In vitro simulation of spiral waves in cardiomyocyte networks using multi-electrode array technology

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corrected tail current density at 120 mV was decreased by 62±10% for Dupl and by 74±9% for the heterozygous condition. Steady-state inactivation showed no significant difference, but activation curve displayed a hyperpolarizing shift of ~ 5 mV. Cell surface biotinylation experiments showed a ~50% decrease of the mature form of Dupl in the membrane fraction, whereas the immature form was not affected. Incubation of the cells at lower temperature (28 °C,36h) resulted in partial rescue of current for the heterozygous and Dupl conditions. Low-temperature rescue was also observed in biotinylation experiments where mature/immature hERG ratios of heterozygous and Dupl conditions were restored to WT-values.

Conclusions — This hERG 343-363dup mutation causes a reduction of the peak current density and hastening of deactivation process. Both alterations lead to a loss-of-function of the channel. Furthermore, amplitude of current measured in the heterozygous model suggests dominant negative effect of the mutation. The shift towards hyperpolarizing voltages in the activation curve may reflect a gain-of-function; however, we speculate that its contribution is negligible. Biotinylation experiments showed that only the mature form of hERG Dupl is affected by the mutation, unveiling a likely defective maturation/trafficking process that can be rescued at lower incubation temperature. Our findings illustrate that N-terminally located mutations can also lead to severe ventricular arrhythmias.

G004
TRANSCRIPTIONAL PROFILING OF ION CHANNEL GENES IN RIGHT-VENTRICULAR MYOCARDIAL DISEASES: PARTICULAR SIGNATURE FOR BRUGADA SYNDROME

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Brugada syndrome is an inherited arrhythmia syndrome associated with sudden cardiac death. Na+-current dysfunction is central in Brugada syndrome, but mutations in the cardiac Na+-channel gene SCN5A are present in only ~20% of probands. Since only a minority of Brugada patients has detectable mutations, we considered alternative disease mechanisms involving a consistent pattern of variations in cardiac electrogenenic processes. We specifically hypothesized that a common pattern of cardiac ion channel and transporter gene-expression might contribute to the pathophysiology of Brugada syndrome by producing a phenotypic background that facilitates expression of characteristic ECG abnormalities and arrhythmogenesis in the context of appropriate genotypic and/or environmental factors. To test this notion, we applied high-throughput real-time PCR, which permits accurate quantification of up to hundreds of transcripts in minute biological samples, to obtained full profiling of ion-channel expression in right-ventricular septal endomyocardial biopsies from 10 patients with Brugada syndrome in comparison with biopsies from 11 non-diseased organ donors, 7 heart transplant recipients, 10 patients with arrhythmogenic right-ventricular cardiomyopathy and 9 with idiopathic right-ventricular outflow-tract tachycardia. Brugada patients showed distinct and reproducible clustering differences versus the two control and two ventricular tachyarrhythmia groups, including 14 of 77 genes encoding important ion-channel/ion-transporter subunits. Nav1.5, Kv4.3 and Kir3.4 were more weakly-expressed, while Nav2.1 and TWIK1 were more strongly-expressed, in Brugada syndrome. Important differences were also seen in transcripts involved in Ca2+-homeostasis, including stronger expression of RYR2 and NCX1. The molecular profile of five Brugada patients with SCN5A mutations did not differ from Brugada patients without SCN5A mutations. Brugada patients exhibit a common ion-channel molecular expression signature, irrespective of the culprit gene. This finding has potentially important implications for our understanding of the pathophysiology of Brugada syndrome, with possible therapeutic and diagnostic implications.

G005
IN VITRO SIMULATION OF SPIRAL WAVES IN CARDIOMYOCYTE NETWORKS USING MULTI-ELECTRODE ARRAY TECHNOLOGY

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Atrial and ventricular fibrillations are thought to be caused by multiple chaotically wandering wavelets of excitation of unknown origin. We aimed thus to provide new insights into the cellular origin of the fibrillation phenomenon by exploring the impulse propagation between cardiac myocytes in confluent monolayers of cultured cardiomyocytes (CM), which beat spontaneously and synchronously. Multisite field potentials have been recorded using microelectrode arrays (MEA) technology in basal conditions and in proarrhythmic conditions. CM were grown on multi-electrode arrays (MEA) allowing non-invasive synchronous multifocal field potential (FP) recordings. The MEA consists of 60 substrate-integrated microelectrode arrays (8 x 8 matrix, 30 µm electrode diameter, 200 µm inter-electrode distance). Data were real time acquired and analyzed with a customized platform programmed with MATLAB (Mathworks) in order to provide two-dimensional electrophysiological maps derived from these multisite FP recordings, in particular the contour of the FP propagation wavefront during each period. The sets of activation maps were then used to reconstruct videos revealing multicycle spatiotemporal patterns. In basal condition, the observation of these activation maps indicated that the spontaneous FP spikes propagated following linear path, with very stable periodic characteristics. Cardiomyocytes were then stimulated by an external electrical signal, consisting in a stimulation train (burst of 200 mV at 100 Hz during 5 min) in one point at the edge of the MEA. After this stimulation protocol, the recorded electrical activities became irregular, as confirmed by Poincaré maps of the FP periods. Moreover, spiral waves (SW) appeared in the cardiac cell network. These SW were unstable in location and could move inside or outside the recording area. SW had a mean radius of 400x100 µm and a mean angular velocity of 225x30 rotations per minute. Unstable reentrant and colliding wavefronts were also observed. To conclude, SW can be precisely characterized using a MEA data acquisition system. Therefore, within the limitations inherent to the preparation used, the cultured CM monolayer is a controlled experimental model that may be useful for further studies on the basic aspects of fibrillation and defibrillation.