Enhanced sodium channel intermediate inactivation in Brugada syndrome

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Brugada syndrome is a genetically inherited cardiac disease causing sudden death related to idiopathic ventricular fibrillation in a structurally normal heart. The biophysical properties of the SCN5A mutation T1620M associated with Brugada syndrome were examined for defects in intermediate inactivation (IM), a gating process in sodium channels with kinetic features intermediate between fast and slow inactivation. Cultured mammalian cells expressing T1620M sodium channels in the presence of the human β1 subunit exhibit enhanced intermediate inactivation as compared to wild-type recombinant human heart sodium channels. Our findings support the hypothesis that Brugada syndrome is caused, in part, by functionally reduced sodium current in the myocardium due to an increased proportion of sodium channels that enter and remain in the IM state.

Brugada syndrome is a form of idiopathic ventricular fibrillation that exhibits a characteristic ECG pattern consisting of ST elevation in the right precordial leads and an apparent right bundle branch block. Disease producing mutations have been identified in several cardiac ion channel genes including SCN5A which encodes the voltage-gated sodium channel α subunit. Chen et al. reported the discovery of mutations in SCN5A in families with Brugada syndrome. Subsequently, additional SCN5A mutations have been identified and characterized. The most well studied missense mutation is a substitution of threonine 1620 by methionine (T1620M). Previous studies have demonstrated subtle changes in the voltage-dependence of steady-state inactivation and enhanced recovery from fast inactivation when the mutant was expressed in Xenopus oocytes and in cultured mammalian cells. These gating defects were surprisingly inconsistent with a loss of function phenotype. But these studies were carried out in the absence of the β1 subunit which our studies suggest may be critical for full expression of the T1620M gating defect. Recently, Veldkamp et al. described an unusual SCN5A insertion mutation (1795 insD) associated with both clinical phenotypes of congenital long QT syndrome and Brugada syndrome. This mutation exhibits sustained sodium current during long depolarizations. In addition, they show the mutation enhances a slow inactivation process with intermediate kinetics. Enhanced intermediate inactivation was argued to represent an additional biophysical mechanism by...
which sodium channels may have reduced function in the setting of Brugada syndrome. The question remains whether other Brugada syndrome missense mutations exhibit this novel biophysical mechanism, or whether this is unique to the 1795insD allele. Based on these studies, we examined the T1620M Brugada syndrome SCN5A mutation with the intent to understanding its intermediate inactivation properties.

Method:
Cells (tsA201) were transiently transfected with pRc/CMV-hH1 or pRc/CMV-T1620M with or without the human β1 subunit (hβ1). Sodium currents were recorded using the whole-cell patch clamp technique. The extracellular solution contained (in mM): 145 NaCl, 4 KCl, 1.8 CaCl2, 1 MgCl2, 10 HEPES; and 10 Glucose, pH 7.35. The intracellular solution contained (in mM): 10 NaF, 110 CsF, 20 CsCl, 10 EGTA, and 10 HEPES, pH 7.35.

Results:
To study the onset of slow inactivation, cells were depolarized to -10 mV for 1-1000 msec from a holding potential of -120 mV and then repolarized to -120 mV for 20 msec, followed by a test pulse to determine available current. During prepulses to -10 mV of variable duration, both WT-hH1 and T1620M develop an "Intermediate" kinetic component of slow inactivation (I_m, time constant ≈ 100 msec). Our results indicate that, at 22°C, the onset of I_m was enhanced in T1620M compared to the WT-hH1 in the presence of hβ1 subunit; The amplitude of I_m (1 sec prepulse duration) was 16.3±2.0% (n=35) for T1620M+hβ1, versus only 5.4±1.0% (n=30) for WT-hH1+hβ1 (P<0.01). Recovery from I_m (1 sec prepulse duration) was also slower and slow steady-state inactivation (1 sec prepulse duration) was enhanced in T1620M compared with WT-hH1 in the presence of hβ1 subunit. At 32°C, T1620M still exhibited enhanced slow inactivation when hβ1 was present. In contrast to previously published work on this mutation, we found no differences in recovery from fast inactivation either with or without hβ1, at 22°C or 32°C.

Discussion:
In this study, we used mammalian cells as the heterologous model and performed experiments predominantly in the presence of the human β1 subunit which is known to be expressed in heart and likely interacts with the cardiac sodium channel α subunit. We present new evidence that enhancement of a form of slow inactivation may also be an important characteristic of Na channel dysfunction in this syndrome. Our results suggests that a small but significant shift in the voltage dependence of steady-state fast inactivation occurs in cells expressing T1620M, but there is little evidence of any substantial effect on the kinetics of fast inactivation or recovery from fast inactivation. Our findings indicate that a Brugada syndrome Na channel mutant has abnormal slow inactivation kinetics, which could decrease sodium channel availability during the cardiac cycle. This may contribute to the in vivo cardiac arrhythmogenesis in patients with Brugada syndrome.

References: