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Differential modulation of late sodium current by protein kinase A in R1623Q mutant of LQT3

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ABSTRACT

Aims: In the type 3 long QT syndrome (LQT3), shortening of the QT interval by overdrive pacing is used to prevent life-threatening arrhythmias. However, it is unclear whether accelerated heart rate induced by β-adrenergic agents produces similar effects on the late sodium current (I_{Na}) to those by overdrive pacing therapy. We analyzed the β-adrenergic-like effects of protein kinase A and fluoride on I_{Na} in R1623Q mutant channels.
Main methods: cDNA encoding either wild-type (WT) or R1623Q mutant of $hNa_v1.5$ was stably transfected into HEK293 cells. I_{Na} was recorded using a whole-cell patch-clamp technique at 23 °C.
Key findings: In R1623Q channels, 2 mM pCPT-AMP and 120 mM fluoride significantly delayed macroscopic current decay and increased relative amplitude of the late I_{Na} in a time-dependent manner. Modulations of peak I_{Na} gating kinetics (activation, inactivation, recovery from inactivation) by fluoride were similar in WT and R1623Q channels. The effects of fluoride were almost completely abolished by concomitant dialysis with a protein kinase inhibitor. We also compared the effect of pacing with that of β-adrenergic stimulation by analyzing the frequency-dependence of the late I_{Na}. Fluoride augmented frequency-dependent reduction of the late I_{Na}, which was due to preferential delay of recovery of late I_{Na}. However, the increase in late I_{Na} by fluoride at steady-state was more potent than the frequency-dependent reduction of late I_{Na}.
Significance: Different basic mechanisms participate in the QT interval shortening by pacing and β-adrenergic stimulation in the LQT3.

Introduction

Congenital long QT syndrome (LQTS) is a hereditary cardiac disorder characterized by prolonged ventricular repolarization, which causes syncope and sudden cardiac death due to life-threatening ventricular tachyarrhythmias. Type 3 of the long QT syndrome (LQT3) is caused by mutations in *SCN5A*, the gene that encodes the α-subunit of the human voltage-dependent cardiac Na^+ channel ($hNa_v1.5$) (Jiang et al., 1994; George et al., 1995; Wang et al., 1995). Previous functional studies of *SCN5A* mutants indicated that most LQT3 mutations cause increased late sodium current (I_{Na}) that results in action potential prolongation and QT lengthening on the surface ECG.

Genotype–phenotype relationships are important for the diagnosis and therapeutic strategy in the LQTS. Ventricular tachyarrhythmias and sudden cardiac death in patients with LQT3 tend to occur during sleep or at rest when the heart rate is slow (Schwartz et al., 1995, 2001). Enhanced shortening of the QT interval by rapid pacing was observed in an experimental model of LQT3 (Priori et al., 1996; Shimizu and Antzelevitch, 1997; Fabritz et al., 2003). The biophysical properties related to this important clinical finding have been reported in particular LQT3 mutations (Clancy and Rudy, 1999; Veldkamp et al., 2000, 2003; Rivolta et al., 2001; Clancy et al., 2002; Nagatomo et al., 2002; Oginosawa et al., 2005). However, it is not clear at this stage whether the increase of heart rate by β-adrenergic agents has effects on the late I_{Na} similar to those of overdrive pacing.

Cardiac voltage-dependent Na^+ channels are modulated by activation of β-adrenergic receptors acting through both direct and indirect pathways (Schubert et al., 1989; Matsuda et al., 1992). The cAMP-dependent protein kinase (protein kinase A, PKA), which is activated by β-adrenergic agents, is one of the major signaling pathways that regulate cardiac Na^+ channel function. In the LQT3 mutant channels, PKA stimulation has little or no effect on the late I_{Na} in Y1795C and Y1795H channels but enhances the late I_{Na} in ΔKPQ and D1790G channels (Chandra et al., 1999; Tateyama et al., 2003b). Thus, the effects of cAMP on the late I_{Na} in LQT3 mutant channels are variable.

