Introduction

The human-ether-a-go-go-related gene (HERG) codes the pore-forming α-subunits of channels that conduct the rapid delayed rectifier K⁺ current $I_{Kr}$ (1, 2). For the treatment of LQTS (long QT syndrome) and the safety of clinical application of drugs, it is particularly important to study the molecular mechanism of how drugs act on the HERG channel and the functional structure of the HERG channel. The HERG channel possesses six transmembrane segments, and between S5 and S6 domain, a pore of the selective filtration can be formed. Available evidence suggests HERG channels have a relatively larger pore cavity than other voltage-gated K⁺ (Kv) channels (3, 4). Moreover, particularly the aromatic amino acid residues lining the inner cavity of HERG are identified by using alanine-scanning mutagenesis to be essential for high-affinity block of the HERG channel for drugs. Two aromatic residues on the inner (S6) helix (Tyr-652 and Phe-656) that are absent from the Kv channel family are critical for binding of most HERG channel blockers (5–8). All of these physicochemical features place the HERG channel in an important position in LQTS research and also provide a plausible explanation for why the HERG channel is readily blocked by structurally diverse drugs: aromatic residues in S6 are required for high-affinity binding of the drugs (9).

Local anesthetics are well known to produce neuromuscular block and increase the neuromuscular responses to non-depolarizing muscle relaxants. The voltage-gated sodium channel is considered to be the primary target of this effect of local anesthetics (10–12). However, with the widespread use of local anesthetics, their diverse toxicity in the clinic is gradually being investigated and found. Especially, in the cardiovascular system, local anesthetic drugs like procaine, bupivacaine, procain-
amide, lidocaine, levobupivacaine, and ropivacaine, affect more than one electrical property of cardiac membranes. This mainly results in depression of cardiovascular system, such as reducing the maximum rate of rise ($V_{max}$) of the upstroke of the action potential and the action potential duration at 50% repolarization (APD50), decreasing myocardial contractility, prolongation of QRS duration in electrocardiogram, bradycardia or standstill, suppression of pacemaker potentials and action potentials (APs) in high concentrations, and profound hypotension (13–17). In addition, Burdyga and Magural indicated that the increased APD of the plateau and amplitude of the phasic contraction within the first 1–2 min of administration of local anaesthetics was greatly reduced in the presence of tetraethylammonium (TEA) (18).

Since the exact mechanisms by which these agents exert their therapeutic effects are not clearly understood, and taking into account that they often affect more than one ion channel in cardiac tissues (19), a considerable amount of work has been devoted recently to the study its actions in many different conditions and preparations (20). For many years, procaine has been one of most commonly used local anesthetics. It is extremely important to ensure the safety of its clinical use. However, the effect of procaine on HERG channels have not been investigated as yet. Hence we chose the wild-type HERG channel and HERG channel with mutations in Y652A and F656A expressed in Xenopus oocytes to investigate the inhibitory action of procaine and determine whether mutations in the S6 region are important for the inhibition of $I_{HERG}$ by procaine.

**Materials and Methods**

**Animals**

Female *X. laevis* were purchased from Nasco (Atkinson, WI, USA). These frogs were reared in three pools filled with chlorine-free water under suitable conditions, with temperature controlled strictly at 18°C – 20°C. The use of animals in this investigation was approved by the Institutional Animal Care and Use Committee of Wuhan University of Science and Technology and conformed to the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (NIH publication No. 85-23, revised 1996) and the Guide for the Care and Use of Laboratory Animals of Hubei Province, China.

**In vitro transcription and expression of HERG in Xenopus oocytes**

HERG gene-wild type, Y652A and F656A, were gifts from Professor Michael C. Sanguinetti of the University of Utah (Salt Lake City, UT, USA). It was known that HERG channel mutations (Y652A and F656A) were introduced by site-directed mutagenesis as described previously (5), subcloned into the pSP64 plasmid expression vector (Promega, Madison, WI, USA), respectively. Complementary RNA (cRNA) for WT, Y652A, and F656A was synthesized by in vitro transcription using mMESSAGE mMACHINE® SP6 Kit (Ambion, Austin, TX, USA) after linearization of the HERG construct with EcoR I restriction enzyme and stored in nuclease-free water at −80°C.

In brief, *Xenopus laevis* oocytes were surgically isolated from frogs anesthetized by a 0.5 – 1 h exposure to crushed ice. Ovarian lobes were digested with 1.5 mg/ml type IA collagenase (Sigma Chemical, St. Louis, MO, USA) in Ca²⁺-free ND96 solution for 0.5 – 1 h to remove follicle cells. Stages IV and V oocytes were injected with 45 nl (0.5 to 1 μg/μl) of HERG cRNA solution using a Nanoinject microdispenser (Drummond Scientific, Broomhall, PA, USA) and incubated at 17°C in ND96 solution.

**Cell culture and solutions**

Oocytes were cultured in ND96 solution at 17°C after injection. ND96 solution contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 2 mM MgCl₂, 5 mM HEPES (pH adjusted to 7.5 with NaOH), especially supplemented with 100 units/ml of penicillin, 100 units/ml of streptomycin, 2.5 mM of pyruvate, and fetal calf serum (concentration 1:500) after high-pressure steam sterilization.

**Solutions and voltage-clamp recordings from oocytes**

Recordings were performed from the 2nd to 10th day after oocyte injection. Normal ND 96 solution contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 2 mM MgCl₂, 5 mM HEPES (pH 7.5). High concentration–K⁺ ND 96 solution contained 68 mM NaCl, 30 mM KCl, 1.8 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES (pH 7.5). The oocytes were suffused with normal (WT and Y652A) or high concentration–K⁺ ND 96 (WT and F656A) solution for at least 15 min as control groups, before dealing with different concentrations of drugs. Solution exchanges were completed within 3 min, and the HERG currents were recorded 5 min after the solution exchange. The effects of several concentrations of drugs on the HERG currents were determined after the currents showed reversibility when washed with ND96 solution. It took about 15 min to wash out ≤ 20 μM of procaine, while it required about 30 min to wash out ≤ 80 μM. If the oocyte did not recover a current to its initial 80% amplitude after 30 min of washing with a normal ND96 solution, it was not used further. In general, each oocyte was treated with five concentrations of procaine.

Currents were measured at room temperature (20°C –
23°C) with a two-microelectrode voltage-clamp amplifier (Warner OC-725 C; Warner Instruments, Hamden, CT, USA). The glass microelectrodes were both filled with 3 M KCl and had a resistance of 2 – 4 MΩ for voltage-recording electrodes.

Data analyses

Clampfit 10.0 software (Axon Instruments, Foster City, CA, USA) was used to measure current amplitudes and fit current tracings to exponential functions. Fractional blockade was calculated using the following equation: Fractional blockade = 1 – (I_{drug} / I_{control}), where I_{control} and I_{drug} are the current amplitudes in the absence and presence of procaine respectively. Concentration–effect curves were fitted with the Hill equation: (I_{control} − I_{drug}) / I_{control} = \frac{B_{\text{max}}}{1 + (IC_{50}/D)^n}, where B_{\text{max}} is the maximum blockade of currents, IC_{50} is the drug concentration for half-maximum blockade, D is the drug concentration, and n is the Hill coefficient. The voltage-dependent current activation and inactivation was determined by fitting the values of the normalized tail currents to a Boltzmann function: y = \frac{A}{1 + \exp[(V_{1/2} − V_m) / K]}, where A is the amplitude term, V_{1/2} is the half-maximal voltage, V_m is the test potential, and K represents the slope factor of the curve.

Statistical evaluations

All data are expressed as the mean ± S.E.M. The “n values” in the text indicate the number of cells used in voltage-clamp experiments. Unpaired or paired Student’s t-tests or one-way analysis of variance (ANOVA) were used for statistical comparisons when appropriate, and differences were considered significant at P-value < 0.05.

Results

Concentration-dependent block of WT and mutant HERG channels by procaine in Xenopus oocytes

From the holding potential of −90 mV, the membrane potential was depolarized to 0 mV for 2 s and then repolarized to −60 mV for 3 s before returning to −90 mV. End-pulse currents of WT and Y652A were recorded during depolarizing, while tail currents (I_{tail}) were induced at −60 mV. The representative current traces from two Xenopus oocytes before and after sequential exposure to 20 and 40 µM (for WT) or 400 and 800 µM (for Y652A) procaine are shown in Fig. 1A. Data from different cells were pooled to obtain the mean (± S.E.M.) fractional blockade values, which were then plotted against the corresponding procaine concentration, and finally the concentration–effect curve was obtained by fitting the data (Fig. 1B). The amplitude of I_{tail} indicated a concentration-dependent decrease with increasing procaine. The following values were obtained: for the WT, IC_{50} = 34.79 ± 7.77 µM, Hill coefficient = 1.10 ± 0.11 and for Y652A, IC_{50} = 402.14 ± 40.34 µM, Hill coefficient = 1.36 ± 0.11; P < 0.05, n = 6.

