18β-Glycyrrhetinic acid preferentially blocks late Na current generated by ΔKPQ Nav1.5 channels

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Aim: To compare the effects of two stereoisomeric forms of glycyrrhetinic acid on different components of Na+ current, HERG and Kv1.5 channel currents.

Methods: Wild-type (WT) and long QT syndrome type 3 (LQT-3) mutant ΔKPQ Nav1.5 channels, as well as HERG and Kv1.5 channels were expressed in Xenopus oocytes. In addition, isolated human atrial myocytes were used. Two-microelectrode voltage-clamp technique was used to record the voltage-activated currents.

Results: Superfusion of 18β-glycyrrhetinic acid (18β-GA, 1–100 μmol/L) blocked both the peak current (I_{Na,P}) and late current (I_{Na,L}) generated by WT and ΔKPQ Nav1.5 channels in a concentration-dependent manner, while 18α-glycyrrhetinic acid (18α-GA) at the same concentrations had no effects. 18β-GA preferentially blocked I_{Na,L} (IC50 = 37.2±14.4 μmol/L) to I_{Na,P} (IC50 = 100.4±11.2 μmol/L) generated by ΔKPQ Nav1.5 channels. In human atrial myocytes, 18β-GA (30 μmol/L) inhibited 47% of I_{Na,P} and 87% of I_{Na,L} induced by Anemonia sulcata toxin (ATX-II, 30 nmol/L). Superfusion of 18β-GA (100 μmol/L) had no effects on HERG and Kv1.5 channel currents.

Conclusion: 18β-GA preferentially blocked the late Na current without affecting HERG and Kv1.5 channels.

Keywords: anti-arrhythmia agent; 18β-glycyrrhetinic acid; Nav1.5 channel; HERG channel; Kv1.5 channel; human atrial myocyte; Anemonia sulcata toxin; long QT syndrome

Introduction

Activation of the cardiac voltage-gated sodium channel (Nav1.5) generates two types of inward currents, a large peak transient current (<3 ms) (I_{Na,P}) and another current of weak intensity that spans the action potential (>300 ms). The transient current initiates the rapid upstroke of the action potential, whereas the late persistent sodium current (I_{Na,L}) does not have a well-defined function, although it has been shown to affect the duration of the action potential. Recently, the role of I_{Na,L} in controlling cardiac action potential repolarization and its importance in arrhythmogenesis has received increased attention[1].

An abnormal increase in I_{Na,L} current, produced by the delayed opening of Na+ channels, prolongs action potential repolarization and can lead to failed repolarization (early after-depolarizations) and Na+-induced Ca2+ overloading that triggers delayed after-depolarizations, calcium oscillations, and rapid tachyarrhythmia such as ventricular tachycardia (VT) or fibrillation[3]. The reduction of I_{Na,L} would therefore be expected to have therapeutic potential[2–4]. For example, ranolazine, a relatively selective inhibitor of I_{Na,L}, has been shown to be effective in reducing angina and the incidence of non-sustained VT in patients with ischemic heart disease[5–7].

Pronounced I_{Na,L} was also observed in the congenital long QT syndrome (LQTS) caused by the mutation of Nav1.5. The most severe defect observed to date has been associated with the ΔKPQ mutation. The loss of three amino acids in the intercellular linker between domains 3 and 4 has been associated with LQTS. In the clinic, LQTS can most commonly be produced as an adverse effect to the drug due to blockade of the rapid component of the delayed rectifier potassium current, I_{Kr}[8]. HERG expresses a rapid delayed rectifier current (I_{Kr}), and the testing of potential drugs for their ability to block HERG is required for drug approval[9]. Kv1.5 conducts ultra-rapid delayed rectifier current (I_{Kur}) in the human atria[6, 10], and the loss-of-function mutation might also result in LQTS and cardiac arrest[11].
Recently, we reported that glycyrrhetinic acid (GA), an active ingredient of licorice[12], blocks both $I_{\text{Na,P}}$ and $I_{\text{Na,L}}$[13]. However, GA exists in two different stereoisomeric forms, the trans form and the cis form. The two forms have different physical and chemical properties and pharmacological effects[14]. Therefore, the purpose of the present study was to determine the action of 18β-GA and 18α-GA on $I_{\text{Na,P}}$ and $I_{\text{Na,L}}$ using wild-type (WT) and mutant ΔKPQ Nav1.5 channels expressed in *Xenopus* oocytes. Our results indicate that 18β-GA blocked WT and ΔKPQ Nav1.5 channels; however, 18α-GA had no significant effect on either channel. We further characterized the inhibition of WT and ΔKPQ Nav1.5 channels by 18β-GA and have demonstrated that 18β-GA preferentially blocks the $I_{\text{Na,L}}$ produced by ΔKPQ Nav1.5 channels in a concentration-dependent tonic manner. Moreover, we investigated the effects of 18β-GA on HERG and Kv1.5 channels and found that 18β-GA had no obvious effects on either channel. Finally, we evaluated the blockage effects of 18β-GA on $I_{\text{Na,P}}$ and $I_{\text{Na,L}}$ induced by ATX-II in human atrial myocytes, to further determine the prospects of this drug for treatment in human cardiovascular disease.

**Materials and methods**

**Drugs**

18α-GA and 18β-GA were purchased from Sigma (USA) and prepared initially as a 100 mmol/L stock solution by dissolving in 100% DMSO. Before use in an experiment, the stock solution was diluted with ND96 solution to reach the desired final concentration. The percentage of DMSO in the final solution was ≤0.1%, which alone showed no detectable effect on the sodium current. ATX-II was purchased from Sigma (USA) and dissolved in distilled water.

**In vitro transcription of cRNA and functional expression in *Xenopus* oocytes**

**In vitro** transcription of cRNAs and the isolation of oocytes were performed as previously described[15, 15, 16]. The plasmid pTracer-SV40 containing WT or ΔKPQ human Nav1.5 genes was a kind gift from Dr Thomas ZIMMER of Friedrich Schiller University Jena. HERG was subcloned into the pSp64 plasmid, which was a kind gift from Prof Michael C SANGIUVENNETTI (University of Utah, USA). The human Kv1.5 gene (a gift from Dr Maria L GARCIA, Merck & Co, Inc, USA) was subcloned into a pCI-neo vector. Stage IV and V oocytes were injected with cRNA and then incubated in ND96 solution supplemented with 100 U/mL penicillin, 100 U/mL streptomycin and 2.5 mmol/L sodium pyruvate at 18°C for 3 to 7 d before use in voltage-clamp experiments. ND96 solution contains (in mmol/L) 96 NaCl, 2 KCl, 1.8 CaCl$_2$, 2 MgCl$_2$, and 5 HEPES; the pH was adjusted to 7.5 with NaOH. The amount of cRNA injected was varied according to the purpose of the experiment. For $I_{\text{Na,P}}$, the amount of cRNA was adjusted to yield peak currents in the range of 4–8 µA to minimize space clamp heterogeneities and series resistance errors; $I_{\text{Na,L}}$ measurements required peak current amplitudes of greater than 10 µA to maximize the signal.

**Two-microelectrode voltage clamp**

Standard two-microelectrode voltage clamp techniques and a TEV-200A amplifier (Dagan Corporation) were used to record currents at room temperature (22-24°C). Glass microelectrodes were filled with 3 mol/L KCl, and their tips were broken to obtain resistances of 0.5 to 1.5 MΩ. pCLAMP software (version 9.0; Molecular Devices, Union City, CA, USA) and a 1322A analog/digital interface (Molecular Devices) were used to generate voltage commands.

The voltage protocols used to obtain currents are described in the results. The data for activation and steady-state inactivation were fitted with a simple Boltzmann function: $I/I_{\text{max}}=\frac{1+\exp(V-V_1/2)/k}{1+\exp(V-V_1/2)/k}$, where $I/I_{\text{max}}$ is the relative current, $V_1/2$ is the half-maximum voltage of activation or inactivation, and $k$ is the slope factor. The recovery time course was fitted with the biexponential function: $I/I_{\text{max}}=A_n+A_h(1-\exp(-t/\tau_h))+A_1(1-\exp(-t/\tau_1))$, where $\tau$ and $A$ are the time constants and the corresponding relative amplitude, respectively.