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A missense mutation of *SCN5A* (R1623Q), in which a charged arginine residue is substituted for a neutral glutamine at an external position of S4 segment of domain IV (DIV-S4), has been identified in a Japanese girl (Miura et al., 2003). The patient has been also reported to develop recurrent ventricular tachycardia and cardiac arrest during sleep and at rest, and cardiac pacing combined with sodium channel blocker effectively prevented the cardiac events (Miura et al., 2003).

In the present study, we investigated the effects of cAMP and a nonspecific phosphatase inhibitor fluoride, which mimics the effects of β-adrenergic agents (Chandra et al., 1999; Tateyama et al., 2003b), on the late I_{Na} in the R1623Q mutant channels. The late I_{Na} was preferentially enhanced compared with peak I_{Na} by continuous application of pCPT-AMP or fluoride. Although frequency-dependent reduction for the late I_{Na} was augmented by fluoride, the overall amplitude of the late I_{Na} was increased by fluoride.

Materials and methods

Clones and construction of R1623Q mutation

Amino acid substitution of glutamine for arginine-1623 (R1623Q) of human cardiac Na^+ channel α-subunit ($hNa_v1.5$) was performed by an overlap extension polymerase chain reaction (PCR). The 459-bp cDNA of $hNa_v1.5$ was amplified using oligonucleotide primers $hNa_v1.5$-4418F (5′-TCAACCAACAGAAGAAAAAGT-3′) and R1623Q-R (5′-GGATGACTT-GGAA-GAGCGTCGG-3′). Similarly, the 165-bp cDNA of $hNa_v1.5$ was amplified using oligonucleotide primers R1623Q-F (5′-CTCTTCCAAGT-CATCCGCCTG-3′) and $hNa_v1.5$-5006R (5′-GCCAAAGATGGAGTAGATGA-3′). Subsequently, the two PCR products were purified and combined in a second round of PCR with the primer pair hH-4418-F and $hNa_v1.5$-5006R. A 608-bp PCR product was digested with BamHI/BstEII and subcloned back into WT-$hNa_v1.5$ to assemble the R1623Q-$hNa_v1.5$ construct as reported previously (Makita et al., 1998). The entire PCR generated region was sequenced completely. We confirmed the mutation and the lack of any unwanted changes in the channel.

Cell preparation and transfection

Approximately 5×10^5 cells from a transformed HEK293 were seeded on a 60-mm diameter plate with 3 ml of culture medium one day before the transfection. The culture medium was MEM complete medium containing minimum essential medium (Eagle's salts and L-glutamine), 10% of fetal bovine serum, 2 mM L-glutamine, 0.1 mM MEM non-essential amino acids solution, 1 mM MEM pyruvate solution, 10,000 U penicillin and 10,000 μg streptomycin. Transfection for WT-$hNa_v1.5$ was carried out by the cationic liposome method, as described previously (Nagatomo et al., 1998). The cDNA for R1623Q-$hNa_v1.5$ was transfected into HEK293 cells using LipofectAMINE™2000 (Invitrogen, San Diego, CA) as directed by the manufacturer. To select stably transfected cells, geneticine (G418 sulfate) at a concentration of 800 μg/ml was added for approximately 15 days, at which time surviving single colonies were isolated and cultured with 400 μg/ml geneticine for 1–3 weeks.

Electrophysiological recordings

Macroscopic sodium current (I_{Na}) was recorded using the whole-cell patch-clamp technique at room temperature (23±1 °C). The bath solution contained (in mM): NaCl 140, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.75 and HEPES 5 (pH 7.4 set with NaOH). The pipette solution contained (in mM): CsF 120, CsCl 20, EGTA 5 and HEPES 5 (pH 7.4 set with CsOH). Pipettes had resistances between 1.0 and 1.2 MΩ when filled with the above electrode solution. Membrane currents were recorded with an Axopatch 200A amplifier (Axon Instruments Inc., Union City, CA). Data were acquired using Clampex ver. 9.2 (Axon Instruments Inc.), then digitized at 100 kHz and low-pass filtered at 10 kHz. The methods used to achieve and verify voltage control methods were those published previously (Nagatomo et al., 1998). Selective activator of cAMP dependent protein kinase, 8-(4-chlorophenylthio) adenosine 3′:5′-cyclic mono phosphate (pCPT-cAMP), and the cAMP-dependent protein kinase inhibitor (PKI) were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in bath solution (2 mM) and pipette solution (20 μM), respectively. The effects of pCPT-cAMP were examined in the presence of intrapipette fluoride (120 mM) because the current recordings were not stable in the absence of intrapipette fluoride.

