European Journal of Pharmacology 791 (2016) 168–178

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Full length article

Propofol inhibits hERG K^+ channels and enhances the inhibition effects on its mutations in HEK293 cells

Sheng-Na Han [a], Ying Jing [b], Lin-Lin Yang [a,d], Zhao Zhang [c,*], Li-Rong ZHANG [a,*]

[a] Department of Pharmacology, Basic Medical College, Zhengzhou University, Zhengzhou 450001, China
[b] Department of Physiology and Neurobiology, Basic Medical College, Zhengzhou University, Zhengzhou 450001, China
[c] Jiangsu Key Laboratory for Molecular & Medical Biotechnology, College of Life Science in Nanjing Normal University, Nanjing 210046, China
[d] Drug Discovery and Design Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 7 June 2016
Received in revised form
12 August 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
Propofol
QT prolongation
Human *ether-a-go-go*-related gene (hERG)
Protein trafficking
Gene mutation

Chemical compounds studied in this article:
propofol (PubChem CID: 4943)
penicillin (PubChem CID: 5904)
streptomycin (PubChem CID: 19649)
HEPES (PubChem CID: 23831)
EDTA (PubChem CID: 6049)
DMSO (PubChem CID: 679)
midazolam (PubChem CID: 4192)
deoxycholate (PubChem CID: 222528)
Triton X-100 (PubChem CID: 5590)

ABSTRACT

QT interval prolongation, a potential risk for arrhythmias, may result from gene polymorphisms relevant to cardiomyocyte repolarization. Another noted cause of QT interval prolongation is the administration of chemical compounds such as anesthetics, which may affect a specific type of cardiac K^+ channel encoded by the human *ether-a-go-go*-related gene (hERG).

hERG K^+ current was recorded using whole-cell patch clamp in human embryonic kidney (HEK293) cells expressing wild type (WT) or mutated hERG channels. Expression of hERG K^+ channel proteins was evaluated using western blot and confirmed by fluorescent staining and imaging. Computational modeling was adopted to identify the possible binding site(s) of propofol with hERG K^+ channels. Propofol had a significant inhibitory effect on WT hERG K^+ currents in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC_{50}) of 60.9 ± 6.4 μM. Mutations in drug-binding sites (Y652A or F656C) of the hERG channel were found to attenuate hERG current blockage by propofol. However, propofol did not inhibit the trafficking of hERG protein to the cell membrane. Meanwhile, for the three selective hERG K^+ channel mutant heterozygotes WT/Q738X-hERG, WT/A422T-hERG, and WT/H562P-hERG, the IC_{50} of propofol was calculated as 14.2 ± 2.8 μM, 3.3 ± 1.2 μM, and 5.9 ± 1.9 μM, respectively, which were much lower than that for the wild type.

These findings indicate that propofol may potentially increase QT interval prolongation risk in patients via direct inhibition of the hERG K^+ channel, especially in those with other concurrent triggering factors such as hERG gene mutations.

1. Introduction

Long QT syndrome (LQTS) is a disorder of myocardial electrical conduction, which renders the organism vulnerable to the development of torsades de pointes (TdP), leading to syncope and sudden death (Roden, 2008). LQTS can be congenital, caused by inherited abnormalities in cardiomyocyte sodium or potassium channels, or acquired, triggered by administration of certain medications, myocardial electrical abnormalities, or metabolic disorders (Saenen and Vrints, 2008). Mutations in 16 different genes have been identified to be associated with inherited LQTS (Schwartz et al., 2013). The human *ether-a-go-go*-related gene (hERG) encodes the α-subunit of the rapidly activating delayed rectifier K^+ channel (I_{Kr}), which is essential for the repolarization of the action potential in mammalian ventral myocytes (Craft, 1986). hERG mutations associated with LQT2 have been identified as a principal cause of congenital LQTS (Mathews et al., 1991). Currently, more than 300 different hERG mutations have been discovered (http://www.fsm.it/cardmoc/). A variety of mechanisms have been suggested to be responsible for hERG K^+ channel dysfunction in these mutants. For example, trafficking problems would disrupt the transportation of mutant channels to the cell membrane, while defective gating or permeation, dysfunctional channels, or dominant-negative mutations would suppress wild type protein activities (Anderson et al., 2006).

