L'angiotensine II et le TNFα ont été impliqués dans la progression de l'insuffisance cardiaque. Nous avons observé dans le myocarde défaillant post-infarctus une surexpression de l'angiotensinogène et du TNFα selon un gradient régional correspondant à celui de l'expression de Kir6.1. Ainsi l'expression de l'angiotensinogène et du TNFα corrélait positivement avec celle de Kir6.1 et négativement avec celle de Kir6.2 dans les différentes zones du myocarde post-infarctus. Nous avons observé dans des cardiomyocytes isolés normauxexposé à de l'angiotensine II ou du TNFα un profil d'expression des sous-unités KATP similaire à celui observé dans le myocarde défaillant post-infarctus, caractérisé par une surexpression de Kir6.1 et des sous-unités SUR et une répression de Kir6.2. Les cardiomyocytes exposé à de l'angiotensine II ou du TNFalpha montraient en réponse au diazoxide un courant KATP prononcé et un raccourcissement du potentiel d'action.

Nous avons confirmé que l'angiotensine II induisait in vitro l'expression du TNFα dans les cardiomyocytes. Qui plus est, l'expression régionale de l'angiotensinogène dans le myocarde défaillant post-infarctus corrélait positivement avec celle du TNFα. Enfin, la plupart des effets de l'angiotensine II sur l'expression des sous-unités KATP étaient réduits en présence d'un anticorps neutralisant le TNFα.

En conclusion nous avons identifié l'angiotensine II et le TNFα comme des médiateurs du remodelage des KATP dans la défaillance cardiaque. Ce modèle sera utile pour analyser les mécanismes moléculaires régissant l'expression des sous-unités KATP dans la défaillance cardiaque.

G017
FACTORS ASSOCIATED WITH THE INDUCTION OF ANTIDROMIC TACHYCARDIA IN THE WOLFF-PARKINSON-WHITE SYNDROME

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Atrioventricular reentrant tachycardia (AVRT) is the most frequent inducible tachycardia in patients with a Wolff-Parkinson-White syndrome (WPW). The incidence and the causes of the induction of antidromic tachycardia (ATD) are unknown. The purpose of the study was to determine the data of patients with a WPW and with inducible ATD.

Methods — 605 patients had a WPW and tachycardias (n=312) or syncope (n=85); other patients were asymptomatic (n=208). Electrophysiological study (EPS) was systematic. In control state (CS), the higher rate conducted through accessory pathway (AP) was measured; programmed atrial stimulation with 1, 2 extrastimuli was performed to induce a tachycardia. Isoproterenol (0.02 to 1 µg. min-1) was infused and the protocol was repeated.

Results — ATD was induced in 44 patients (7%) (group I). Their data were compared to those of remaining patients (group II). Group I differed from group II by the following data: Female sex was less frequent in group I (29.5%) than in group II (47%); AP was more frequently left sided in group I (54.5%) than in group II (38%) ($p<0.05$). AVRT was induced less frequently in group I (34%) than in group II (57%) ($p<0.01$); maximal rate conducted through AP was higher in group I (215±52 b/min) than in group II (189±61) in control state, and after isoproterenol (281±57 in group I vs 236±61 in group II) ($p <0.001$). Some data were similar: Age was not different in group I (33.5±20 years) and II (34.5±17); the indications of EPS were similar (syncope, reentrant tachycardia, atrial fibrillation (AF) or asymptomatic WPW were the reasons for 16%, 43%, 11% and 25% of group I patients and 14%, 46%, 5.5% and 35% of group II patients); posteroseptal and right AP locations were similar in both groups; AF was induced as frequently in group I (27%) as in group II (23%).

Conclusions — antidromic tachycardia was induced more frequently in men than in women, with a left lateral AP which conducted more rapidly than in other patients.

G018
EVALUATION OF IKR BLOCKING PROPERTIES OF DIFFERENT MOLECULES WITH OR WITHOUT TORSADOGENIC PROPERTIES

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Currently, industrials and regulatory authorities are worried by Torsades de Pointes (TdP), a type of ventricular tachycardia, which can lead to sudden death. The most recent guidelines from the International Committee of Harmonization, recommend to assess properly the risk of TdP, by different approaches, among others an in vitro method. This method consists on studying the blockade of a voltage-dependant potassium channel, called hERG. Indeed, hERG channel, responsible for the IKr current, seems to be blocked by the majority of the torsadogenic molecules and, is thus considered as an important marker of pro arrhythmic risk.

CERB developed a bio-computerized database named TdPScreen® to predict the risk of TdP. Known molecules are classified according to their pro arrhythmic potential, from group A to C. The group A corresponds to drugs with numerous or several reports of TdP, the group B to compounds causing QT prolongation with TdP at very low frequency; and in group C to drugs with no report of TdP or QT prolongation. This database suggests that other factors than a single blockade of IKr could be involved in the genesis of drug-induced TdP.

We performed experiments in patch-clamp using HEK cells expressing stably the hERG channel. Different compounds from the different groups, above mentioned, were evaluated for their IKr blocking potency and compared to the TdPScreen® database.

Results show some torsadogenic drugs might exhibit very low IKr blocking properties (e.g. D-sotalol), whereas other non-torsadogenic drugs are potent IKr inhibitors (e.g. verapamil, diltiazem…).

These results, and others, indicate that drugs can block hERG current without any influence on TdP appearance.

