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Block effect of capsaicin on hERG potassium currents is enhanced by S6 mutation at Y652

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ABSTRACT

The objectives of this study were to investigate the inhibitory action of capsaicin on wild-type (WT) and mutation human *ether-a-go-go*-related gene (hERG) potassium channel currents (I_{hERG}), and to determine whether mutations in the S6 region are significant for the inhibition of I_{hERG} by capsaicin. The hERG channel (WT, Y652A and F656A) was expressed in *Xenopus* oocytes and studied using standard two-microelectrode voltage-clamp techniques. The results show that capsaicin blocks WT hERG in a concentration-dependent manner, with an IC_{50} of 17.45 μM and a negative shift in the steady-state inactivation curve. Characteristics of blockade were consistent with capsaicin causing components of block in both the closed and open channel states. However, mutating the Y652 residue to Ala enhances the blockade effect of capsaicin with an IC_{50} of 4.11 μM, whereas mutation of F656A does not significantly alter drug potency. Simultaneously, for Y652A, the steady-state activation parameter is shifted to a more positive value by 5 mV and the inactivation parameter is shifted to a more negative value by −29 mV in the presence of 25 μM capsaicin. In conclusion, capsaicin blocks hERG channels by binding to both the closed and open channel states.Y652 was important as a molecular determinant of blockade. Mutation Y652A enhances the drug block, which may cause some patients to be particularly sensitive to capsaicin clinically.

1. Introduction

The human ether-a-go-go-related gene (hERG or KCNH2) (Warmke and Ganetzky, 1994) encodes the ion channel underlying the rapid component of the cardiac delayed rectifier potassium current, I_{Kr} (Sanguinetti et al., 1995). Reduction of hERG currents due to mutations in hERG or via excessive drug-induced blockade may cause acquired long QT syndrome (LQTS), a potentially lethal cardiac repolarization disorder (Napolitano et al., 1994; Viskin, 1999; Thomas et al., 2003).

Because of their potential proarrhythmic effects, a number of non-cardiac drugs have been withdrawn from the market (e.g. terfenadine, cisapride and thioridazine) and many others have been labeled for restricted use (e.g. mesoridazine, ziprasidone, droperidol, astemizol and arsenic trioxide) (Thomas et al., 2003; De Ponti et al., 2002). Along with ongoing research, more and more drugs which have not been reported to lead to LQTS, however are discovered to inhibit the hERG channel. This presents a latent disaster for the development and the clinical uses of them. To fully understand and prevent unwanted hERG channel block, there is intense interest in understanding the molecular and structural basis of gating in hERG K^+ channels. The hERG channel appears to have a larger pore cavity than other (Kv) six transmembrane domain K^+ channels and possesses particular aromatic amino acid residues in the S6 region of the channel (Mitcheson etal., 2000a; Mitcheson and Perry, 2003; Lees-Miller et al., 2000). At present, mutagenesis studies show that whereas the equivalent positions of the inner helices of most voltage-gated K^+ channels have aliphatic isoleucine or valine residues, that of the hERG channel contains two aromatic residues (Y652 and F656) that are important structural determinants of binding for drugs (Mitcheson et al., 2000a; Lees-Miller et al., 2000; Kamiya et al., 2001; Sanchez-Chapula et al., 2002). Mutation of these amino acid residues to alanine dramatically reduces the potency of most drugs tested to date except a few drugs whose blockade of hERG potassium currents is only partially attenuated at concentrations causing profound blockade of wild-type hERG. (e.g. BIM I, vesnarinone, fluvoxamine) (Mitcheson and Perry, 2003; Lees-Miller et al., 2000; Kamiya et al., 2001; Sanchez-Chapula et al., 2002; Sanchez-Chapula et al., 2000).

However, our previous drug screening studies detected a blocking effect of capsaicin on hERG potassium currents was enhanced by S6 mutation at Y652. Capsaicin is a pungent irritant present in peppers of the *Capsicum* family. Because of widespread distribution of its receptors in vivo, which participate in multitudinous physiological and pathological processes (Caterina et al., 1997), capsaicin is used in analgesia, anti-inflammation, inducing apoptosis of tumor cell, lowering blood pressure and treating gastrointestinal functional

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diseases (Chow et al., 2007; O'Sullivan et al., 2004). In recent years, increasing attention has been paid to capsaicin as a potential anticancer agent (Zhang et al., 2003; Lo et al., 2005; Lee et al., 2004). In addition to the above mentioned effects, capsaicin has also been reported to affect voltage-gated Na^+, K^+ and Ca^{2+} channels (Petersen et al., 1989). There are no reports about the electrophysiological characteristic of capsaicin interacting with the hERG channel. Therefore, the aim of the present study was to investigate the potential interaction of capsaicin with cloned hERG potassium channels heterologously expressed in *Xenopus laevis* oocytes. This approach revealed detailed insights into the biophysical mechanism of hERG channel block by capsaicin.

2. Materials and methods

2.1. Animals

Female *X. laevis* were purchased from the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (Beijing, China). These frogs were reared in three pools filled with chlorine-free water under suitable conditions, with temperature controlled strictly at 18–20 °C. Animals used in this investigation conformed to the "the guide for the care and use of laboratory animals regulated by the Administrative Regulation of Laboratory Animals of Hubei Province".