Data analysis

Passive leak subtraction of peak and late I_{Na} was performed as previously described (Nagatomo et al., 1998). Data were fit to model equations using non-linear regression with pClamp ver. 9.2 (Axon Instruments Inc.) or Sigma Plot ver. 9.0 (SPSS Science, Chicago, IL). Goodness of fit was judged both visually and by the sum of squares errors. The time course of macroscopic current decay after 90% of peak was fit with double-exponential function: $I_{Na}(t)=1-[A_f\times\exp(-t/\tau_f)+A_s\times\exp(-t/\tau_s)]+\text{offset}$, where t is time, τ_f and τ_s represent the time constants of the fast and slow components, and A_f and A_s are fractions of each component, respectively. Steady-state inactivation and activation data were fit with the Boltzmann function: Normalized $I_{Na}=[1+\exp(V-V_{1/2})/\kappa]^{-1}$; Normalized $G_{Na}=[1+\exp(V_{1/2}-V)/\kappa]^{-1}$, where $V_{1/2}$ and κ are half-maximum voltage and the slope factor, respectively. For activation curve, conductance (G_{Na}) was calculated from peak I_{Na} divided by the driving force and normalized to the peak conductance. Recovery from inactivation was analyzed by fitting data with double-exponential function: Normalized $I_{Na}=A_f\times\exp(-t/\tau_f)+A_s\times\exp(-t/\tau_s)+(\text{offset})$, where t is a recovery time interval, τ_f and τ_s are the fast and slow time constants, A_f and A_s are the fractions of recovery components, and offset is a non-inactivating component. Data are expressed as mean±standard error (SEM) with n representing the number of cells. Differences between two groups were examined for statistical significance using the Student's t-test, while those among multiple groups were examined by one-way ANOVA. A P value of <0.05 was considered statistically significant.

Results

Effects of cAMP on WT and R1623Q channels

Fig. 1A shows representative current recordings from HEK293 cells expressing wild-type (WT) and R1623Q mutant channels at baseline and 10 min after perfusion of cells with pCPT-cAMP (2 mM) in the presence of intrapipette fluoride. Currents were elicited by 250 ms step pulse to −20 mV from a holding potential of −150 mV. In R1623Q channels, the macroscopic current decay was delayed and the late I_{Na} was significantly increased in the presence of pCPT-cAMP. However, the effects of pCPT-cAMP on late I_{Na} in WT were negligible. The peak I_{Na} was not affected by pCPT-cAMP in both WT and R1623Q channels. Table 1 lists the time constants for fast (τ_f) and slow (τ_s) components of the current decay. The late I_{Na} in R1623Q channels measured as the mean value of the current amplitude between 39 and 41 ms was increased in a time-dependent manner and more than doubled to the baseline amplitude at 15 min after the application of pCPT-cAMP (Fig. 1B). Fig. 1C shows changes in fraction of late I_{Na} (normalized to peak I_{Na}) at baseline and at 10 min after application of pCPT-cAMP. The fraction of the late I_{Na} in R1623Q channels was significantly higher than that at baseline ($P<0.05$). These results suggest that cAMP-dependent phosphorylation preferentially increases the late I_{Na} in R1623Q channels.

Effects of fluoride on WT and R1623Q channels

Since intracellular fluoride acts as a nonspecific phosphatase inhibitor, we tested whether it could mimic the effect of pCPT-cAMP