

Topic 19 – Electrophysiology: signaling – D

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0301

Ca²⁺ handling in intact sinoatrial node in a mice model of catecholaminergic polymorphic ventricular tachycardia

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a lethal genetic disease characterized by exercise/stress-induced syncope and/or sudden death in young individuals with structurally normal hearts. More than 150 identified mutations located in the cardiac Ca²⁺ release channel (type-2 ryanodine receptor, RyR2) gene are related to CPVT. Besides ventricular tachycardia sinoatrial node (SAN) dysfunction is frequently observed in CPVT patients. However, the cellular mechanism remains unknown. Here we analysed intracellular Ca²⁺ handling in SAN dissected from mice bearing a mutation in RyR2 found in CPVT patients (RyR2R420Q) and compared to wild type littermates (WT). Animals were implanted with Holter telemetry captors. One week after surgery, ECGs were recorded in basal conditions and after epinephrine/caffeine injection (2/120 mg/kg). All RyR2R420Q mice presented sustained bidirectional ventricular tachycardia, characteristic of CPVT, thus validating the model. SAN were dissected, loaded with the fluorescence Ca²⁺ dye Fluo-4 and spontaneous Ca²⁺ variations viewed with by confocal microscopy using a white light laser and resonant scanning. The cycle length of spontaneous [Ca²⁺]_i transients was significant longer in RYR2R420Q mice, but [Ca²⁺]_i transient amplitudes were similar between RyR2R420Q and WT. Besides [Ca²⁺]_i sparks in each spontaneous beats was also longer in RYR2R420Q mice. Addition of 20 nM isoprenaline dramatically increased the heart rate in both RYR2R420Q and WT SAN, but the effect was proportionally higher in RyR2R420Q SAN. Thus, our research shows that RYR2R420Q SAN mutation: 1) induce CPVT phenotype in mice, 2) decreases the SAN rhythm, suggesting a SAN dysfunction, 3) has more [Ca²⁺]_i leak during diastole as [Ca²⁺]_i sparks, 4) has more effect to β adrenergic agonists, which may contribute to supraventricular arrhythmia in CPVT.

0038

SCN5A/ Δ QKP mice: new model of long QT syndrome associated with ventricular arrhythmias and dilated cardiomyopathy

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Deletion of QKP1507-1509 amino-acids in the Nav1.5 voltage-gated Na⁺ channel is associated with a large phenotypic spectrum of LQT3, conduction disorder, dilated cardiomyopathy (DCM) and high incidence of youth sudden death. This mutation does not affect the peak Na⁺ current but rather increases the late Na⁺ current. In order to identify the mechanism of DCM in these patients, a knock-in mouse model presenting the equivalent to human QKP1507-1509 mutation, has been generated (Scn5a/ Δ QKP).

Four groups of mice were studied: wild-type (Scn5a/+), heterozygous flp deleter (Scn5a/+flp), heterozygous neomycin- Δ QKP (Scn5a/neo) and Scn5a/ Δ QKP mice. Six-lead ECG were recorded on 3-week-old to 10-week-old mice. Acute Ranolazine treatment (30 mg/kg) was performed on 3-week-old mice. Action potential recording was performed on left atrium and right ventricle of 4-week-old mice. Echocardiography and histological studies were also performed on 4-week-old mice.

Scn5a/ Δ QKP mice exhibited high early mortality with 50% of death at the age of 5-6 weeks. ECGs showed that 24/56 Scn5a/ Δ QKP exhibited ventricular extrasystoles and/or non-sustained ventricular tachycardia. Scn5a/ Δ QKP mice in sinus rhythm showed a prolonged QT interval (QTc = 78 \pm 6 ms, versus 46 \pm 2 ms in controls, n=22). Atrial and ventricular action potential recordings displayed action potential prolongation in Scn5a/ Δ QKP mice. In 4-week-old Scn5a/ Δ QKP mice, echocardiographic and histological analysis displayed cardiac diastolic dysfunction compared to control mice with right ventricle enlargement. Ten-week-old Scn5a/ Δ QKP mice showed signs of hypertrophic remodeling. Treatment with Ranolazine suppressed arrhythmias and QT heterogeneity in Scn5a/ Δ QKP mice.

Scn5a/ Δ QKP mice reproduce the phenotype of the human mutation carriers, i.e. long QT syndrome, heart failure and increased risk of sudden death at a young age. We could hypothesize that part of early mortality could be attributed to arrhythmias.

0097

Role of cAMP phosphodiesterases in the regulation of BK (Ca) channels in rat coronary arteries

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Objective: 3'-5'-cyclic adenosine monophosphate (cAMP) is an important mediator of vasorelaxation. In vascular smooth muscle, subcellular cAMP concentrations are mainly regulated by type 3 and 4 phosphodiesterases (PDE3 & PDE4). Large conductance Ca²⁺-activated K⁺ channels (BK_{Ca}) channels are reported to mediate cAMP-induced relaxation. Here, we sought to clarify whether BK_{Ca} channels are regulated by PDE activities in rat coronary arteries (CA) following β -adrenergic stimulation.

Material and methods: CA were rapidly isolated from euthanized adult male Wistar rats, cut in 2 mm length rings and mounted on a wire myograph. Cumulative concentration-relaxation experiments were conducted on rings contracted with the thromboxane analogue U46619 (0.1-3 μ M).

Results: The BK_{Ca} inhibitor iberiotoxin (IBTX, 0.1 μ M) did not alter pD₂ of isoproterenol (ISO) response. However, IBTX significantly reduced maximal relaxation induced by ISO, from 97 \pm 0.3% to 42 \pm 0.1% (N=10-12 animals, P<0.001). In rings without a functional endothelium, IBTX still significantly reduced ISO maximal response (36 \pm 3%, N=5, P<0.001) compared to vehicle (100 \pm 1%, N=6). Endothelium denudation of rCA rings slightly reduced ISO potency compared to intact rings, and this was observed either in the presence (N=6) or in the absence (N=5) of IBTX. In intact rings, inhibition of PDE3 using 1 μ M cilostamide (Cil) and of PDE4 using 10 μ M Ro20-1724 (Ro) increased ISO potency (N=9, P<0.01; N=6, P< 0.001, respectively, compared to vehicle: pD₂=7.7 \pm 0.1, N=14). However, Cil and Ro were without effect in the presence of IBTX (N=6-9).

Conclusion: These results show that, in rat CA, i) BK_{Ca} channels are important effectors of ISO-induced relaxation; ii) BK_{Ca}-dependent component of the response to ISO is not affected by endothelium removal; and iii) inhibition of PDE3 or PDE4 potentiates the response to ISO, an effect that requires active BK_{Ca} channels.

0150

PDZ domain proteins interacting with Nav1.5 differentially regulate Nav1.5 channel pools in mouse cardiomyocytes

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