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Inhibition of HERG potassium channels by domiphen bromide and didecyl dimethylammonium bromide

Yan Long [a], Wanjuan Chen [b], Zuoxian Lin [a], Hongmao Sun [c], Menghang Xia [c], Wei Zheng [c], Zhiyuan Li [a,*]

[a] *Key Laboratory of Regenerative Biology, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Kaiyuan Road 190, Guangzhou Science Park, Guangzhou 510530, China*
[b] *The School of Life Sciences, Anhui University, Hefei 230027, China*
[c] *National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD 20892, USA*

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A B S T R A C T

Domiphen bromide and didecyl dimethylammonium bromide were widely used environmental chemicals with potent activity on blockade of human ether-a-go-go related gene (HERG) channels. But the mechanism of their action is not clear. The kinetics of block of HERG channels by domiphen bromide and didecyl dimethylammonium bromide was studied in order to characterize the inhibition of HERG currents by these quaternary ammonium compounds (QACs). Domiphen bromide and didecyl dimethylammonium bromide inhibited HERG channel currents in a dose-dependent manner with IC_{50} values of 9 nM and 5 nM, respectively. Block of HERG channel by domiphen bromide and didecyl dimethylammonium bromide was voltage-dependent and use-dependent. Domiphen bromide and didecyl dimethylammonium bromide caused substantial negative shift of the activation curves, accelerated activated process, but had no effects on the deactivation and reactivation processes. The docking models implied that these two compounds bound to PAS domain of HERG channels and inhibited its function. Our data demonstrated that domiphen bromide and didecyl dimethylammonium bromide blocked the HERG channel with a preference for the activated channel state.

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1. Introduction

Domiphen bromide (DB) and didecyl dimethylammonium bromide (DDB), two members of quaternary ammonium compounds (QACs), are widely used in clinical and industrial fields. Domiphen bromide is used in the treatment of acute infectious oral diseases (Scaglione et al., 1983). Didecyl dimethylammonium bromide is being used in various industrial fields including biochemical industries (Kuo and Yu, 2011a, b). The chloride form of DDB is authorized for use in food industries (Mechin et al., 1999). These two compounds, similar to the well-known voltage-gated potassium channel blocker tetraethylammonium (TEA), have four ethyl groups attached to a central nitrogen atom. Previous electrophysiological studies demonstrated that QA's binding site was located inside the channel pore, and it accessed this binding site through open potassium channel pore (Armstrong, 1969, 1971). Moreover, findings have been verified that TEA could be trapped inside the channel pore by closure of the activation gate. On the other hand, large QA compounds were reported to block K^+ channels by a foot in the door mechanism (Armstrong, 1969, 1971). These two mechanisms may reflect that different compounds cause various alterations on HERG channels kinetics.

The human ether-a-go-go related gene (HERG) potassium channel, a member of voltage-gated potassium channels, plays a pivotal role in cardiac rhythm regulation, especially in the repolarization of the cardiac action potential. Drugs selectively inhibiting HERG channels may reduce the repolarizing cardiac potassium currents, causing the prolonged cardiac action potential and producing long QT syndromes. Thus, the HERG channel has been subjected to a routine test for compound cardiac toxicity in the drug development process. Recently, several QACs including benzethonium chloride, domiphen bromide, and tetra-n-octylammonium bromide have been found to block the HERG channel (Long et al., 2013; Xia et al., 2011). To further investigate the potential mechanisms for the efficacy of HERG inhibition of domiphen bromide and didecyl dimethylammonium bromide, two QACs, we performed detailed studies to explore the effects of domiphen bromide and didecyl dimethylammonium bromide on the use-dependence, voltage-dependence and state-dependence of HERG channels expressed in Chinese hamster ovary (CHO) cells.

* Corresponding author. Tel.: +86 20 32015241; fax: +86 20 32015299.
E-mail address: li_zhiyuan@gibh.ac.cn (Z. Li).

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2. Materials and methods

2.1. Materials

The two quaternary ammonium compounds domiphen bromide and didecyl dimethylammonium bromide as well as other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Cell culture

HERG K^+ channels stably transfected CHO cell line was purchased from ChanTest (Cleveland, OH, USA). The cells were cultured in 35 mm plastic dishes with culture medium of HAMS F-12 (Invitrogen, Carlsbad, CA, USA), supplemented with 1 mM L-glutamine and 10% fetal bovine serum (Hyclone, Logan, UT, USA) in a humidified, 5% CO_2 incubator at 37 °C.

2.3. General electrophysiologic recordings

HERG potassium current was recorded with the method published previously (Long et al., 2013). Briefly, a whole-cell patch clamp technique was conducted at room temperature (22 °C). The extracellular solution contained (mM): NaCl 137; KCl 4; $CaCl_2$ 1.8; $MgCl_2$ 1.0; glucose 10; HEPES 10; pH was adjusted to 7.4. An Axopatch 200B patch clamp amplifier in conjunction with a Digidata 1400 interface (Axon Instruments) was used for recording. Using a Flaming/Brown micropipette puller (P-97; Sutter Instruments, Co.), patch pipettes were pulled and had resistances of 2–4 MΩ when filled with the internal pipette solution, which contained (mM): KCl 130; $MgCl_2$ 1; EGTA 5; Mg-ATP 5; HEPES 10; pH was adjusted to 7.2. Cell and pipette capacitances were nulled and series resistance was compensated (85–95%) before recording. Data were acquired using pCLAMP programs (10.0; Axon Instruments).