The amplitudes of $I_{\text{Na,L}}$ were measured as maximal amplitudes during the first 5 ms of the depolarizing pulse and the $I_{\text{Na,L}}$ as the mean current amplitude of the last 10 ms of the pulse. The concentration required for a 50% block of current ($IC_{50}$) was determined by fitting the data to a Hill equation using five concentrations of drug (6–27 oocytes/point).

**Human atrial myocyte isolation and whole-cell patch clamp**

Human atrial myocytes were enzymatically dissociated as described previously[17]. Right atrial appendage tissues were obtained during atroiyotomy in patients undergoing coronary artery bypass grafting. All patients were free of supraventricular tachyarrhythmia and symptomatic congestive heart failure, and all atrial tissues were grossly normal at the time of cardiac surgery. Using a patch-clamp amplifier (Axon-200 B, Molecular Devices), the whole-cell patch clamp technique was used to record the $I_{\text{Na}}$. The series resistance averaged 1.6±0.4 MΩ after compensating for approximately 80% of the initial value. The pipette solution contained (in mmol/L) 120 CsCl, 1 CaCl$_2$, 5 MgCl$_2$, 5 Na$_2$ATP, 10 TEACl, 11 EGTA and 10 HEPES (pH 7.3 with CsOH). The bath solution for $I_{\text{Na,P}}$ recording contained (in mmol/L) 25 NaCl, 105 CsCl, 1.8 CaCl$_2$, 1 MgCl$_2$, 0.05 CdCl$_2$, 10 HEPES and 10 glucose (pH 7.4 with CsOH). The bath solution used to measure the $I_{\text{Na,L}}$ induced by ATX-II recording contained (in mmol/L) 135 NaCl, 1.8 CaCl$_2$, 1 MgCl$_2$, 0.05 CdCl$_2$, 10 HEPES and 10 glucose (pH 7.4 with NaOH).

**Data analysis**

pCLAMP 9.0 and Origin 7.5 (Microcal Software, Northampton, MA, USA) software were used for data acquisition and analysis. Values are expressed as the mean±SD. Analyses of variance (ANOVA) for repeated measures and the Holm-Sidak multiple comparison post-test or Student’s t-test (InStat 2.04; GraphPad Software) were employed to determine statis-
tical significance \((P<0.05)\).

**Results**

**Effects of 18α-GA and 18β-GA on WT and ΔKPQ Nav1.5 channels**

We first compared the effects of 18α-GA and 18β-GA on \(I_{Na}\) produced by WT (Figure 1) and ΔKPQ (Figure 2) Nav1.5 channels expressed in *Xenopus* oocytes. Currents were elicited by a series of 500-ms depolarizing steps from a hold potential of -120 mV with an interpulse interval of 10 s. Original \(I_{Na,P}\) traces of WT Nav1.5 channel-mediated current before and after superfusion with 100 µmol/L 18α-GA or 30 µmol/L 18β-GA are shown in Figures 1A and 1C, respectively. Figures 2A and 2C show one typical current trace of ΔKPQ Nav1.5 channel current elicited by a depolarizing pulse to -20 mV, which demonstrates the incomplete inactivation of \(I_{Na,L}\) and the effects of 100 µmol/L 18α-GA and 100 µmol/L 18β-GA, respectively. Application of 18β-GA significantly inhibited the \(I_{Na}\) produced by both WT and ΔKPQ Nav1.5 channels (Figures 1D and 2D), while 18α-GA had no obvious effects on WT and ΔKPQ Nav1.5 channels (Figures 1B and 2B).