To increase the current amplitude of poorly expressed
F656A, 30 mM extracellular K⁺ were used for both WT and F656A, and tail currents were recorded at −90 mV (Fig. 1C) (21). The concentration–effect curve is shown in Fig. 1D. For the WT, IC₅₀ = 45.14 ± 7.01 μM, Hill coefficient = 1.30 ± 0.15 and for F656A, IC₅₀ = 813.08 ± 85.43 μM, Hill coefficient = 1.56 ± 0.19; P < 0.05, n = 6. In conclusion, the mutations to Ala of Y652 and F656 located on the S6 domain produce about 11-fold and 18-fold increases in IC₅₀ for IᵢHERG blockade, respectively.

Voltage-dependent block of WT and Y652A HERG channels by procaine in Xenopus oocytes

The procaine-induced decrease in Iᵦₜₐᵢ at different potentials was compared in order to determine if the effect of procaine was voltage-dependent (Fig. 2). From a holding potential of −90 mV, the depolarizing pulses for 2 s from −60 to +40 mV in 10-mV increments were applied to record the end-pulse currents, and then Iᵦₜₐᵢ were evoked by repolarizing to −60 mV for 3 s. The recorded WT and Y652A current traces in absence and presence of procaine and the protocol are respectively shown in Fig. 2, A and B. Current–voltage plots of step currents measured at the end of 2 s pulses (Iᵦₑₛₜₑ) and peak tail currents (Iᵦₜₐᵢ) following the step to −60 mV of WT and Y652A HERG channels are depicted in Fig. 2, C and E, D and F, respectively. Figure 2C shows that the I-V curve for WT HERG slightly moves to the negative after applying procaine, but Fig. 2D obviously shows a small positive shift for Y652A, suggesting a positive shift in the voltage dependence of activation. Furthermore, Iᵦₑₛₜₑ and Iᵦₜₐᵢ amplitude of WT and Y652A were all dramatically reduced by procaine. These were consistent with tail current analysis Fig. 2, E and F. Tail currents of WT and Y652A in the control and in the presence of procaine were calculated using Boltzmann equation (Fig. 2: G and H) to obtain the half-maximal activation.

**Fig. 2.** Voltage-dependent blockade of procaine to WT and Y652A HERG K⁺ currents in Xenopus oocytes. A, B: Representative current records before and after incubation with procaine, 20 and 40 μM for WT (A), 400 and 800 μM for Y652A (B). C, D: I–V relationships for WT and Y652A end-pulse currents measured at the end of the 2-s test pulses with and without application of procaine (n = 6). E, F: I–V relationships for WT and Y652A tail currents with and without application of procaine (n = 6). Currents were normalized to the peak in the control condition for each oocyte. G, H: Steady state activation curves for WT and Y652A peak tail currents respectively in the control and in the presence of procaine. Tail currents were normalized to the peak currents under each condition and the data were fitted with the Boltzmann function. I, J: Voltage-dependent blockade of the end-pulse currents and tail currents after application of procaine, 40 μM procaine (G) for WT and 800 μM procaine for Y652A (J). Fractional blockade was defined as the current amplitude reduced by drug divided by the control current amplitude, which is described in the Materials and Methods.
Procaine Inhibits $I_{HERG}$ by Y652 and F656

Proline Inhibits $I_{HERG}$ by Y652 and F656

Voltage ($V_{1/2}$) and the slope factor. For WT HERG channels, the $V_{1/2}$ and the slope factors were $-27.51 \pm 1.46$ mV and $6.69 \pm 1.27$ mV in the control, $-28.92 \pm 1.44$ mV and $6.25 \pm 1.26$ mV at $20 \mu M$ procaine, and $-28.36 \pm 1.55$ mV and $6.77 \pm 2.27$ mV at $40 \mu M$ procaine (both $P > 0.05$, $n = 6$); and for Y652A HERG channel, the $V_{1/2}$ and the slope factors were $-28.71 \pm 2.64$ mV and $9.49 \pm 2.27$ mV in the control, $-19.50 \pm 2.39$ mV and $10.05 \pm 2.22$ mV at $400 \mu M$ procaine and $-14.87 \pm 2.61$ mV and $11.15 \pm 2.52$ mV at $800 \mu M$ procaine (both $P < 0.05$, $n = 6$).