2.4. Recording HERG tail currents

To determine the concentration-response of domiphen bromide and didecyl dimethylammonium bromide on HERG tail currents (I_{HERG}), cells were depolarized from a holding potential of −80 mV to 20 mV for a period of 4 s, followed by 2 s repolarization to −40 mV; this cycle was repeated for 5 min in the absence of drugs (control) until the HERG current became stable. Thereafter, the test compound was added to the bath solution from low concentrations to high concentrations cumulatively and each concentration was recorded for 5 min.

2.5. Recording voltage-dependent inhibition of HERG channels

To determine the current–voltage (I–V) relations of domiphen bromide and didecyl dimethylammonium bromide on I_{HERG}, cells were assessed with a standard I–V protocol. With the standard I–V protocol, the activating currents were elicited by 4 s depolarizing pulses ranging from −70 to +50 mV and the tail currents by 2 s repolarizing pulses to −40 mV. The voltage steps were delivered from a holding potential (HP) of −80 mV at an inter-pulse interval of 10 s.

2.6. Recording HERG channel kinetics

The activation curves were constructed with the standard I–V protocols. Activation time courses were obtained by fitting the currents elicited by a step to 0 mV from a holding potential of −80 mV to the double exponential function (fast and slow time courses). To construct the inactivation curves, the following voltage protocols were employed: a 2 s depolarizing pulse to +40 mV to inactivate the HERG channels followed by varying repolarizing pulses to potentials between −140 and +30 mV for a short period to allow full recovery of channels from inactivation at more negative potentials and rapid inactivation at less negative potentials. Deactivation time course was measured using a double pulse protocol consisting of a depolarization to +20 mV followed by a test pulse of −40 mV. The decaying phase of the tail current was fitted to the double exponential function and calculated fast and slow components. Recovery from inactivation was measured using a double pulse protocol consisting of a depolarization to +50 mV followed by a test pulse of −140 mV. The time constants derived from the fits for the first phase (descending phase or increasing inward current) with the single exponential function.

2.7. Recording use-dependent inhibition

The use-dependent inhibition was recorded as followings first, 4 s duration voltage steps to +20 mV were applied from the holding potential of −80 mV, and then reporalized to −40 mV with an interval of 30 s in the absence of compounds (Control run). Next, 3 nM domiphen bromide or didecyl dimethylammonium bromide was added to the bath solution. After every 10 min, the same protocol was repeated with the same cycle lengths (the second, third and fourth runs).

2.8. Data analysis

All data were presented as means and standard error of the mean (S.E.M.). The statistical significance of data was analyzed with Student's t-test or one-way analysis of variance followed by the Dunnett's test using SPSS software. A probability of $P < 0.05$ was considered as indicating a statistically significant difference.

3. Results

3.1. Concentration-dependent effects on HERG tail currents

The voltage clamp protocol and the representative traces are shown in Fig. 1; fitting of the data with the Hill equation indicated that the calculated half-maximal inhibitory concentration (IC_{50}) value for domiphen bromide block of peak tail I_{HERG} was 9 nM with a Hill coefficient of 0.24 (Fig. 1A and C). The calculated IC_{50} value for didecyl dimethylammonium bromide block of peak tail I_{HERG} was 5 nM with a Hill coefficient of 0.45 (Fig. 1B and C). In addition, the inhibitory effects of these two compounds did not recover after 10 min of washing.

3.2. Voltage-dependent inhibition of HERG channels

The I–V relationships in the absence and presence of about IC_{50} concentration of domiphen bromide and didecyl dimethylammonium bromide (3 nM) on I_{HERG} are shown in Fig. 2. In the absence of drug, the standard I–V curves at the pulse end demonstrated a strong inward rectification at potentials positive to 0 mV, whereas domiphen bromide and didecyl dimethylammonium bromide caused a significant shift of the curves to the negative direction (Fig. 2C and G). Domiphen bromide at concentration of 3 nM substantially diminished the currents at varying test potentials: the blockade was significantly increased between −70 mV and +20 mV (0–39.2%), then slightly declined at more positive potentials up to +50 mV (39.2–27.4%), whereas the blockade of tail current by 3 nM domiphen bromide was persistently raised between −70 mV and +20 mV (0–40.4%), then maintained in a stable level (Fig. 2D).

Compared with domiphen bromide, didecyl dimethylammonium bromide at concentration of 3 nM caused a weaker inhibition on the currents elicited at the pulse end, with a blockade of 0–22.4% between −70 mV and +10 mV, then slightly declined at

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