**Concentration-dependent tonic block of WT and ΔKPQ Nav1.5 channels by 18β-GA**

We then examined the dose-dependent and tonic blockage effects of 18β-GA on WT and ΔKPQ Nav1.5 channels. Oocytes were held at -120 mV, and currents were evoked by depolarization to -20 mV every 10 s. This infrequent pulsing protocol should minimize the effects of a frequency-dependent block, therefore providing a reasonable estimate of the extent to which 18β-GA induces a tonic block of \(I_{Na}\). The upper panel of Figure 3A shows the experimental recordings of \(I_{Na,P}\) from WT Nav1.5 channels before and after the successive application of 1, 30, and 100 µmol/L 18β-GA. The upper panels of Figures 3B and 3C show the superimposed \(I_{Na,P}\) and \(I_{Na,L}\) traces of ΔKPQ Nav1.5 channels and the blocking effects of 18β-GA at 1, 30 and 100 µmol/L, respectively.

Because there was no detectable \(I_{Na,L}\) in WT Nav1.5 channels, only the suppression of \(I_{Na,P}\) was analyzed. The lower panels of Figure 3 show the concentration-response curves for 18β-GA after application to the WT and ΔKPQ Nav1.5 channels. The smooth lines represent the best fits of the data using the Hill equation, with the parameters of the fits shown in Figure 3. The Hill coefficients of 18β-GA binding to WT and ΔKPQ Nav1.5 channels were not significantly different from 1, suggesting that only one drug molecule is necessary to block the channel. The 18β-GA block of \(I_{Na,P}\) and \(I_{Na,L}\) in ΔKPQ Nav1.5 channels exhibited IC\(_{50}\) values of 100.4±11.2 µmol/L and 37.2±14.4 µmol/L, respectively. These results demonstrated

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**Figure 1.** Effects of 18α-GA and 18β-GA on \(I_{na}\) mediated by the WT Nav1.5 channel. (A) and (C) Representative current traces elicited by 500-ms test pulses from -70 mV to 30 mV with 10 mV increments at 0.1 Hz in typical oocytes perfused before and after in 100 µmol/L 18α-GA (A) or 30 µmol/L 18β-GA (C). The membrane potential was held at -120 mV. (B) and (D) Averaged current-voltage relationships of \(I_{na,p}\) before and after perfusion with 100 µmol/L 18α-GA (B) or 30 µmol/L 18β-GA (D); \(n=6\) per group; **\(P<0.05\).
that 18β-GA exhibited greater degrees of tonic inhibition of $I_{Na,L}$ compared with $I_{Na,P}$ when applied to ΔKPQ Nav1.5 channels.

**Rate-dependent block of WT and ΔKPQ Nav1.5 channels by 18β-GA**

To study the rate-dependent block (phasic block), a series of 30 repetitive impulses to -20 mV from a holding potential of -120 mV were applied at 1, 2, and 4 Hz, according to the method proposed by Rajamani et al. The amplitude of currents was normalized to the current during the first impulse and plotted as a function of the pulse number. Lidocaine is a clinically used class I anti-arrhythmic agent, and its action on Nav1.5 channels has been studied extensively (reviewed by Sheets et al.\(^\text{[19]}\)). In the presence of 18β-GA (30 µmol/L), $I_{Na,P}$ of WT Nav1.5 channels did not change after 30 repetitive depolarizing events at 4 Hz (approximately 0.93 at the 30th pulse, $n=6$ in each group, Figure 4A). On the contrary, lidocaine (100 µmol/L) caused a significant rate-dependent reduction in the current conducted by the WT Nav1.5 channel ($P<0.05$ at 1 Hz and 2 Hz, $P<0.01$ at 4 Hz, $n=5$, Figure 4B), which is similar to other reports\(^\text{[20, 21]}\). The $I_{Na,P}$ mediated by the WT Nav1.5 channel (Figure 4A) shows that the administration of 18β-GA at 100 µmol/L caused an additional phasic block of $I_{Na,P}$ and $I_{Na,L}$ at ΔKPQ Nav1.5 channels ($P<0.05$, $n=6$, Figures 4C and 4D).

**Voltage dependence of the activation and inactivation of WT and ΔKPQ Nav1.5 channels in the absence and presence of 18β-GA**

Voltage-dependent activation and steady-state inactivation of WT and ΔKPQ Nav1.5 channels were measured and fitted with Boltzmann equations. The former was evaluated as normalized conductance-voltage relationships, and the latter was determined using a double-pulse as indicated in the protocol diagram in Figure 1S. The parameters are summarized in Table 1. Treatment with 18β-GA significantly shifted the $V_{1/2}$ of activation curves in the positive direction and $V_{1/2}$ of the steady-state inactivation curves in the negative direction; these shifts were completely recovered after washout. These results confirmed and extended our previous study on GA\(^\text{[13]}\) and suggested that 18β-GA alters the gating function of both WT and ΔKPQ Nav1.5 channels.