To investigate the blockade of procaine to the end-pulse currents and tail currents of WT and Y652A channel at different test potentials, two histograms were drawn, respectively (Fig. 2: I and J). For both channels, it was observed that the blockade was voltage dependent after application of $40 \mu M$ procaine for WT and $800 \mu M$ procaine for Y652A, where the blockade increased significantly when the channels were activated and saturated at a range of voltages where the activation was maximal. Especially, for the Y652A channel, the blockade increased rapidly to the maximum at more positive voltage. Otherwise, tail currents showed little difference from the end-pulse currents in that the fractional block almost showed no further changes with the voltage protocol once it increased to the peak level.

**Time-dependent blockade of WT and Y652A HERG channels by procaine in Xenopus oocytes**

A protocol containing a single long test pulse (4 s) was applied. Membrane potential was held at $-90$ mV to ensure that all the HERG channels were in the closed state, followed by a single long depolarizing pulse (4 s) to 0 mV. After the control measurement, $40 \mu M$ (for WT) or $800 \mu M$ (for Y652A) procaine was applied to oocytes and equilibrated (> 3 min) at the holding potential of $-90$ mV. The records are shown in Fig. 3. A and C. The drug-induced fractional block, whose function had been mentioned in the previous study, fitted with the time of the pulse (Fig. 3: B and D).

The time course of development of WT and Y652A $I_{HERG}$ blockade by procaine was also assessed using an “envelope of tail” protocol (22, 23). Membrane potential was voltage-clamped at a holding potential of $-90$ mV and stepped to $+40$ mV for increasing durations between 20 and 700 ms in 20-ms increments, after which $I_{HERG}$ tails were evoked on repolarization to $-60$ mV. Original current records before and after application of $40 \mu M$ procaine for WT and $800 \mu M$ procaine for Y652A are shown in Fig. 4, A and B, respectively. The amplitude of envelope tail current with procaine was normalized relative to the control (Fig. 4: C and D), and the relative tail current decayed in a pulse duration–dependent manner. The time course of this decay was fitted to a mono-exponential function with a time constant of $167.04 \pm 2.77$ ms in the control and $117.18 \pm 6.19$ ms at $40 \mu M$ procaine ($P < 0.05$, $n = 6$) for WT HERG channel.

![Fig. 3](image-url)
and 136.72 ± 2.09 ms in the control and 100.97 ± 4.17 ms at 800 μM procaine (P < 0.05, n = 6) for Y652A.

Figure 4: E and F show the time-dependence of fractional block of the I\text{HERG} tails fitted with a mono-exponential function, yielding the τ of 32.28 ± 1.41 ms and 98.60 ± 1.01 ms, respectively, for the WT and Y652A HERG channel block. The inhibition of HERG channels increased rapidly to the peak over the first 200 ms, but there is no obvious change after that.

To investigate the issue further, the cell membrane potential was held at −100 mV (which greatly favors the closed-channel state); then differently from 10, 200, and 2000 ms, depolarizing to +40 mV; and repolarizing to −40 mV to obtain the currents (24). The protocol was applied first in the control and then after 3 min equilibration in procaine at −100 mV, which was important to maintain all HERG channels in the closed state during the addition and equilibration of the drug-containing perfusate, and activated until the depolarization step to +40 mV. Moreover, 200 μM and 10-fold lower concentrations of procaine were respectively applied to obtain the concentration-dependent data (Fig. 5).

