4 regions: Endocardium, Epicardium, roof and PV. Cells were re-suspended into a 1.8mm Ca2+ solution by steps. Ca2+ transients were recorded (Fura-2, field stimulation) using an IonOptix system and cell membrane was stained with di-8 ANNEPS and visualized under confocal microscopy.

Ca2+ tolerant myocytes were obtained from the 4 LA regions. Ca2+ amplitude was similar across all regions, however the time to peak and the time to decay showed significant differences: Epicardium vs roof and PV. Confocal microscopy study showed the presence of t-tubules in all regions. Those results suggest regional differences in Ca2+ transient may play a major role in the development of atrial arrhythmias. This study will be completed by the development of a sheep model in persistent AF.

0443

In vitro reconstruction of an arrhythmic phenotype using cardiac-pacemaker-like cells differentiated from muscle derived stem cells

Mattia Lorenzo DiFrancesco (1), Romain Davaze (2), Pietro Mesircia (1), Daria Mamaeva (2), Isabelle Bidaud (1), Violeta Mitutova (2), Ned Lamb (2), Anne Fernandez (2), Matteo Elia Mangoni (1)

(1) Institut de Génomique Fonctionnelle, Montpellier, France – (2) Institut de Génétique Humaine, Montpellier, France

Skeletal Muscle Derived Stem Cells (MDSC), spontaneously differentiate in vitro into multiple cell types such as myocytes, adipocytes, smooth muscle, neuronal and cardiac cells. We characterize here a sub-population of an in vitro differentiated MDSC, hereby named Muscle Derived Pacemaker-like cells (MDP/Mc) displaying cardiac phenotype, spontaneous automaticity and contractile activity. We used patch-clamp recordings of ionic currents, imaging of spontaneous intra-cellular calcium transients, and measurements of contraction velocity and immuno-fluorescence detection of ion channel proteins to determine the functional properties shared by MDP/Mc and native cardiac pacemaker cells. Functional analysis of MDP/Mc differentiated from knockout mice lacking L-type Cav1.3 Ca2+ channels or G-protein activated K+ (GIRK4, underlying the muscarinic activated KACh) currents showed that MDP/Mc display similar functional properties to native sino-atrial mutant cells. Similarly to what observed in native sino-atrial Cav1.3-/- cells, MDP/Mc differentiated from Cav1.3-/- mice displayed reduction in the spontaneous beating rate of 26.6% ± 9.91 (n=9) compared to that recorded in MDP/Mc differentiated from wild-type mice. Acetycholine (10nM) decreased the contraction velocity of wild-type MDP/Mc by 62.47% ± 13.75 (n=12), but only by 14.95% ± 5.96 (n=8) in MDP/Mc derived Girk4-/- mice, values that were comparable to those observed in native Girk4-/- sino-atrial cells. MDP/Mc expressed the hyperpolarization-activated HCN4 channel, a key marker of pacemaker cells. The HCN channel inhibitor ivabradine 1μM slowed the spontaneous beating rate of MDP/Mc by 53,05% ±11,67 (n=19) demonstrating that these cells exhibited automaticity similar to that of native pacemaker cells. Finally, we were able to derive MDP/Mc also from the primate mouse lemur. In conclusion, we show that MDP/Mc may be used as a new cellular model recapitulating dysfunction of heart automaticity and arrhythmias.

0235

In vivo overexpression of a cardiac sodium channel mutant in mice

Nicolas Doisne, Marta Grauso, Nathalie Mougenot, Michel Clergeau, Alain Coulombe, Pascale Guicheny, Nathalie Neyroud

Faculté de Médecine, INSEM U1166, Paris, France

Loss-of-function mutations in the cardiac Na+ channel α-subunit gene, SCN5A, cause Brugada syndrome (Brs), a hereditary disease characterized by ventricular fibrillation and sudden cardiac death. We previously evidenced, in HEK cells, the dominant-negative effect of the R104W Brs mutation in Nav1.5, inducing the retention of the wild-type (WT) channel and the proteasomal degradation of the mutant protein. To explore this dominant-negative effect in vivo, we created a murine model using adeno-associated viruses (AAV).

