changed of APD after application of HG; 20 s and 3 min refer to the time of HG perfusion. n = 5–7, *P < 0.05 and **P < 0.01 *vs* CON.

3.2. Time-dependent activation of $I_{Ca\text{-}L}$ and I_{Ks} by HG

As described, HG increased $I_{Ca\text{-}L}$ and I_{Ks}, the two antagonizing currents during repolarization in cardiomyocytes. Based on the above results, we found that I $I_{Ca\text{-}L}$ and I_{Ks} had different sensitivity to HG. Here, we investigated time-dependent increases in $I_{Ca\text{-}L}$ and I_{Ks} in the presence of HG (1 μM). Fig. 5 shows that $I_{Ca\text{-}L}$ and I_{Ks}were recorded simultaneously, consistent with the previous report [12], and both $I_{Ca\text{-}L}$ and I_{Ks} increased in a time-dependent manner. Fig. 5A shows the representative traces of $I_{Ca\text{-}L}$ and I_{Ks}. Curve fitting analysis for the $I_{Ca\text{-}L}$ and I_{Ks} with one exponential function resulted in a time-constant of 34.5 s for the $I_{Ca\text{-}L}$ increase and 40.2 s for I_{Ks} increase (Fig. 5B). A similar time course was found between $I_{Ca\text{-}L}$ and I_{Ks} in the increase by HG.

3.3. Effects of HG on the APD in left ventricular myocytes

In mammalian hearts, the APD is determined by the delicate balance of outward and inward currents during the repolarization phase. We examined the effect of HG on the APD in single left ventricular myocytes. HG induced bidirectional effects on the APD. At a stimulation frequency of 0.5 Hz, HG slightly prolonged the APD at low concentrations (Fig. 6A), but significantly shortened the APD and induced the delayed after depolarization (DAD) at high concentrations (Fig. 6B and C, **see arrows**), showing some pro-arrhythmogenic effects. The incidence of inducing DAD by HG was higher at 1 Hz than that seen at 0.5 Hz (Fig. 6D). Fig. 6E shows that HG first prolonged the APD, and then shortened the APD after adding 0.5 μM HG *via* the bath perfusion system. However, HG alone produced the APD shortening effect in the cell-side delivery system (Fig. 6F). These results indicated that the bidirectional effects of HG on the APD was concentration dependent. Although HG markedly affected the APD, it did not influence the amplitude of the action potential and the resting potentials.

3.4. Effects of HG on ECG in isolated heart

The effects of HG on ECG parameters were determined in the Langendorff-perfused isolated guinea-pig hearts. Representative ECG traces are illustrated in Fig. 7A in the absence or presence of HG. HG significantly increased the heart rate by affecting in sinoatrial node cells (Fig. 7B) with an EC_{50} value of 0.13 μM (Fig. 7C). HG shortened the QTc