2.2. In vitro transcription and functional expression in Xenopus oocytes

Point mutations were introduced into hERG gene (Y652A and F656A) and subcloned into the pSP64 plasmid expression vector (Promega, Madison, WI), which was a gift from Professor Michael. C. Sanguinetti from the University of Utah. Fig. 1 shows the electrophoregram before this construct was used in this experiment. The positions of the electrophoresis bands are consistent with the construction of pSP64-hERG plasmid vector. Complementary RNAs (cRNAs) for injection into oocytes were prepared with mMESSAGE mMACHINE® SP6 Kit (Ambion, Austin, TX, USA) after linearization of the expression construct with *EcoR* I (Takara, Kyoto, Japan). *Xenopus* frogs were anesthetized by cooling on crushed ice for 30–40 min. Ovarian lobes were digested with 1.5 mg/ml type IA collagenase (Sigma Chemical, St Louis, MO, USA) in Ca^{2+}-free ND96 solution for 1 h to remove follicle cells. Stages IV and V *X. laevis* oocytes were injected with 45 nl (1 μg/μl) of HERG cRNAs per oocyte using a Nanoject microdispenser (Drummond Scientific, Broomhall, PA, USA) and then cultured in ND96 solution supplemented with 100 units/ml of penicillin, 100 units/ml of streptomycin and 2.5 mM of pyruvate at 17 °C for 1–3 days before being used in voltage-clamp experiments. ND96 solution contained (in mM): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 2, HEPES 5, titrated to pH 7.5 with NaOH.

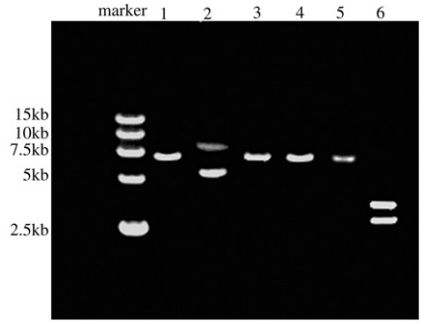

Fig. 1. Electrophoresis identification of pSp64-hERG plasmid vector. Lane 1, WT plasmid with EcoR I restriction enzyme digestion; Lane 2, WT pSP64-hERG plasmid vector ; Lane 3, WT plasmid with HindIII restriction enzyme digestion; Lanes 4,5, mutant Y652A and F656A plasmid with EcoR I restriction enzyme digestion , respectively; Lane 6, WT plasmid with EcoR I and HindIII restriction enzyme digestion.

2.3. Electrophysiological recording

Recordings were performed 2–10 days after oocyte injection. A standard two-microelectrode voltage-clamp technique was used to record currents at 21–23 °C. The glass microelectrodes were both filled with 3 M KCl and their tips were bevelled using a 1300 M Micropipette Beveler (World Precision Instruments, Sarasota, FL, USA) to obtain a resistance of 1–3 MΩ. Oocytes were clamped with a standard and advanced two-microelectrode voltage-clamp amplifier (Warner OC-725C; Warner Instruments, Hamden, CT, USA) and PLAMP software (Axon Instruments, Foster City, CA, USA). All currents were digitally sampled at 5 kHz and low pass filtered at 500 Hz. Leak and capacitive currents were not corrected. Oocytes were superfused with ND96 solution at a rate of 2.0 ml/min. Control currents were recorded repeatedly at 5 min intervals, capsaicin being applied until the control peak currents achieved a stable level.

2.4. Drugs

Capsaicin was obtained from Aldrich. The supply contained approximately 65% capsaicin and 35% dihydrocapsaicin. On the day of experiment, capsaicin was dissolved in dimethylsulphoxide to give a 120 mM stock solution from which test solutions were made. Dimethylsulphoxide was added when necessary to equalize its concentration in control and test solutions. The stock solution was diluted with cell external solution to reach the desired final concentration before experiments and the drug was applied in cumulative doses during experiments. All other chemicals were obtained from Sigma. In order to keep the concentrations of the various types of ions constant, we strictly controlled the perfusion rate by using the perfusion device BPS-4 (ALA Scientific Instruments, Inc., Westbury, NY, USA) and a constant-flow pump.

2.5. Data analysis

Clampfit 10.0 software (Axon Instruments, USA), Origin 7.0 software (Origin Laboratory, Northampton, MA, USA) and Excel (Microsoft, Redmond, WA, USA) software were used for data acquisition and analysis. Fractional blockade was defined as follows: $f = 1 - (I_{drug}/I_{control})$, where $I_{control}$ and I_{drug} are the current amplitudes in the absence and presence of capsaicin respectively. Dose–response curves were fitted by the Hill equation: $(I_{control} - I_{drug})/I_{control} = B_{max}/[1 + (IC_{50}/D)^n]$, where B_{max} is the maximum blockade of currents, IC_{50} is the concentration of capsaicin for half-maximum blockade, D is the concentration of capsaicin, and n is the Hill coefficient. The activation curve was approximated by the normalized conductance–voltage relationship and fitted with a Boltzmann function: $y = A/\{1 + \exp[(V_{1/2} - V_m)/K\}$, where A is the amplitude term, $V_{1/2}$ is the midpoint of activation, V_m is the test potential, and K represents the slope factor of the curve. The corrected steady-state inactivation curves were also fitted with a Boltzmann equation. Data were presented as means ± S.D., and the number of cells (n) in each group was given. A student t-test for paired and unpaired data was used to compare the control group with the treated group. A value of $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Concentration-dependence blockade of WT and mutant hERG channels by capsaicin

In our studies, the data analysis for the tail currents measured on return to −60 mV showed that both WT and Y652A hERG channel currents were reduced by capsaicin in a concentration-dependent manner, but to different extents. Representative traces were recorded in an experiment in which a range of concentrations of capsaicin were