TOPIC 16 – Electrophysiology, arrythmias and pacing – D

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Aberrant splicing of SCN5A and cardiac sodium current dysfunction in DMSXL transgenic mouse model of Myotonic Dystrophy

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Background: Myotonic dystrophy type 1 (DM1) cardiac manifestations include commonly cardiac arrhythmias and rarely dilated cardiomyopathy. Beyond the CTG expansion, the mechanisms involved are unknown. Similar phenotypes have been described in cardiac sodium channelopathies and referred as overlap syndromes. Here we tested the hypothesis that abnormalities in the cardiac sodium current are involved DM1.

Methods: In a transgenic mouse model of DM1 reproducing the CGT expansion, we performed at 3 and 8 months: i) surface electrocardiograms (ECG); and ii) cardiac echocardiography with analysis of tissular velocities and strain rate before and after administration of the sodium current blocker flecainide. Ventricular action potentials were recorded using microelectrode technique, and sodium current was recorded using whole cell patch-clamp technique. Tissular slices of ventricles were stained with Nav1.5 antibody, and picrosirius red to measure cardiac fibrosis. We studied SCN5A splicing on myocardial tissue.

Results: At 3months DM1 and WT mice had similar ECG and cardiac echo at baseline. Flecainide injection lowered significantly more the tissular velocities and strain rate in DM1 mice. At 8 months, tissular velocities and strain rate were slower in DM1 mice than in WT at baseline and flecainide injection induced bradyarrhythmias in 60% of DM1 mice vs none in WT ($p< 0.05$). In 8 months mice AP recordings showed that the maximum upstroke velocity was slower in DM1 ventricular myocytes (dV/dtmax: 99±32 vs 116±34V/s; p=0.03). The kinetics of sodium current inactivation were faster in DM1 (T1/2, (-20 mV): 1.4±0.5 vs 1.8±0.4 ms; p=0.01). Ventricular staining showed similar Nav1.5 distribution in DM1 and WT, with no significant fibrosis. We found abnormal splicing of SCN5A exon 18, which was truncated.

Conclusions: In an original mouse model of DM1, we provided evidences for alterations of sodium current that could contribute to both electrical disturbances and ventricle dysfunction and may be related to missplicing of SCN5A. DM1 could be another example of overlap syndrome due to sodium channelopathy.

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Multiple roles of TRPM4 channel in heart

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Aim: TRPM4 is a Ca^{2+}-activated non selective channel expressed in atrium, conduction tissue but not in ventricle. Recently, several studies demonstrated that gain-of-function mutations in TRPM4 induced conduction disturbances in human. Nevertheless, the role of the TRPM4 channel in heart physiology remains to be elucidated. Here, we took advantage of a mouse in which Trpm4 gene is invalidated (*Trpm4*$^{-/-}$) to investigate the effect of TRPM4 channel in heart physiology in comparison with *Trpm4*$^{+/+}$ mice.

Results: We demonstrated that *Trpm4*$^{-/-}$ mice displayed increased left ventricle (LV) wall thickness and dilation, associated to better stroke volume and cardiac output, suggesting eccentric hypertrophy. This higher LV mass was due to an increase of cellular density. To link myocellular density to proliferation, hyperplasia was assessed in neonate hearts. Positive mitotic nuclei were 3-fold increased in *Trpm4*$^{-/-}$ mice in ventricle but not in auricle. In order to determine the effect of eccentric hypertrophy in electrical propagation, we performed electrocardiograms (ECGs) analysis in freely moving mice. *Trpm4*$^{-/-}$ mice exhibited multilevel blocks in surface ECGs, validated by intracardiac explorations, associated to ectopic atrial activities. While the increase in cellular density could explain infrahisian conduction lengthening, it did not in suprahisian level. However, we showed a mRNA level increased of connexin 30.2, known to decelerate influx. We also demonstrated that atropine reduced Luciani-Weckenbach atrio-ventricular blocks, suggesting parasympathetic overdrive. We finally performed action potential (AP) measurement in cardiomyocytes from atria and left ventricle using the patch-clamp technique. We showed that *Trpm4*$^{-/-}$ atrial cardiomyocytes displayed shorter AP whereas there is no difference in ventricular cardiomyocytes.