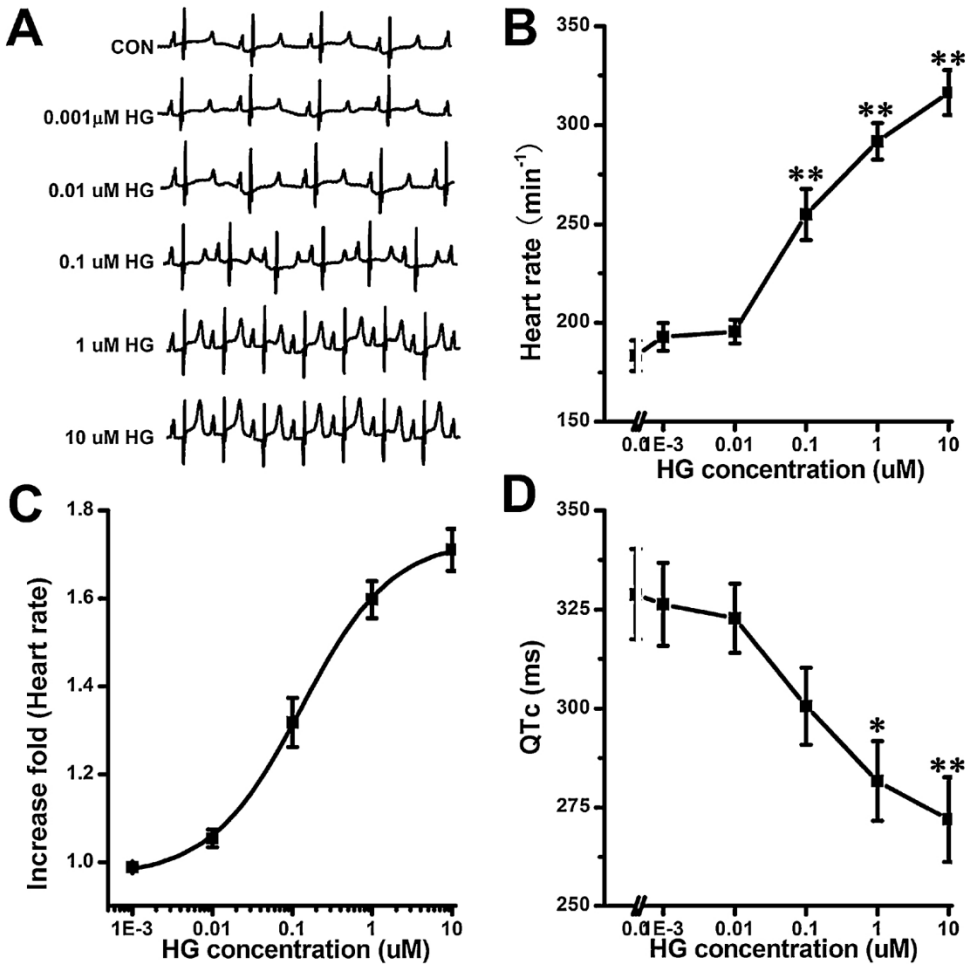

Fig. 7. The effect of HG on ECG in isolated guinea pig perfused hearts. (A) the traces of ECG were recorded in a representative perfused guinea pig heart in absence and presence of 0.001, 0.01, 0.1. 1 and 10 μM HG in a cumulative manner (each concentration for approximately 5 min); (B) Mean values of heart rate interval in different HG concentration; (C) The concentration-dependent relationship curve of heart rate was fitted to Hill equation; (D) Mean values of QTc interval in different HG concentration. n = 6, *P < 0.05 and **P < 0.01 *vs* CON.

interval only when the concentration was over 1 μM (Fig. 7D), and its effect on other ECG parameters was not seen. HG did not induce cardiac arrhythmias even when the concentration was higher (10 μM).

3.5. HG stimulates β1-AR to modulate cardiac electrophysiology

Based on the results above, we realized that the sensitivity to HG was different between ventricular myocytes and sinoatrial node cells. HG can activate β-adrenergic receptors (β-AR) in the heart [3], and exerts inotropic and chronotropic effects by β1-AR [4,5]. To investigate whether, or not, the cardiac electrophysiology response to HG is mediated through β-AR, we determined the effect of HG on the APD in single myocytes and on the heart rate in isolated hearts in the presence of different β-AR blockers. As illustrated in Fig. 8**A** and **B**, pretreatment of ventricular myocytes with the non-selective β-AR blockade propranolol (Pro, 10 μM) almost totally abolished the effect of HG (0.5 μM) on the APD and heart rate (Fig. 8). We then examined the β-AR subtypes that might be involved in the HG-induced effect in the APD and heart rate. The results showed that the β1-AR antagonist, CGP20712 A (CGP, 300 nM), comparably abolished the effect of HG on the APD and heart rate as well. Then, we also assessed the role of β2-AR stimulation in the response to HG. The effect of HG on the APD and heart rate was not affected by ICI-118,551 (ICI, 100 nM, a selective β2-adrenergic receptor antagonist), suggesting that β1-adrenergic stimulation is the primary modulation of HG on the APD and heart rate. Thus, HG might regulate the cardiac electrophysiology mainly through β1-AR activation.

4. Discussion

In this study, we examined the direct effect of HG on cardiac electrophysiology. We found three important points regarding the direct effects of HG on cardiac electrophysiology. First, HG increased $I_{Ca\text{-}L}$ and I_{Ks} in concentration- and voltage-dependent manners, and potentiated the $I_{Ca\text{-}L}$ and I_{Ks} simultaneous synchronization. There was no effect of HG on I_{Na} and I_{Kr}. Second, HG activated $I_{Ca\text{-}L}$, I_{Ks} and heart rate with unidentical EC_{50} values. The sinoatrial node cells were more sensitive to HG compared to ventricular cells. HG significantly increased the heart rate by effects on sinoatral node cells and the EC_{50} value was 0.13 μM. However, the EC_{50} values were 0.27 μM and 0.64 μM for $I_{Ca\text{-}L}$ and I_{Ks}, respectively. Third, the effects of HG on ventricular cells and sinoatrial node cells were both mediated through stimulation of β1-AR. This agrees with the previous report [4], showing that HG is a beta 1-adrenoceptor agonist and that it had positive inotropic and chronotropic effects on isolated murine atria.