We used a dual AAV vector strategy combining viral DNA recombination and trans-splicing. One week old mice were injected with two AAV serotypes capsid 9: one, packaging the cardiac specific troponin-T promoter, the 5′ half of hSCN5A, the 5′ acceptor site of a synthetic intron and a highly recombinogenic sequence; and another, packaging the same recombinogenic sequence, the 3′ acceptor site of the synthetic intron, the 3′ half of hSCN5A, the gfp gene as a reporter, and the SV40 polyA signal. Six weeks after injection, the hSCN5A full-gene expression and the percentage of transduced cardiac cells were assessed by qPCR, western blot (WB) analysis and immunohistochemistry on transduced heart tissues. The Na+ current was recorded by the patch-clamp technique in isolated cardiomyocytes. Both WT and mutant human Nav1.5 transcripts and proteins were observed by RT-qPCR, WB and immunohistochemistry on injected-mice heart tissues. Patch-clamp recordings in WT-channel injected mice evidenced a two-fold increase of the Na+ current. In contrast, the cardiac Na+ current of R104W- injected mice was impaired (i.e. the current density was decreased by 45% and the activation was shifted by -4mV).

Our data suggest that the trans-splicing and viral DNA recombination strategy using AAV9 serotype and a cardiac-specific promoter is successful to overexpress WT or mutant Na+ channels in mouse hearts. This approach allowed us to modulate the cardiac Na+ current in adult mice.

0167

Dependence of the ratio of co-expressed connexin43 and connexin45 on the connexin composition of gap junction channels

Sébastien Dupuis, Sébastien Chaigne, Marion Constantin, Thomas Desplanze

Université de Bordeaux, IJU LIRYC – L’Institut de Rythmologie et Modélisation Cardiaque, Pessac, France

In the heart, the propagation of the action potential (AP) is regulated by several factors, including gap junction channels (GJCs) made of connexins (Cxs). The ventricular myocardium expresses large amount of Cx43 and traces of Cx45 at distinct co-expression ratios that change in physiological and pathological conditions.

This study aims to understand whether and how the ratio Cx43/Cx45 regulates the formation of GJCs.

Rat Liver epithelial cells that express endogenous Cx43 and stably transfected with Cx45 to induce accurate Cx43/Cx45 ratios are used. Electrical macroscopic and unitary properties of GJCs are determined by applying the dual voltage clamp method.

We previously showed that induction of Cx45 decrease the electrical coupling, increase the voltage dependence and accelerate or decelerate the kinetics of deactivation and recovery. To date, our single channel recordings permit to estimate the Cxs composition of GJCs. Induction of Cx45 leads to the formation of one dominant GJCs population made of mixed Cx43/Cx45 composition, whereas GJCs composed with only Cx43 or Cx45 are present in minor proportions. Interestingly, this finely regulated GJCs make-up with Cx43 and Cx45 seems to be independent on the ratios Cx43/Cx45.

Further experiments will be performed to mimic cardiac border zone which have different ratios of Cxs co-expression. This will provide a better understanding of the regulation of the cardiac impulse propagation in the healthy heart, and the pro-arrhythmic behavior of Cxs dysfunction and remodeling in the diseased heart.

0134

Using cardiomycocytes differentiated from urine-derived hiPSCs to recapitulate electrophysiological characteristics of LQT2 syndrome

Mariam Jouni (1), Karim Si-Tayeb (1), Zeineb Es-Salah-Lamoureuxa (1), Xenia Martin Latypova (1), Benoite Champion (1), Anais Rungoat (1), Flavien Charpentier (1), Gildas Loussouarn (1), Kazem Zibara (2), Patricia Lemarchanda (1), Nathalie Gaborita (1)

(1) CHU Nantes, Institut du Thorax, INSERM UMR 1087-CNRS UMR 6291, Nantes, France – (2) Doctoral School of Science and Technology (DSSST-PRASE), Lebanese university (UL), Beirut, Liban

Rationale: Human genetically inherited cardiac diseases have mainly been studied in heterologous systems or animal models, independently of the patients’ genetic background. Because sources for human cardiomyocytes are extremely limited, the use of urine samples to derive cardiomyocytes would be a non-invasive method to identify cardiac dysfunctions that lead to pathologies within the patients’ specific genetic background.

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