Effect of procaine on WT and Y652A HERG channel inactivation in Xenopus oocytes

We used a 3-step protocol (25), shown in Fig. 6A. In this protocol, the membrane potential was held to −90 mV. After a 2-s pulse to +40 mV, the repolarizing pulses were applied between −120 mV and +40 mV in 10-mV increments for 20 ms to allow for the channel inactivation, followed by a return step to +40 mV. The peak currents were elicited by the second step to +40 mV (Fig. 6A). Peak current amplitudes in the presence of 40 μM (for WT) and 800 μM (for Y652A) procaine were normalized and plotted against the test pulse potential, giving the steady-state inactivation curve by fitting with the Boltzmann function (Fig. 6: B and C). For WT HERG channels, the half-point inactivation values were −48.92 ± 1.3 mV and −43.65 ± 1.41 mV before and after application of 40 μM procaine (P > 0.05, n = 6), respectively, with no significant change in slope factors, which were 26.31 ± 1.36 V and 29.53 ± 1.76 mV in the control and 40 μM procaine (respectively, P > 0.05, n = 6). For Y652A HERG channel, in the presence of 800 μM procaine the steady-state inactivation curve was also positively shifted: from −19.18 ± 1.55 mV (control) to −9.67 ± 1.89 mV (procaine) (P < 0.05, n = 6), again with no significant change in slope factors, 28.01 ± 1.52 mV (control) to 26.85 ± 1.53 mV (procaine) (respectively, P > 0.05, n = 6).

In Fig. 6, B and C, it was obviously observed that the inhibition level of procaine decreased with the

[Fig. 4. Time-dependence of wild-type (A, C, and E) and Y652A (B, D, and F) HERG channel blockade by procaine. A, B: Representative current recordings evoked by an “envelope of tails” protocol in the same cell before and after procaine. C, D: Peak tail currents after return to −60 mV were plotted as a function of test pulse duration. E, F: Fractional blockade of tail current produced by procaine as a function of test pulse duration.]
Fig. 5. State-dependence of wild-type HERG channel blockade by procaine. A: Representative current traces recorded using a 200-ms activating step prior to and after treatment with procaine, while the membrane potential was always held at −100 mV to make sure all the channels closed. B: Fractional blockade following 10-, 200-, and 2000-ms depolarizing to +40 mV in 20 and 200 μM procaine is summarized (P < 0.05, n = 6).

Fig. 6. Effect of procaine on voltage-dependent inactivation of WT and Y652A HERG channel. After a 2-s pulse to +40 mV, the repolarizing pulses were applied between −120 mV and +40 mV in 10-mV increments for 20 ms to allow for the channel inactivation, followed by a return step to +40 mV. The membrane potential was held to −90 mV. A: Typical recordings before and after equilibration of 40 and 800 μM procaine on the steady-state inactivation of WT and Y652A HERG channels. B, C: Normalized steady-state inactivation curves of WT and Y652A fHERG. D: Representative WT current traces in the control and in 40 μM procaine after it was applied with a modified version of the protocol. E: Mean fractional blockade with the time pulse development in the presence of 40 μM procaine.
development of depolarization, suggesting that inactivation reduced drug block (24). Therefore, a modified version of the protocol was used for the WT HERG channel in Fig. 6D. From a holding potential of −80 mV, a step to +80 mV was imposed from and back to 0 mV. The reduction in \( I_{\text{HERG}} \) during the phase at +80 mV reflects the increased number of inactive channels at this potential. The maximum fractional blockade during each phase of test pulse before and after application with 40 \( \mu \text{M} \) procaine was calculated (Fig. 6D). Mean fractional blockade with the time pulse development in the presence of 40 \( \mu \text{M} \) procaine is shown in Fig. 6E. After application of procaine, the level of inhibition was remarkably decreased on stepping from 0 mV to +80 mV and also back to 0 mV from +80 mV. The results suggest that increasing inactivation reduced the drug block.

**Discussion**

*Molecular determinants of HERG-channel block by procaine*

This study reports the inhibition of HERG channel expressed in *Xenopus* oocytes and the molecular determinants of block by procaine for the first time. We find that procaine is an inhibitor of HERG channel, displaying an \( IC_{50} \) value of 34.79 ± 7.77 \( \mu \text{M} \). However, the \( IC_{50} \) values of channels with the mutation to Ala of Y652 and F656 are 402.14 ± 40.34 and 813.08 ± 85.43 \( \mu \text{M} \) procaine, respectively, which produce about 11-fold and 18-fold increases in \( IC_{50} \) for \( I_{\text{HERG}} \) blockade, respectively (Fig. 1). It suggests that the mutants decreased the blockade of procaine. Most HERG-blocking drugs that have been examined for their molecular determinants of block have been shown to be dependent on a variety of amino-acid residues in the pore-S6 region (the inner lining of the pore). Alanine-scanning mutagenesis has previously been used to determine residues important for block of the HERG channel (5, 26), which showed that drug-induced blockade was dependent on a variety of amino-acid residues in the S6 helices. Presumably, the residues of S6 that face into the vestibule and those residues deep in the selectivity filter might trap drugs within the vestibule and contribute to their efficacy of block. Hence our results indicated that the mutation Y652A and F656A decreased the sensitivity to procaine.

