Selective inhibition of late sodium current suppresses ventricular tachycardia and fibrillation in intact rat hearts

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BACKGROUND Enhanced late inward Na current (I_{Na-L}) modulates action potential duration (APD) and plays a key role in the genesis of early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs) and triggered activity.

OBJECTIVE The purpose of this study was to define the influence of selective block of I_{Na-L} on EAD- and DAD-mediated triggered ventricular tachycardia (VT) and ventricular fibrillation (VF) in intact hearts using (GS967), a selective and potent ($IC_{50} = 0.13 \pm 0.01$ μM) blocker of I_{Na-L}.

METHODS VT/VF were induced either by local aconitine injection (50 μg) in the left ventricular muscle of adult (3–4 months) male rats (N = 21) or by arterial perfusion of 0.1 mM hydrogen peroxide (H_2O_2) in aged male rats (24–26 months, N = 16). The left ventricular epicardial surface of the isolated-perfused hearts was optically mapped using fluorescent voltage-sensitive dye, and microelectrode recordings of action potentials were made adjacent to the aconitine injection site. The suppressive and preventive effects of GS967 (1 μM) against EAD/DAD-mediated VT/VF were then determined.

RESULTS Aconitine induced VT in all 13 hearts studied. Activation map (N = 6) showed that the VT was initiated by a focal activity arising from the aconitine injection site (cycle length [CL] 84 ± 12) that degenerated to VF (CL 52 ± 8 ms) within a few seconds. VF was maintained by multifocal activity with occasional incomplete reentrant wavefronts. Administration of GS967 suppressed the VT/VF in 10 of 13 hearts ($P < .001$). Preexposure to GS967 for 15 minutes before aconitine injection prevented initiation of VT/VF in 5 of 8 additional hearts ($P < .02$). VF reoccurred within 10 minutes on washout of GS967. Microelectrode recordings (N = 7) showed that VT/VF was initiated by EAD- and DAD-mediated triggered activity at CL of 86 ± 14 ms (NS from VT CL) that preceded the VF. GS967 shortened APD, flattened the slope of the dynamic APD restitution curve, and reduced APD dispersion from 42 ± 12 ms to 8 ± 3 ms ($P < .01$). H_2O_2 perfusion in eight fibrotic aged hearts promoted EAD-mediated focal VT/VF, which was suppressed by GS967 in five hearts ($P < .02$).

CONCLUSION The selective I_{Na-L} blocker GS967 effectively suppresses and prevents aconitine and oxidative stress-induced EADs, DADs, and focal VT/VF. Suppression of EADs, DADs, and reduction of APD dispersion make GS967 a potentially useful antiarrhythmic drug in conditions of enhanced I_{Na-L}.

KEYWORDS Optical mapping; Late I_{Na}; Aconitine; Oxidative stress; Ventricular tachycardia; Ventricular fibrillation; Early afterdepolarization; Late afterdepolarization

ABBREVIATIONS **AP** = action potential; **APA** = action potential amplitude; **APD** = action potential duration; **CL** = cycle length; **DAD** = delayed afterdepolarization; **EAD** = early afterdepolarization; **H_2O_2** = hydrogen peroxide; **IC_{50}** = concentration for 50% inhibition; **I_{Na-L}** = late inward Na current; **I_{Kr}** = delayed rectified potassium current; **LV** = left ventricle; **VF** = ventricular fibrillation; **VT** = ventricular tachycardia

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Introduction

An emerging new strategy to suppress the initiation of triggered ventricular tachycardia (VT) and ventricular fibrillation (VF) is to prevent pathologic increases in the late inward Na current (I_{Na-L}).[1–4] Enhanced I_{Na-L} seen in human heart failure,[5,6] patients with long QT syndrome type 3,[7] and diverse animal models including aconitine[8,9] and oxidative stress[10,11] plays an important role in promoting early depolarizations (EADs) and delayed afterdepolarizations (DADs).[12] These afterpotentials initiate triggered activity[1,2,12–14] that may cause focal VT[15,16] and VF[17] maintained by multifocal mechanisms.[2,18] Previous studies have shown that inhibition of I_{Na-L} with sodium channel–blocking drugs such as ranolazine, flecainide, and mexiletine suppresses EAD- and DAD-mediated triggered arrhythmias.[1,2,19]

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Table 1 IC_{50} of Drugs for Peak I_{Na}, Late I_{Na}, and I_{Kr}

Drug	Peak I_{Na}	Late I_{Na}	I_{Kr}
Flecainide	84 ± 4 μM	3.4 ± 0.5 μM	1.5 ± 0.1 μM
Ranolazine	1329 ± 114 μM	17 ± 1 μM	13 ± 1 μM
GS967	7.5% at 10 μM	0.13 ± 0.01 μM	17% at 10 μM

Note that the inhibitory effect of GS967 on peak I_{Na} and I_{Kr} becomes manifest only at 10 μM (i.e., 10 times higher than the concentration used in this study).

Numbers are given as mean ± SEM.