The recovery time course of WT and ΔKPQ Nav1.5 channels from inactivation had fast ($t_f$) and slow components ($t_s$) (Figure 1S and Table 2). The application of 18β-GA significantly slowed the recovery of WT (30 µmol/L) and ΔKPQ (100 µmol/L) Nav1.5 channels. Table 2 summarizes the above parameters of WT and ΔKPQ Nav1.5 channels. These results suggest that 18β-GA could act on both fast and slow inactiva-
Our previous study demonstrated that GA had no significant effect on L-type calcium current (I_{Ca,L}) or hyperpolarization-activated inward current (I_{f}) in rabbit sinoatrial node pace-maker cells[13]. In this study, we extended our previous study to investigate the effects of 18β-GA on HERG and Kv1.5 channels. Figures 5A and 5B show the experimental current recordings obtained from HERG and Kv1.5 channels before and after the application of 100 µmol/L 18β-GA. The voltage protocols are presented in the inset of Figure 5. 18β-GA at 100 µmol/L had no inhibitory effects on either HERG (n=6) or Kv1.5 channels (n=7). No significant effect of 18α-GA (100 µmol/L) was observed on either HERG or Kv1.5 channels (n=7). Table 2 summarizes the recovery inactivation parameters of WT (at 30 µmol/L) and ΔKPQ (at 100 µmol/L) I_{Na,P} in the absence (control) and presence of 18β-GA. Mean±SD. n=6 per group. *P<0.05 vs control.

**Table 2.** Recovery inactivation parameters of WT (at 30 µmol/L) and ΔKPQ (at 100 µmol/L) I_{Na,P} in the absence (control) and presence of 18β-GA. Mean±SD. n=6 per group. *P<0.05 vs control.

|        | WT                  | ΔKPQ                |
|--------|---------------------|---------------------|
|        | Activation          | Inactivation        | Activation          | Inactivation        |
|        | V_{1/2} K           | V_{1/2} K           | V_{1/2} K           | V_{1/2} K           |
| Control| -33.8±1.8 6.7±1.6   | -81.8±0.4 7.2±0.3   | -36.2±1.8 7.0±1.5   | -75.1±0.4 4.8±0.4   |
| 18β-GA | -25.1±1.9b 7.7±1.7  | -88.1±0.3b 6.9±0.2b | -28.6±1.6b 7.8±1.3b | -80.5±0.5b 5.1±0.4b |
| Washout| -31.6±1.7 7.6±1.5   | -83.0±0.4 7.2±0.4   | -33.2±1.8 7.0±1.5   | -75.4±0.4 4.7±0.4   |

**Table 1.** Comparative activation and inactivation parameters of WT (at 30 µmol/L) and ΔKPQ (at 100 µmol/L) I_{Na,P} in the absence (control) and presence of 18β-GA. Mean±SD. n=6 per group. *P<0.05 vs control.

|        | Activation          | Inactivation        |
|--------|---------------------|---------------------|
|        | V_{1/2} K           | V_{1/2} K           |
| Control| -33.8±1.8 6.7±1.6   | -81.8±0.4 7.2±0.3   |
| 18β-GA | -25.1±1.9b 7.7±1.7  | -88.1±0.3b 6.9±0.2b |
| Washout| -31.6±1.7 7.6±1.5   | -83.0±0.4 7.2±0.4   |

**Figure 3.** Concentration-dependent block by 18β-GA of WT I_{Na,P} (A), ΔKPQ I_{Na,P} (B), and I_{Ca,L} (C). I_{Na,P} was elicited by a depolarizing pulse from a holding potential of -120 mV to -30 mV for 500 ms at 0.1 Hz. Representative traces (upper panels) were superimposed before (control) and during perfusion of 18β-GA (1–100 µmol/L). (B, C) are traces from the same oocyte on an expanded scale. Summarized dose-response data (lower panels) fitted with the Hill equation. IC_{50} values and the Hill coefficient are provided in the figure. n=6–27 oocytes/point.
Inhibition of $I_{Na,P}$ and $I_{Na,L}$ induced by ATX-II in human atrial myocytes after exposure to 18β-GA