**Biophysical mechanism of HERG-channel block by procaine**

We also firstly studied the biophysical mechanism for the procaine block of HERG. The results indicate that WT HERG is blocked in a concentration- and voltage-dependent manner by procaine. The steady state activation curves slightly move to the negative; the blockade increased significantly when the channels were activated and saturated at a range of voltages where the activation was maximal. Otherwise, tail currents showed little difference with the end-pulse currents in that the fractional block showed almost no further change with the voltage protocol once it increased to the peak value (Fig. 2).

The time-dependent test revealed that voltage-dependent \( I_{\text{HERG}} \) blockade increases rapidly to reach a plateau (< 200 ms) by using a single long test pulse (4 s) and an “envelope of tail” protocol (Figs. 3 and 4) as previously reported for imipramine (27), canrenoic acid (28), and fluvoxamine (24). This may suggest that the development of blockade by procaine on channel gating was extremely rapid (29) or a component of closed HERG channel likely contributed to the development of blockade with the long step protocol and envelop tail protocol. However, it could be difficult to distinguish between closed- and extremely rapid open-state-dependent channel block. Meanwhile, the rate of development of a rapid open channel blockade should be dependent upon drug concentration (30). To investigate the issue further, the protocol was applied first in the control and then after 3-min equilibration with procaine at −100 mV, which was important to maintain all HERG channels in the closed state during the addition and equilibration of the drug-containing perfusate, and activated until the depolarization step to +40 mV. Thus for procaine, binding site–dependency upon channel activation would be expected in the duration of depolarization. Moreover, 200 \( \mu \text{M} \) and 10-fold lower concentration of procaine were, respectively, applied to obtain the concentration-dependent data. As observed in Fig. 5, A and B, the level of inhibition exerted by procaine was concentration-dependent, but didn’t increase with the development of depolarization. Especially, as shown in Fig. 5B, different duration of depolarization could produce the same inhibition in the presence of 200 or 20 \( \mu \text{M} \) procaine.

The inactivation parameters move to the positive in the presence of procaine (Fig. 6: A – C). To further investigate the effect of inactivation, a modified version of the protocol is used for the WT HERG channel in Fig. 6D. After procaine application, the level of inhibition was remarkably decreased on stepping from 0 mV to +80 mV and also back to 0 mV from +80 mV. The reduction in \( I_{\text{HERG}} \) during the phase at +80 mV reflects the increased number of inactive channels at this potential. Figure 6E shows that the fractional blockade is decreased during the phase at +80 mV. The results suggest that increasing inactivation reduced the drug block.
Collectively, the data suggest that part of the block of $I_{HERG}$ by procaine may result from drug binding to a component of the closed HERG channel state. Pure closed-channel-state-dependent drug binding might be expected to result in a decrease in channel inhibition with time during sustained depolarization or in an inverse dependence of inhibition on test voltage. The data in Figs. 3 and 4 show that procaine modified the voltage dependence of current activation. It seemed likely that the drug interacted also with the activated channel states. Figure 6E suggested that increasing inactivation reduced the drug block. Furthermore, the mutant Y652A and F656A just slightly reduced the inhibitory effects of procaine (Fig. 1), which suggest that the aromatic amino acid residues Y652 and F656 in the S6 transmembrane domain are not the high affinity binding sites for procaine. Reference to the drug binding site model of HERG channels by Thomas et al. (29), Fig 7 indicates that the binding site of procaine is located outside the pore of HERG channels.

Simultaneously, for Y652A, the steady state activation and inactivation parameters are shifted to more positive values after perfusion of procaine.