However, none of these drugs, including ranolazine, are sufficiently selective inhibitors of the $I_{Na\text{-}L}$ as they also affect the conductances of other ion channels. As a result, the exclusive role played by enhanced $I_{Na\text{-}L}$ in the genesis of focal VT and multifocal VF in intact hearts remains unclear. The recent synthesis of GS-458967 (GS967), a highly selective and potent (IC_{50} = 0.13 μM) blocker of $I_{Na\text{-}L}$, which completely eliminates $I_{Na\text{-}L}$ while having minimal or no effect on the conductances of other ion channels,[1,20] made the study of the exclusive role of enhanced $I_{Na\text{-}L}$ in the genesis of focal VT/VF possible. Table 1 (modified from Belardinelli et al[1]) describes the comparative potencies of GS967 against $I_{Na\text{-}L}$ relative to ranolazine and flecainide. In this study, we hypothesize that focal EAD/DAD-mediated VT/VF associated with aconitine and oxidative stress is suppressed by selective inhibition of $I_{Na\text{-}L}$ with GS967. Preliminary data have been reported in abstract form.[21]

Materials and methods

Surgical preparation and electrophysiologic recordings

We studied 21 adult (~3 months) and 16 aged (23–25 months) male Fisher344 rats. This investigation conforms to the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No.85-23, revised 1996) and was approved by the Animal Research Committee of UCLA. The hearts of the anesthetized rats were removed and cannulated and perfused through the aorta at 5 mL/min in a Langendorff setting. Simultaneous ECG, ventricular and atrial electrograms, and single-cell glass microelectrode recordings of transmembrane action potentials (APs) were made from the left ventricular (LV) epicardium. To enhance $I_{Na\text{-}L}$ and EAD/DAD-mediated VT/VF, we injected 50 μg aconitine directly into the LV muscle at a lateral LV site midway between the base and the

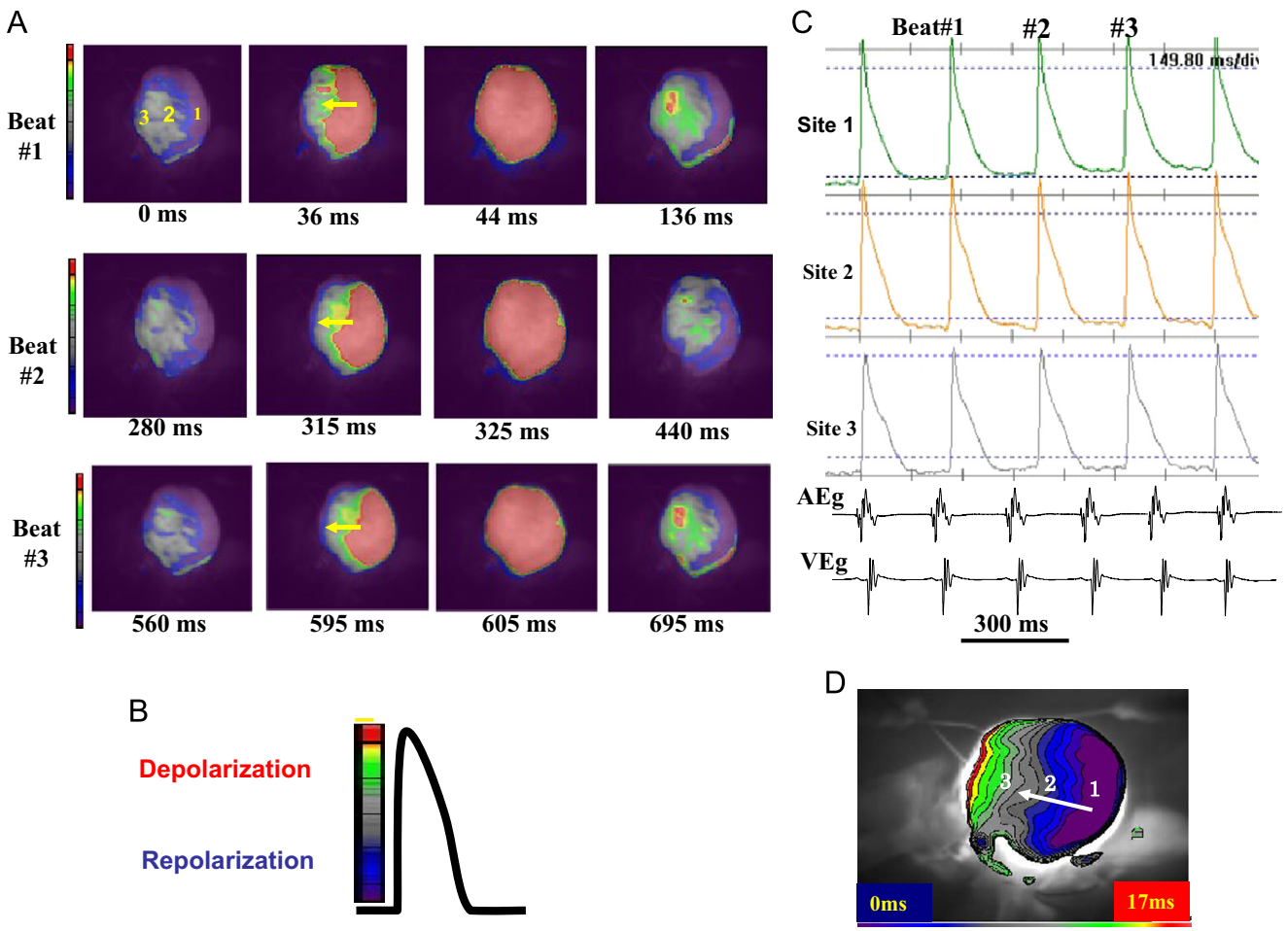

Figure 1 Optical snapshots, action potentials, and isochronal activation map during sinus rhythm in an adult rat heart. **A:** Three consecutive sinus beats (beats # 1 to #3), with *red* indicating depolarization and *blue/purple* repolarization (**B**). *Yellow arrows* indicate the direction of wave propagation. **C:** Selected action potentials recorded from sites 1, 2, and 3 indicated in A. **D:** Isochronal map, with *blue* indicating time zero. Note that no conduction block is present.