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Enhanced late sodium current underlies pro-arrhythmic intracellular sodium and calcium dysregulation in murine sodium channelopathy

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ABSTRACT

Background: Long QT syndrome mutations in the *SCN5A* gene are associated with an enhanced late sodium current ($I_{Na,L}$) which may lead to pro-arrhythmic action potential prolongation and intracellular calcium dysregulation. We here investigated the dynamic relation between $I_{Na,L}$, intracellular sodium ($[Na^+]_i$) and calcium ($[Ca^{2+}]_i$) homeostasis and pro-arrhythmic events in the setting of a *SCN5A* mutation.
Methods and results: Wild-type (WT) and *Scn5a*$^{1798insD/+}$ (MUT) mice (age 3–5 months) carrying the murine homolog of the *SCN5A*-1795insD mutation on two distinct genetic backgrounds (FVB/N and 129P2) were studied. $[Na^+]_i$, $[Ca^{2+}]_i$ and Ca^{2+} transient amplitude were significantly increased in 129P2-MUT myocytes as compared to WT, but not in FVB/N-MUT. Accordingly, $I_{Na,L}$ wassignificantly more enhanced in 129P2-MUT than in FVB/N-MUT myocytes, consistent with a dose-dependent correlation. Quantitative RT-PCR analysis revealed intrinsic differences in mRNA expression levels of the sodium/potassium pump, the sodium/hydrogen exchanger, and sodium calcium exchanger between the two mouse strains. The rate of increase in $[Na^+]_i$, $[Ca^{2+}]_i$ and Ca^{2+} transient amplitude following the application of the Na^+/K^+-ATPase inhibitor ouabain was significantly greater in 129P2-MUT than in 129P2-WT myocytes and was normalized by the $I_{Na,L}$ inhibitor ranolazine. Furthermore, ranolazine decreased the incidence of pro-arrhythmic calcium after-transients elicited in 129P2-MUT myocytes.
Conclusions: In this study we established a causal link between the magnitude of $I_{Na,L}$, extent of Na^+ and Ca^{2+} dysregulation, and incidence of pro-arrhythmic events in murine *Scn5a*$^{1798insD/+}$ myocytes. Furthermore, our findings provide mechanistic insight into the anti-arrhythmic potential of pharmacological inhibition of $I_{Na,L}$ in patients with LQT3 syndrome.

1. Introduction

Gain-of-function mutations in *SCN5A*, the gene encoding the alpha subunit of the cardiac sodium channel, cause increased late sodium current ($I_{Na,L}$) which prolongs the action potential (AP). This is thought to underlie arrhythmic events in LQT3 patients [1]. In addition to AP prolongation, enhanced $I_{Na,L}$ may also increase intracellular sodium ($[Na^+]_i$) concentration, and consequently cytosolic calcium ($[Ca^{2+}]_i$) levels due to a decrease of forward and increase of reverse mode of the sodium calcium exchanger [2–5]. Increased $[Ca^{2+}]_i$ may in turn trigger calcium-dependent pro-arrhythmic events and furthermore activate calcium-dependent signaling pathways within the cardiomyocyte, including pro-hypertrophic pathways [4,6]. In this respect, we have previously shown that attenuation of the increase in $[Na^+]_i$ normally seen in failing hearts can prevent and regress development of heart failure [7,8]. Understanding sodium and calcium dysregulation secondary to increased $I_{Na,L}$ is clinically relevant when considering anti-arrhythmic therapeutic options aimed at preventing both direct pro-arrhythmic effects and possible long term cardiomyopathic remodeling. Previous studies have demonstrated increased sarcoplasmic reticulum Ca^{2+} load in addition to spontaneous diastolic Ca^{2+} transients in isolated cardiomyocytes from mice carrying the LQT3 mutation *Scn5a*-deltaKPQ [9,10]. However, a detailed investigation of the dynamic

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relation between $I_{Na,L}$, $[Na^+]_i$ and $[Ca^{2+}]_i$ homeostasis and pro-arrhythmic events in the setting of a *SCN5A* mutation has yet to be performed.

We have previously generated and characterized two mouse models of distinct genetic backgrounds (FVB/N and 129P2), both carrying the mouse homolog ($Scn5a^{1798insD/+}$) of the human *SCN5A-1795insD*