Two major currents modulated by adrenergic stimulation are $I_{Ca\text{-}L}$ and I_{Ks}. Although the electrophysiological mechanism of HG was similar to that of isoproterenol (Iso), which promotes the influx of Ca2+ and increases repolarization K^+ current, we found that the two currents had different response times to HG and Iso stimulation. Iso activated $I_{Ca\text{-}L}$ in a manner that was faster than that for I_{Ks} [12]. β adrenergic (β-AR) signaling stimulated by Iso potentiated the L-type Ca current ($I_{Ca\text{-}L}$) faster than the slow delayed rectifier potassium current (I_{Ks}), which transiently prolongs the action potential duration (APD) and promote

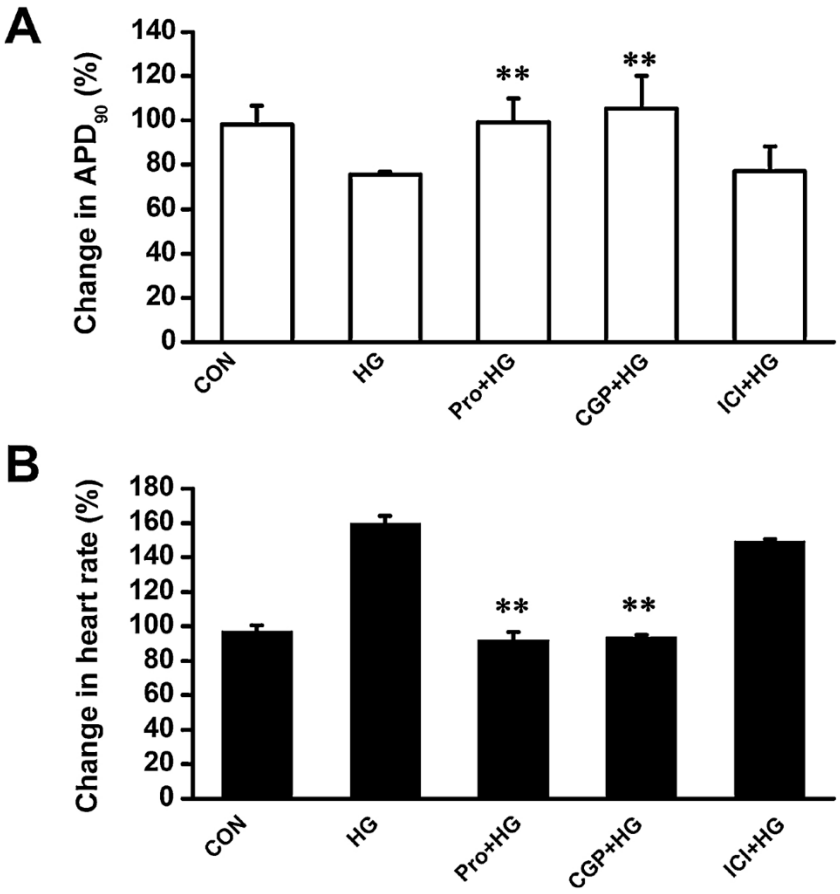

Fig. 8. Effects of HG on the APD_{90} and heart rate in the presence of β-AR blockers.(A) Summary data of APD_{90} (n = 5–8). (B) Summary data of heart rate. n = 5, **P < 0.01 *vs* HG.

EAD in single ventricular myocytes [12]. However, we found that HG increases $I_{Ca\text{-}L}$with a time course similar to that of I_{Ks}, and HG only induced DAD in single ventricular myocytes. The effect of HG on the APD in guinea pig ventricular myocytes was different from that of Iso. HG slightly prolonged the APD in lower concentrations, and shortened the APD in higher concentrations, but Iso prolonged the APD in guinea pig ventricular myocytes [13,14]. In addition, Yu et al. [9] showed that HG was able to shorten the action potential duration of the right ventricular papillary muscle of dogs *in vitro*, but increased the effective refractory period. However, Iso shortened the APD and shortened the effective refractory period, which might lead to cardiac arrhythmias [15]. Comparing the effects of HG with Iso, we found that the modulation mechanisms on the cardiac electrophysiology might be different between HG and Iso, although their effects were both mediated through β1-AR.