Conclusion: TRPM4 seems to play multiple roles in the heart, including the regulation of heart size, conduction and cellular electrical activity.

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CASK, a new partner of sodium channel Nav1.5 in the heart

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MAGUK proteins are implicated in the anchoring and scaffolding of macromolecular complex in the sarcolemma. CASK differs from other MAGUK by an N-terminal calcium/calmodulin-dependant protein kinase domain, a single PDZ domain and its nuclear translocation. However, little is known on CASK in the heart except that the gene is expressed. We found that CASK was expressed at a protein level in rat and human cardiomyocytes. Immunostainings of myocardial cryosections revealed that CASK belongs to the costamere at the lateral membrane where it colocalized with syntrophin/dystrophin but not with vinculin, two components of adhesion costamere complexes. In MDX mice hearts which lacks dystrophin, CASK was no longer expressed at the lateral membrane, confirming that CASK is a part of the syntrophin/dystrophin costameric complex. Moreover, CASK and dystrophin co-precipitated. We previously showed that two pools of Nav1.5 channels are present in cardiomyocytes: one at the intercalated discs interacting with the MAGUK, SAP97 and a second at the lateral membrane interacting with the syntrophin/dystrophin complex. Here, we examined whether CASK could be implicated in the targeting of Nav1.5 channels at the lateral membrane. First, GST pull down experiments performed on heart lysat showed that CASK interacts with Nav1.5. Second, immunostainings revealed that CASK and Nav1.5 colocalizes at the lateral membrane of myocytes. Third, in a HEK cell line stably expressing Nav1.5 channels, CASK silencing enhanced sodium current (INa) while CASK overexpression decreased INa. In cultured rat atrial myocytes silenced for CASK, INa was also enhanced.

Conclusion, CASK shows unique cell localization among the MAGUK family at the costamere level where it interacts with dystrophin. It is a new partner of the subpopulation of Nav1.5 channels of lateral membrane that inhibits their functional expression.

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In vivo role of the PDZ domain-binding motif of the cardiac sodium channel Nav1.5: mouse and human findings

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Background: The sodium channel $Na_V1.5$ initiates the cardiac action potential (AP) and is essential for conduction. The last three residues of $Na_V1.5$ (**S**er-**I**le-**V**al) constitute a PDZ domain-binding motif (SIV) that interacts with PDZ proteins such as syntrophin and SAP97, at different locations of the cardiomyocyte, defining distinct subsets of $Na_V1.5$ multi-protein complexes. Here, we investigated the *in vivo* role of this $Na_V1.5$ SIV motif by characterizing mice with a truncation of this motif (ΔSIV), and screening for genetic variants in arrhythmia patients.

Methods and Results: Using a proximity ligation assay, we observed loss of interaction between $Na_V1.5$ and syntrophin at lateral membranes of cardiomyocytes that was verified in immunostainings to result from loss of lateral membrane $Na_V1.5$ expression. This was consistent with a 60% decrease in sodium current (I_{Na}), recorded at the lateral membrane of cardiomyocytes. However, $Na_V1.5$ staining remained at intercalated discs (ID) and T-tubules, and no decrease was observed in I_{Na} recorded at the ID. Western blots of ΔSIV hearts displayed reduced levels of $Na_V1.5$ which corresponded to a 35% decrease in cardiomyocyte whole-cell I_{Na} and AP upstroke velocity. Epicardial mapping of ΔSIV hearts displayed decreases in conduction velocity (CV) that manifested as prolongation of the QRS interval in ECGs. Transversal CV was preferentially affected. Furthermore, an $Na_V1.5$ mutation in the SIV motif (p.V2016M), which was found in a Brugada Syndrome patient, displayed decreased I_{Na} and surface expression in transfected HEK293 cells. Pull-down experiments showed that V2016M disrupts interaction of $Na_V1.5$ with SAP97.