Figure 6A shows superimposed typical recordings of $I_{Na,P}$ in the absence and presence of 30 µmol/L 18β-GA at 4 Hz (A) ($n=6$) and 100 µmol/L lidocaine at 1, 2, and 4 Hz (B) ($n=5$); for ΔKPQ, 100 µmol/L 18β-GA of $I_{Na,P}$ (C) and $I_{Na,L}$ (D) at 4 Hz ($n=6$). $^bP<0.05$, $^cP<0.01$.

Discussion

In the present study, we compared the electrophysiological effects of two stereoisomeric forms of glycyrretinic acid, 18α- and 18β-GA, on WT and ΔKPQ Nav1.5 channels expressed in Xenopus oocytes. The key findings were that 18β-GA inhibited both channels, while 18α-GA had no significant effects on...
In contrast, 18β-GA, the hydrogen atom and the carboxyl chain are in the same plane. Consistent with our results, different potencies of 18α-GA and 18β-GA have been observed in other pharmacological contexts\cite{22, 23}. For example, 18α-GA blocks voltage-gated potassium channels in vascular smooth muscle cells, while 18β-GA does not\cite{24}.

Our previous study showed that GA blocked the \( I_{Na,P} \) mediated by the WT Nav1.5 channel (approximately 33% at 90 µmol/L) and the \( I_{Na,L} \) mediated by the ΔKPQ Nav1.5 channel (IC\(_{50}=67±7.08\) µmol/L)[13]. In this study, 18β-GA inhibited the \( I_{Na,P} \) mediated by the WT Nav1.5 channel with an IC\(_{50}\) of 40 µmol/L and blocked \( I_{Na,P} \) and \( I_{Na,L} \) currents mediated by the ΔKPQ Nav1.5 channel at IC\(_{50}\) values of 100 µmol/L and 37 µmol/L, respectively. Therefore, 18β-GA was a more potent \( I_{Na,P} \) and \( I_{Na,L} \) blocker than GA; its potency to block \( I_{Na,L} \) was approximately three-fold greater than its potency in blocking the \( I_{Na,P} \) mediated by the ΔKPQ Nav1.5 channel.

Following tonic block, 30 depolarizing pulses were delivered to determine the extent of phasic block, the rate-dependent block, at different frequencies (1, 2, and 4 Hz). For the WT Nav1.5 channel, at 4 Hz, the \( I_{Na,L} \) remaining at the 30th pulse was 94% of the 1st pulse in the presence of 30 µmol/L 18β-GA. In contrast to 18β-GA, lidocaine caused a much greater reduction at the 30th pulse vs the 1st pulse (~21%). This result implies that 18β-GA caused much less phasic block than lidocaine. Similar results were also found for the \( I_{Na,P} \) and \( I_{Na,L} \) currents of the ΔKPQ Nav1.5 channel. Our results suggest that 18β-GA caused a potent tonic block with little additional phasic block at WT and ΔKPQ Nav1.5 channels, which was different from the effect of lidocaine as well as the reported effects of mexiletine\cite{25} and ranolazine\cite{18}.