Inhibition of the hERG K^+ channel protein accounts for ninety-five percent of drug-induced LQTS cases, while QT interval

* Corresponding authors: Li-Rong Zhang, Department of Pharmacology, School of Medicine, Zhengzhou University, Zhengzhou 450001, China. Zhao Zhang, Jiangsu Key Laboratory for Molecular & Medical Biotechnology,College of Life Science in Nanjing Normal University, Nanjing 210046, China.
E-mail addresses: zhangzhao@njnu.edu.cn (Z. Zhang), zhanglirongzzu@126.com (L.-R. ZHANG).

http://dx.doi.org/10.1016/j.ejphar.2016.08.028
0014-2999/

prolongation associated with TdP has been recognized as a common indicator for the restriction of drug administration. Drug-induced LQTS may be a pharmacogenomic syndrome predisposed by rare genetic variants (Yang et al., 2002). Propofol is a short-acting intravenous anesthetic widely used to induce and maintain general anesthesia, or to induce sedation in intensive care units. Despite its commendable record, propofol has been reported in scattered cases to be associated with sudden death (Cannon et al., 2001; Cremer et al., 2001; Ernest and French, 2003; Parke et al., 1992). These cases were presumed to be related to propofol-induced cardiac disorders due to the development of malignant cardiac arrhythmias. However, no causal relation between propofol application and sudden cardiac death has been established, although the effects of propofol on the cardiovascular system are still being evaluated.

Conflicting data have accumulated regarding propofol, which has been documented to prolong (Kim et al., 2008; Saarnivaara et al., 1990), shorten (Higashijima et al., 2010; Kleinsasser et al., 2001), or not alter QT interval (Hanci et al., 2010; Kies et al., 2005; Whyte et al., 2005). In all these studies, propofol was administered simultaneously with other agents, which made it much more difficult to identify the specific effects of propofol alone on the QT interval. Though some studies showed propofol had an effect on hERG K^+ channels in different cells, the results were also different (Heath and Terrar, 1996; Yang et al., 2015). Moreover, there was no reliable clinical data on the effects of propofol in patients with inherited LQTS.

Some reports have shown that hERG K^+ channel mutations increase susceptibility to arrhythmogenic factors (Hisajima et al., 2015; Sakaguchi et al., 2008). Herein, we selected three hERG mutations (Q738X, A422T, H562P) (Guo et al., 2012; Sharma et al., 2004; Yasuda et al., 2010) reported previously in Asian population, each with different mutation sites and mechanisms of dysfunction, to illustrate genetic susceptibility to and molecular mechanisms of propofol-induced QT interval prolongation, which will help provide a theoretical basis for the establishment of individualized clinical administration protocols.

2. Materials and methods

2.1. Cell culture and transfection

The pcgi-EGFP-hERG, pcgi-EGFP-A422T-hERG, and pcgi-EGFP-H562P-hERG plasmids were kindly provided by Dr. Zhao Zhang (College of Life Sciences, Nanjing Normal University, Nanjing, China), and the pcDNA3-hERG plasmid was gifted generously by Dr. Gail A. Robertson (University of Wisconsin, Madison, WI, USA).

HEK293 cells (Institute of Biochemistry and Cell Biology, Shanghai, China) were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and maintained in a humidified 5% CO_2 incubator at 37 °C.

Transfections were performed using Lipofectamine™ 2000 according to manufacturer instructions (Invitrogen, Carlsbad, CA, USA). Briefly, HEK293 cells were pre-seeded in culture dishes and reached about 80% confluency prior to transfection. Then, plasmids containing either the WT-hERG K^+ channel gene or heterozygous mutated hERG K^+ channel genes were diluted in Opti-Mem media and added to HEK293 cells drop-wise. After 48 h, cells were observed under a fluorescent microscope for the presence of the GFP reporter, which indicated successful transient transfection and expression of the wild type or mutated hERG K^+ channel genes.