Conclusions: These data reflect the *in vivo* significance of the PDZ domain-binding motif of $Na_V1.5$ in protein expression and cardiac function. The data support the model of distinct pools of $Na_V1.5$ which are differentially regulated by interacting proteins that reside in separate membrane compartments of the cardiac cell.

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Leptin treatement modulates electrophysiological remodeling in experimental heart failure

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Obesity has been established as a risk factor for the development of heart failure (HF). The role of adipose tissue with its secretory products (adipokines) as an active endocrine organ is gaining importance in the investigation of obesity-associated diseases. One of the adipokines that has been postulated as a link between obesity and HF is leptin. Leptin is a 16-kDa adipocyte-derived protein hormone, encoded by the ob (obesity) gene. Investigation into whether leptin exerts beneficial or detrimental effects on cardiovascular function has yielded paradoxical observations. Here we analyzed leptin effects on experimental HF. Mice were subjected to thoracic aortic stenosis (TAC) to induce HF or to sham-operation (controls). 3 weeks after surgery, each group was divided into 2 sub- groups. Osmotic minipumps were implanted subcutaneously to all, half of the animals filled with leptin and the other half with saline solution. 3 weeks after initiation of treatment, animals were echocardiographically analyzed and then sacrificed for patch-clamp experiments. Action potentials were analyzed in current clamp mode and potassium currents in voltage clamp mode. Special attention was given to the appearance of Early After Depolarizations (EADs) and/or Delayed After Depolarizations (DADs). TAC procedure induced a significant decrease in the ejection fraction, which was not ameliorated by leptin treatment. Action potential duration (APD) was prolonged in TAC animals at all repolarization periods, and frequently presented EADs. At 50% of repolarization, Leptin treatment reduced the DPA prolongation in TAC animals. This was correlated with a decrease in the potassium current IKs in HF. Thus although leptin did not ameliorated the cardiac function assessed by echocardiography, it did ameliorated electrophysiological remodeling.

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Oxidation of DHA is responsible for its anti-arrhythmic effects on mouse ventricular myocytes

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Since forty years, it is known that long-chain polyunsaturated fatty acids of the series n-3 (PUFAs) have cardioprotective effects by preventing cardiac arrhythmias. The main PUFAs are eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA) that are highly peroxidable due to the presence of the skipped dienes. The effects of n-3 PUFA on cardiac function are still debated, notably because of the lack of information on the mechanisms involved. For example, it is not really known which the active lipid is: the PUFA or one of its oxygenated metabolites. A diet enriched in n-3 PUFAs (mainly fish-based), leads to enrichment in these fatty acids of cardiac cell membranes. Our hypothesis is that, during an infarct, the oxidative stress and the generation of reactive oxygen species might be responsible for an oxidation of membrane-bound PUFAs and the oxygenated metabolites generated might modulate the activity of ionic channels to exert anti-arrhythmic effects. We thus decided to investigate the influence of the peroxidation of DHA on its potentially anti-arrhythmic properties. In this study, we applied DHA free acid or DHA methyl ester (ME) (less sensitive to peroxidation) on freshly isolated mouse ventricular myocytes without or with α-tocopherol (Vitamin E, to prevent oxidation) or hydrogen peroxide (to enhance oxidation). We investigated, using a Photometric system (IonOptix®), calcium transients (using the ratiometric calcium fluorescent dye Indo-1) and cell shortening of electrically stimulated myocytes. By stimulating β-adrenergic pathways with 10 nM isoproterenol, it is also possible to observe the appearance of arrhythmic events. We observed that DHA free acid reduced the percentage of arrhythmic cells but not DHA ME. These effects of DHA are correlated with the peroxidation of the fatty acid since α-tocopherol prevented the anti-arrhythmic effects while hydrogen peroxide enhanced them. These results suggest thus that rather than DHA itself, it is the oxygenated metabolites derived from DHA that are potentially anti-arrhythmic.

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Reduction of APD90 dispersion and ventricular arrhythmias by aldosterone blockade in an in vitro model of "border zone"

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Background: Sudden cardiac death (SCD) is principally due to ventricular arrhythmia (VA) occurring during the acute phase of ST elevation myocardial infarction (STEMI). In this context, many studies have shown the benefit to use aldosterone blockers. However, actually the mechanisms remain unknown. We propose a new in vitro model using rabbit ventricle, mimicking the border zone between a normal zone (NZ) submitted to normoxia and an altered zone (AZ) submitted to an ischemia followed by a reperfusion, to study electrophysiological effects induced by aldosterone and potassium canrenoate.

Method and Results: During simulated ischemia and on account of an increase of the action potential duration dispersion at 90% (APD90) between NZ and AZ, aldosterone increased VA occurrence. These deleterious effects were prevented by adding potassium canrenoate which also increased conduction block occurrence.

Conclusion: Aldosterone increased APD90 dispersion between NZ and AZ and VA occurrence. These results support recent clinical studies underlying an interest in precocious aldosterone blockade in STEMI context.

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Dronedarone is superior to amiodarone in preventing ventricular arrhythmias in an in vitro model of "Border Zone"

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Background: Ventricular arrhythmias (VA) are the most important causes of mortality in patients with heart failure and within the first days after ST elevation myocardial infarction. To date, amiodarone is the more effective antiarrhythmic drug (AAD) in these contexts but unfortunately, extra-cardiac side effects are limited its use. Dronedarone, a non-iodinated benzofuran derivative of amiodarone, has not been studied on ventricular cardiomyocytes and in the prevention of VA. We propose an *in vitro* model using rabbit ventricle, mimicking the "border zone" existing between normal and ischemic/reperfused regions, to study electrophysiological effects induced by dronedarone and amiodarone.

Method and Results: Like amiodarone, dronedarone has multichannel blocking properties and affected action potential parameters and decreased spontaneous arrhythmias occurrence. Both did not have proarrhythmic effects in this model. In comparison with amiodarone, dronedarone was superior in preventing spontaneous VA occurrence during both simulated-ischemia and reperfusion periods. Both dronedarone and amiodarone systematically induced conduction blocks during simulated ischemia period.

Conclusion: In this *in vitro* model mimicking the "border zone", we have shown that dronedarone has electrophysiological effects comparable to those of amiodarone but was more effective than amiodarone in preventing VA occurrence. Dronedarone did not show proarrhythmics effects. Our data suggest that the acute effects of dronedarone, despite absence of iodine in its molecular structure, seem to be very similar to those of amiodarone in cardiac ventricle and so this AAD could be an interesting alternative to amiodarone in preventing VA, particularly on ischemic cardiomyopathy. These conclusions need to be confirmed by clinical studies.

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Mitochondrial sensitivity to ADP in permeabilized fibers changed as a function of cardiac muscle compartments: left/right atria and ventricles of sheep heart

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Because of the balance between ATP supply and ATP demand, alterations in cardiac energetics potentially affect cardiac work and may explain pathologies. Neverthelesss energetics alterations in each compartment remain unknown. Since mitochondrial oxidative phosphorylation is the main process of energy supply, mitochondrial sensitivity to energetic intermediates (e.g. ADP) is here investigated in a compartment-specific way.

KmADP were obtained in sheep permeabilized fibers of right and left ventricles and atria using O2 consumption measurements (OROBOROS Oxygraph-2k), carried out in Mir05 with glutamate (5 mM) and malate (2 mM), at 25°C.

Basal respiration (V_0) and maximal ADP-stimulated respiration (V_{max}) were similar among atria or ventricles (mean V_0=33±3.9 and mean V_{max}=72 pmol.sec^{-1}.mg^{-1} dry wt). Interestingly, K_mADP were significantly higher in atria, especially in right one, than in ventricles, that were consequently characterized by a 1.5 to 3-fold higher sensitivity to ADP compared to left and right atria.

In beating heart, for a given energy state of the cell, mitochondria are more responsive in ventricles than in atria and more responsive in the left than in the right atrium. Interestingly mitochondrial responsiveness is likely in adequation with requested work in each chamber of the healthy heart and may be closely related to the ventricle ability to withstand problems of arrhythmias.