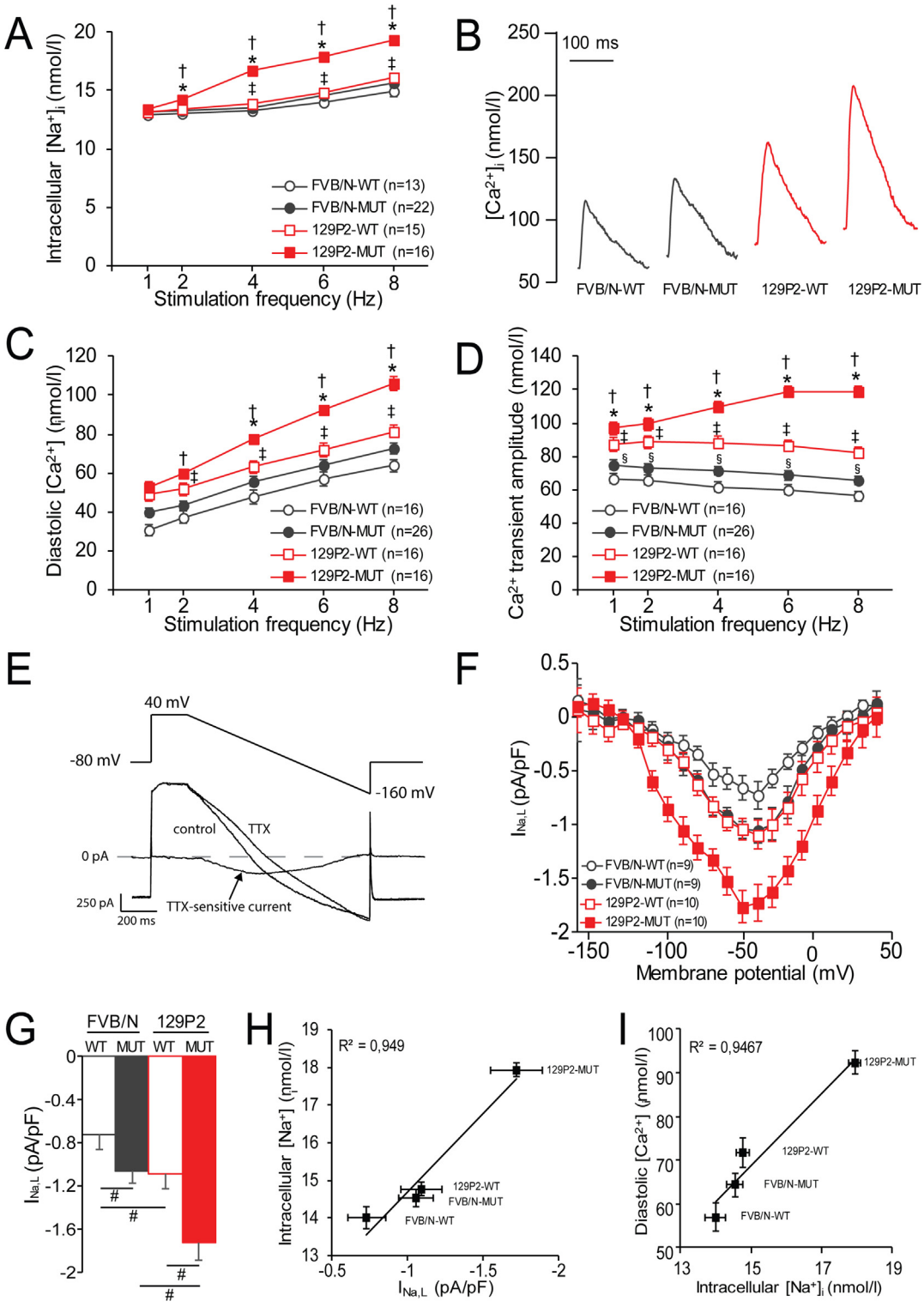

Fig. 1. Strain-dependent intracellular sodium ($[Na^+]_i$) and calcium ($[Ca^{2+}]_i$) abnormalities secondary to the $Scn5a^{1798insD/+}$ mutation correlates with magnitude of late sodium current ($I_{Na,L}$). (A) Increased $[Na^+]_i$ in left ventricular (LV) myocytes from 129P2-MUT as compared to 129P2-WT, FVB/N-WT and FVB/N-MUT. (B) Representative examples of Ca^{2+} transients in LV myocytes. (C, D) Increased diastolic $[Ca^{2+}]_i$ and Ca^{2+} transient amplitude in LV myocytes from 129P2-MUT as compared to 129P2-WT, FVB/N-WT and FVB/N-MUT. (E) Representative example of $I_{Na,L}$ measurements by ramp protocol. (F) Average current-voltage relationships for TTX-sensitive $I_{Na,L}$ in LV cardiomyocytes from WT and MUT mice of the FVB/N and 129P2 strains. (G) Increased average TTX-sensitive $I_{Na,L}$ in MUT cardiomyocytes as compared to WT and larger TTX-sensitive $I_{Na,L}$ in 129P2 cardiomyocytes as compared to FVB/N, measured at a holding potential of -40 mV. #$p < 0{,}05$ (as assessed by paired ANOVA and LSD posthoc testing). (H) Relationship between $I_{Na,L}$ (at -40 mV) and $[Na^+]_i$ (at 6 Hz) (I) Relationship between $[Na^+]_i$ and $[Ca^{2+}]_i$ at 6 Hz. * $p < 0.05$ 129P2-WT vs 129P2-MUT, † $p < 0.05$ 129P2-MUT vs FVB/N-MUT, ‡ $p < 0.05$ 129P2-WT vs FVB/N-WT, § $p < 0.05$ FVB/N-MUT vs FVB/N-WT (as assessed by repeated measures ANOVA and LSD posthoc testing).