HG consistently increased the heart rate as shown in previous reports [4]. In our study, we confirm that HG increased the heart rate in isolated hearts and this is the first time to show that HG had a predominant action on the sinoatrial node. We observed that the sensitivity to HG's regulation in $I_{Ca\text{-}L}$, I_{Ks} and heart rate was different, with heart rate > $I_{Ca\text{-}L}$ > I_{Ks}. It was previously claimed that sympathetic stimulation modulates different cardiac electrophysiology using different signal transduction pathways. This suggests that different mechanism (PKA or PKC pathway) might be involved in the sympathetic stimulation effects of HG on ventricular myocytes and sinoatrial node cells. Although HG induced DAD in single ventricular myocytes and produced arrhythmias, it did not induce cardiac arrhythmias in isolated perfused hearts. The priority response to HG in the sinoatrial node is a possible explanation. This was consistent with the study conducted by Yu et al, in which no ventricular arrhythmia was observed in the rabbits treated with HG [16]. As the pro-arrhythmic action of Iso was found in the perfusion-isolated heart [17], HG seems to be safer than Iso.

Sick sinus syndrome (SSS) comprises a variety of conditions involving sinus node dysfunction (SND) which occurs as a result of anatomical damage to the sinoatrial node of the heart. Abnormalities in this syndrome include sinus bradycardia, sinus arrest or exit block, combinations of sinoatrial and atrioventricular nodal conduction disturbances, and atrial tachyarrhythmias [18]. Currently, no medications are routinely used to treat symptomatic SND. However, acute treatment with the anticholinergic agent atropine and the adrenergic agonist isoprenaline (Iso) may be warranted. HG appears to control the cardiac electrophysiology through its predominant effect on the sinoatrial node cells, and does not induce ectopic activity that might cause cardiac arrhythmias. Indeed, it has been reported that HG has a marked therapeutic effect on SSS in a rabbit model by increasing sinus node self-discipline [16]. HG is also safe in human subjects [19,20]. Thus, HG might be useful in treating bradycardia, as a potential medicine for sick sinus syndrome. The modulation of ionic currents that contribute to the pacemaker activity of HG needs further investigation.

A traditional Chinese formulation, Shenfu decoction (SFD) [21,22], first documented in 1465, has been used to treat heart failure for many centuries. Aconite is one of the two main components of SFD. The daily dose suggested by the Chinese pharmacopoeia (2015) is 3–15 g per day. HG, which could be *de novo* synthesized now, is the active component of Actonite. According to the Chinese pharmacopoeia, the HG content is low in Actonite.However, as its EC_{50} for heart rate is only 0.13 μM, the amount of HG in the herbal preparations of Aconite might be sufficient for clinical "use". To fully understand the cardiac electrophysiology of HG, further investigation is necessary.

5. Conclusions

In summary, we demonstrated, for the first time, that HG produced a predominant action in the sinoatrial node, compared to ventricular myocytes. The effect of HG appears to be different from that of Iso on cardiac electrophysiology, although the effects of both agents are mediated by β1-AR. HG appears to control the cardiac electrophysiology through its predominant effect on the sinoatrial node cells, and it does not induce ectopic activity that might cause cardiac arrhythmias. Thus, HG might be useful for the treatment of bradycardia.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by grant from CAMS Innovation Fund (No. 2016-I2M-1-011, No. 2017-I2M-1-008, No. 2017-I2M-B&R-09), Science and Technology Project (2015ZX09102-023-004, Z151100000115008, 2016YFC1305500), and Beijing Key Laboratory (No. BZ0150, No. Z141102004414062), National Natural Science Foundation (No. 81621064, No. 81703583).We are grateful to Dr. Xu Yanfang from Hebei Medical University for excellent technical assistance.

References

[1] T. Kosugeand, M. Yokota, Letter: studies on cardiac principle of aconite root, Chem. Pharm. Bull. (Tokyo) 24 (1976) 176–178.
[2] N. Zhang, Z. Lian, X. Peng, Z. Li, H. Zhu, Applications of Higenamine in pharmacology and medicine, J. Ethnopharmacol. 196 (2017) 242–252.
[3] K.C. Chang, W.S. Chong, I.J. Lee, Different pharmacological characteristics of structurally similar benzylisoquinoline analogs, papaverine, higenamine, and GS 389, on isolated rat aorta and heart, Can. J. Physiol. Pharmacol. 72 (1994) 327–334.
[4] I. Kimura, M. Makino, Y. Takamura, M.A. Islam, M. Kimura, Positive chronotropic and inotropic effects of higenamine and its enhancing action on the aconitine-induced tachyarrhythmia in isolated murine atria, Jpn. J. Pharmacol. 66 (1994) 75–80.
[5] I. Kimura, M.A. Islam, M. Kimura, Potentiation by higenamine of the aconitine-induced positive chronotropic effect in isolated right atria of mice: the effects of cholera toxin, forskolin and pertussis toxin, Biol. Pharm. Bull. 19 (1996) 1032–1037.
[6] G. Bai, Y. Yang, Q. Shi, Z. Liu, Q. Zhang, Y.Y. Zhu, Identification of higenamine in Radix Aconiti Lateralis Preparata as a beta2-adrenergic receptor agonist 1, Acta Pharmacol. Sin. 29 (2008) 1187–1194.
[7] M.P. Wu, Y.S. Zhang, Q.M. Zhou, J. Xiong, Y.R. Dong, C. Yan, Higenamine protects ischemia/reperfusion induced cardiac injury and myocyte apoptosis through activation of beta2-AR/PI3K/AKT signaling pathway, Pharmacol. Res. 104 (2016) 115–123.
[8] G.R. Yu, B.Y. Wang, D.S. Zheng, M.X. Huang, B.K. Gu, Y.L. Chen, M. Shang, Z.J. Jin, T.O. Cheng, Effect of higenamine on action potential of ventricular myocardial cells, J. Electrocardiol. 18 (1985) 97–103.
[9] G.R. Yu, S.Y. Chia, D.S. Zhen, M.X. Huang, Y.T. Bien, T.O. Cheng, Effect of higenamine on action potential of Purkinje and ventricular myocardial cells, Acta Cardiol. 40 (1985) 409–420.
[10] Y.H. Wang, C.X. Shi, F. Dong, J.W. Sheng, Y.F. Xu, Inhibition of the rapid component of the delayed rectifier potassium current in ventricular myocytes by angiotensin II via the AT1 receptor, Br. J. Pharmacol. 154 (2008) 429–439.
[11] A. Roguin, Henry Cuthbert Bazett (1885–1950)–the man behind the QT interval correction formula. Pacing and clinical electrophysiology, PACE 34 (2011) 384–388.
[12] G.X. Liu, B.R. Choi, O. Ziv, W. Li, E. de Lange, Z. Qu, G. Koren, Differential conditions for early after-depolarizations and triggered activity in cardiomyocytes derived from transgenic LQT1 and LQT2 rabbits, J. Physiol. 590 (2012) 1171–1180.
[13] L. Belardinelliand, G. Isenberg, Actions of adenosine and isoproterenol on isolated mammalian ventricular myocytes, Circ. Res. 53 (1983) 287–297.
[14] M. Rocchetti, V. Freli, V. Perego, C. Altomare, G. Mostacciuolo, A. Zaza, Rate dependency of beta-adrenergic modulation of repolarizing currents in the guinea-pig ventricle, J. Physiol. 574 (2006) 183–193.
[15] S.H. Litovskyand, C. Antzelevitch, Differences in the electrophysiological response of canine ventricular subendocardium and subepicardium to acetylcholine and isoproterenol. A direct effect of acetylcholine in ventricular myocardium, Circ. Res. 67 (1990) 615–627.
[16] F. Yu, L. Kong, S. Wang, Influence of racemic higenamine on the sinus node, Exp. Ther. Med. 5 (2013) 591–595.
[17] A. Tosaki, B. Woodward, F. Yamamoto, D.J. Hearse, Isoproterenol and the genesis of reperfusion-induced arrhythmias in isolated rat heart: adrenoceptor or free radical-mediated mechanisms? J. Cardiovasc. Pharmacol. 15 (1990) 398–407.
[18] V. Adanand, L.A. Crown, Diagnosis and treatment of sick sinus syndrome, Am. Fam. Phys. 67 (2003) 1725–1732.
[19] R.J. Bloomer, J.M. Schriefer, T.A. Gunnels, Clinical safety assessment of oral higenamine supplementation in healthy, young men, Hum. Exp. Toxicol. 34 (2015) 935–945.
[20] S. Feng, J. Jiang, P. Hu, J.Y. Zhang, T. Liu, Q. Zhao, B.L. Li, A phase I study on pharmacokinetics and pharmacodynamics of higenamine in healthy Chinese subjects, Acta Pharmacol. Sin. 33 (2012) 1353–1358.
[21] J. Luo, S. Min, K. Wei, J. Cao, Ion channel mechanism and ingredient bases of Shenfu Decoction's cardiac electrophysiological effects, J. Ethnopharmacol. 117 (2008) 439–445.
[22] H. Wei, H. Wu, W. Yu, X. Yan, X. Zhang, Shenfu decoction as adjuvant therapy for improving quality of life and hepatic dysfunction in patients with symptomatic chronic heart failure, J. Ethnopharmacol. 169 (2015) 347–355.