2.2. Site-directed mutagenesis

Mutations of the hERG K^+ channel gene (Q738X, A422T, H562P, Y652A and F656C) were constructed using the QuickChange site-directed mutagenesis kit (Stratagene, LaJolla, CA, USA) according to the manufacturer's instructions. Each mutation was verified by sequencing, and subsequently subcloned into the full-length pcgi-EGFP vector. Plasmid vectors were then amplified in Escherichia coli DH5α and harvested for eukaryotic cell transfection as described above.

2.3. Electrophysiological recordings

The hERG K^+ channel current in HEK293 cells transfected with wild type or mutated hERG genes was recorded using the whole-cell patch clamp technique at room temperature (18–22 °C) as previously described (Han et al., 2013). EGFP-positive cells were visually selected using an epifluorescence system. The patch clamp apparatus bath was superfused with normal Tyrode's solution containing (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 10, and HEPES 10, which was adjusted to pH 7.4 with 1 M NaOH. The whole-cell configuration involved the use of a glass pipette with tip resistance 2–5 MΩ filled with internal pipette solution (in mM): KCl 130, $MgCl_2$ 1, EGTA 5, Na_2ATP 5 and HEPES 10, which was adjusted to pH 7.2 with 1 M KOH. The electrodes were constructed from borosilicate glass using a micropipette puller (P-87; Sutter Instrument Co., Novato, CA, USA). An EPC-10 patch-clamp amplifier (HEKA electronic, Lambrecht-Pfalz, Germany) was used with Pulse 8.67 software. Cell capacitance and series resistance were routinely compensated to reduce voltage error (limited to 5 mV during an experiment).

After cell membrane rupture, at least 5 min was allowed for cell dialysis prior to any recording. For potency tests, 3–5 min of stable recording was performed before the application of the compound as a baseline control. In the presence of propofol, a steady-state response was achieved before a subsequent concentration was applied.

Time-dependent hERG K^+ channel currents were elicited by depolarizing steps between −50 and +60 mV with a 10-mV step increment from a holding potential of −80 mV, followed by repolarization to −40 mV to produce tail currents.

The voltage dependence of current activation was determined by fitting the normalized tail current (I_{tail}) versus the test potential (V_{test}) to a Boltzmann function expressed by the following equation (Han et al., 2013): $I_{tail}=1/(1+\exp[(V_{1/2}-V_t)/k])$, where $V_{1/2}$ is the voltage at which the current is half maximally activated, and k is the slope factor. For analysis of steady-state inactivation, corrected steady-state inactivation curves were fitted with a Boltzmann function as follows: $I/(I_{max}-I_{min})=1/(1+\exp[(V_t-V_{1/2})/k])+I_{min}$, where I is the amplitude of the inactivating current corrected for deactivation, I_{max} is the maximum of I, I_{min} is the minimum of I, V_t is the prepulse of the test potential, $V_{1/2}$ is the voltage at which I is half of I_{max}, and k is the slope factor.

Concentration-response relationships were fit to a Hill equation of the following form: $I_{drug}/I_{control}=1/(1+[D/IC_{50}]^n)$, where I indicates current densities, D is the drug concentration, n is the Hill coefficient, and IC_{50} is the drug concentration necessary for half-maximal inhibition.

2.4. Western blot analysis

Prior to protein extraction, propofol was diluted in serum- and antibiotic-free DMEM and added to transfected HEK293 cells for 36–48 h at 37 °C. Western blotting was performed as described previously (Han et al., 2013). In brief, cells were solubilized in ice-cold RIPA lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% deoxycholate, 1% Triton X-100, 2 mM EDTA, 0.1% SDS, and 50 mM NaF) containing a protease inhibitor cocktail (Roche, Mannheim, Germany). Protein concentrations were determined using the