Phasic block is a characteristic of most class I anti-arrhythmic drugs\cite{19}, but it also accounts for the unfavorable lethal pro-arrhythmias induced by these drugs\cite{26}. 18β-GA, which exhibits less potent phasic block, may prevent excessive blockage of \( I_{Na} \) in the treatment of tachyarrhythmia and may reduce the frequency of bradycardia. We found that \( I_{Na,L} \) exhibited a significant rate-dependent reduction similar to the observation reported by Guo et al\cite{27}, who suggested that the \( I_{Na,L} \) mediated by the ΔKPQ Nav1.5 channel was more pronounced at low heart rates. This may explain the clinical findings that ventricular tachyarrhythmias and sudden cardiac death in patients with LQT3 tend to occur during sleep or at rest, when the heart rate is slow\cite{28}. Ranolazine has recently been used to treat LQT-3 patents expressing the ΔKPQ channel[29]. Ranolazine exhibits strong rate-dependent inhibition, as ranolazine produced less inhibition when \( I_{Na,L} \) was more pronounced at a slower heart rate\cite{28}. Unlike ranolazine\cite{18}, 18β-GA produced a potent tonic block of \( I_{Na,L} \), which resulted in stronger anti-arrhythmic effects. Recently, a more selective potent \( I_{Na,L} \) blocker, F15845, also characterized by tonic blockade, has been demonstrated to be effective in preventing ischemia-induced arrhythmia\cite{30}.

HERG expresses \( I_{Na} \), and blockage of HERG is believed to cause LQT, which can induce EAD and a Torsades de-Pointe-type of ventricular arrhythmia, as observed after treatment.

In this study, we compared the pharmacological effects of two stereoisomeric forms of GA (18α- and 18β-) on WT and ΔKPQ Nav1.5 channels. We observed that 18α-GA had no significant effects on either WT or ΔKPQ Nav1.5 channels, even at a very high concentration (100 µmol/L). 18β-GA, however, significantly inhibited both WT and ΔKPQ Nav1.5 channels. Our results demonstrated that 18β-GA but not 18α-GA blocked WT and ΔKPQ Nav1.5 channels. One possible explanation regarding the difference in blocking effects between the two stereoisomers is the stereochemical structure. Conformational analysis showed that in 18α-GA, the hydrogen atom of C18 is not in the same plane with the carboxyl chain of C30.
with class I or class III anti-arrhythmic drugs including ranolazine[9, 31, 32]. Kv1.5 conducts \( I_{\text{KNa}} \) in the human atria[9, 10]. This channel is also found in the ventricle, but its role remains unknown. 18β-GA at 100 µmol/L demonstrated no inhibition of HERG or Kv1.5 channels, which suggested that 18β-GA might not increase QT. However, further study is required to investigate the effects of 18β-GA on QT because other potassium currents, such as transient outward current \( (I_{o}) \), slow delayed rectifier current \( (I_{K}) \) and inward rectifier current \( (I_{K1}) \), also determine QT duration.

Up-regulated \( I_{\text{Na,L}} \) has been demonstrated to be a major contributor to intracellular Na+ accumulation during many pathological conditions, such as ischemia or hypoxia, leading to elevated levels of intracellular Ca2+. This has been demonstrated to be a major contributor to intracellular Na+ accumulation during many pathological conditions such as ischemia or hypoxia, leading to elevated levels of intracellular Ca2+. The cell through the reverse-mode Na+-Ca2+ exchanger, which may also affect the subunit composition and additional post-translational modifications of Na+ channels in cardiomyocytes. Through blockade of \( I_{\text{Na,L}} \) by 18β-GA, 18β-GA can alleviate \( I_{\text{Na,L}} \)-associated arrhythmias[37] and cardiac injury after myocardial ischemia[38].

In conclusion, our results show that 18β-GA but not 18α-GA preferentially blocks \( I_{\text{Na,L}} \). 18β-GA has significant potential for development as a novel anti-arrhythmic agent, particularly in \( I_{\text{Na,L}} \)-associated arrhythmias and myocardial ischemia. Care should be taken in applying the results from heterologous expression studies to the clinical management of patients. Temperature, lipid environment, subunit composition and additional post-translational modifications of Na+ channels in cardiomyocytes may also affect the degree of blockade by 18β-GA. Nonetheless, further evaluation of the therapeutic potential of 18β-GA is warranted.

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Author contribution
Jia-hong XIA, Ming LEI, and Yi-mei DU designed the research program; Yi-mei DU, Cheng-kun XIA, Ning ZHAO, and Qian DONG performed the research; Yi-mei DU, Cheng-kun XIA, Ming LEI, and Jia-hong XIA analyzed the data and drafted the article.

Supplementary information
Supplementary figure is available at the Acta Pharmacologica Sinica website.

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