**Clinical significance**

As one of the most commonly used local anesthetics, procaine had been reported to depolarize the cells to around −30 mV, reduce the action potential amplitude (APA) and the maximum rate of depolarization ($V_{max}$), and increase APD$_{50}$ on organ-cultured embryonic chick (2 – 3-day-old) ventricular cells (17). Satoh and Hashimoto also found that procaine reduced APA, the maximum diastolic potential, and $V_{max}$ in a dose-dependent manner. At the same time, APD and the cycle length were prolonged by procaine in rabbit sino-atrial node cells. In voltage clamp experiments, procaine (50 mg/ml) did not affect the slow inward current (Isi), but reduced the time-dependent outward current (Ik) (31). Another study reports that the inclusion of the local anesthetic procaine causes a considerable and clinically important decrease in the incidence of ventricular fibrillation after declamping of the aorta. It is an inexpensive drug with no apparent side effects when added in low concentration (1 mM) to cardioplegia (32). Procaine has been in clinical use for many years and is the main cardioplegic agent, especially in German cardioplegic solutions (33).

Since the plasma concentration of procaine can reach to more than 150 μM, the inhibitory concentration (IC$_{50}$ = 34.79 μM) is lower than the clinically applied dose. We also selected the local anesthetics tetracaine and lidocaine to check their effects on HERG channels in *Xenopus* oocytes. We found that after sequential exposure to 200 μM tetracaine and 100 μM lidocaine, $I_{HERG}$ was obviously inhibited. This needs to be taken seriously. However, others have found that the IC$_{50}$ of procaine for Na$^+$ current block is 60 μM in peripheral nerve, 57 μM in rat dorsal root ganglion neurons, and so on (34, 35). Their concentrations are both a little higher than $I_{HERG}$ block, but lower than the clinically applied dose. Further, another group reported that procaine inhibits the Ca$^{2+}$-release effect of cerivastatin on the sarcoplasmic reticulum of mouse and rat skeletal muscle fibers (36). This kind of multiple-channel block may increase the risk of arrhythmias. Considering the rapid delayed rectifier potassium current, $I_{Ks}$, which flows through HERG channel, is a major determinant of the shape and duration of the human cardiac action potential (APD) (37), we suggest that $I_{HERG}$ suppression plays an extremely important role in the increase of APD and decrease in the incidence of ventricular fibrillation after declamping of the aorta of procaine. However, owing to $I_{Ks}$ playing a major role in ventricular action potential repolarization (1), the suppression of $I_{HERG}$ by drugs can also cause acquired or drug-induced long QT syndrome (LQTS) (38). In recent years, disruption of HERG protein trafficking by drugs has also been shown to cause a drug-induced long QT syndrome that is similar to LQT2 (39, 40). Inhibition of $I_{HERG}$ can affect the delicate balance between inward and outward currents during repolarization to cause prolongation of the APD that may be accompanied by early after-depolarizations (EADs). Although prolongation of APD is the primary mechanism of action for class III anti-arrhythmic agents, APD prolongation often (but not always) leads to pro-arrhythmic events such as EADs, instability, and/or beat-to-beat variability of ventricular repolarization, both harbingers ventricular tachyarrhythmia *Torsades de Pointes* (TdP) (41). It has been documented that drug-induced QT prolongation is mainly due to the drug-mediated inhibition of $I_{Ks}$ (42), although these drugs are

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**Fig. 7.** Model for state-dependent blockade of HERG potassium channels by procaine. The procaine molecules can access the hypothetical binding site located outside the pore when HERG channels are open or closed, consecutively blocking the channels. The binding site is not accessible for the drug molecule when HERG channels are in the inactivated state.
structurally diverse. On the other hand, data showed that HERG activator caused QT shortening in dogs in the patch clamp studies (43). Accordingly, procaine may also have an arrhythmogenic effect accompanied with an antiarrhythmic effect, especially in patients with long QT syndrome.

Since procaine is an inhibitor of variety of ionic channels in cardiac tissues, it is critical to investigate the exactly interaction mechanism and pay attention to strictly control clinical dosage.

In summary, the study is the first to show that procaine, as a closed-channel blocker, inhibits $I_{\text{HERG}}$ with the $IC_{50}$ of 34.79 $\mu$M. The aromatic amino acid Y652 and F656 in the S6 transmembrane domain might play a role in interaction of the drug with the channel.

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References

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