We conclude that assessing pro arrhythmic potential of compounds, only on the blocking effects of IKr, in vitro, can lead to the eviction of interesting molecules.

G019
CHOLESTEROL DEPLETION ENHANCES KV1.5-ENCODED K+ CURRENT BY INCREASING RAB11-MEDIATED RECYCLING

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Membrane lipid composition is a major determinant of protein organisation in the cell membrane. In a previous study, we reported that depletion of membrane cholesterol by methyl-ƒÒ-cyclodextrin (MCD) causes a marked increase in Kv1.5-current (Ikur) in neonatal cardiac myocytes. Here, we examined the mechanisms of the cholesterol effects on potassium current in adult rat cardiomyocytes (ARC). GFP-tagged Kv1.5 channels were transduced in ARC using adenoviral vectors and patch clamp experiments were performed to record whole-cell currents and single channel activity. Fluorescence recovery after photobleaching (FRAP) technique was used to investigate GFP-Kv1.5 channels mobility; 3D-epifluorescence microscopy was conducted to follow Kv1.5 channels trafficking.

In both freshly isolated and cultured ARC over-expressing GFP-Kv1.5 channels, MCD induced a rapid (< 7 min) increase in Ikur but not Ito. On the contrary, incubation with the cholesterol donor LDL reduced Ikur. Single channel experiments revealed that MCD application caused a progressive and drastic increase of the number of active channels. Moreover, FRAP experiments showed that MCD reduced both mobility and recovery of GFP-Kv1.5. Several steps of the trafficking process of ion channels were studied. Blocking SNARE-mediated exocytosis with N-ethylmaleimide prevented the MCD-effect on Ikur. While disruption of Golgi complex/secretion pathway with brefeldine-A had no effect, manipulation of GTP-ases activity with GTP-ƒ×-S suppressed the MCD effect. Transfection with a dominant negative (DN) form of Rab11 effect but not Rab4 DN prevented the MCD. Moreover, Kv1.5 channels co-immunoprecipitated with Rab11 which is stringly expressed in myocardium and ARC (qPCR and western blot). Finally, 3D-microscopy evidenced that Kv1.5 channels association with Rab11-positive recycling endosomes observed in control condition disappeared following cholesterol depletion.

Conclusion — Lowering cholesterol rapidly induces the insertion of Kv1.5 channels by a process that involves vesicle fusion and trafficking processes, particularly the Rab11-associated slow recycling pathway. Given the role of Kv1.5 channel in normal and pathological atrial electrical properties, this study opens news perspectives for therapeutic modulation of cardiac myocytes excitability.

G020
PROPERTIES OF PULMONARY ARTERY SMOOTH MUSCLE FROM TASK-1 KNOCKOUT MICE

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In pulmonary artery (PA), compelling evidence have suggested that TWIK-related Acid Sensitive K+ -1 (TASK-1) channel contribute to the background K+ current that support the membrane potential (Em) of the smooth muscle cells. However, due to poor selectivity of its modulators, the role of TASK-1 channel in the functional regulation of tone in pulmonary arteries remains elusive. The purpose of this study was to investigate the properties of PA isolated from mice in which the TASK-1 and TASK-3 genes have been deleted (TASK1/3 knockout, KO), compared with their wild type controls (C57BL/6 mice, WT).

Intra-PAs were isolated from adult male WT and KO mice. Vessels were mounted on a wire myograph for isometric tension recordings. PAs were also processed for gene expression studies using RT-PCR.

The addition of 10mM KCl, the K+ channel modulators 4-AP (1mM) and levcromakalim (10 µm), or the L-type calcium channel blocker nifedipine (1 µm), did not elicit any significant contractile response in PAs from either KO (N=3) or WT mice (N=3), suggesting that the Em is not depolarised and that artery is fully dilated in both groups. The contractile response of PA to phenylephrine, serotonin and PGF2α did not show any difference between the two strains. Potency (pEC50) of the prostacyclin analog treprostinil to induce relaxation on preconstricted vessels was significantly higher in KO mice (6.7 ± 0.1, N = 3) than in WT mice (7.2 ± 0.1 N = 4).

RT-PCR confirmed TASK-1 expression in PA from WT but not KO mice whereas TASK-3 was not expressed in either WT or KO mouse PA. TASK-2, TASK-5, TWIK-2, Kv1.5 and Kv2.1 RNA levels did not show any significant alteration between WT (N = 3) or KO mice (N = 3).

The PA isolated from KO mice does not display any resting tone or altered responses to the studied agonists. Surprisingly, response to treprostinil was increased, and TASK-1 absence in PA was not compensated by alteration of other K+ channels expression. The results thus far do not support a major physiological role for TASK-1 in mouse PA.

G021
PAINLESS, CANAL TRPA DE LA DROSOPHILE, JOUE UN RÔLE MAJEUR DANS LE COUPLAGE MÉCANO-ÉLECTRIQUE CARDIAQUE

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Le cœur est soumis à différentes tensions qui interviennent au niveau cellulaire pour réguler l'activité cardiaque. Parmi ces forces, on peut noter l'étirement membranaire au cours de la contraction, les forces de cisaillement dues au flux de sang ou l'étirement des membranes lors du gonflement cellulaire osmotiquement induit. Tous ces stimuli mécaniques sont convertis en signal électrique, le plus souvent par une entrée de cations dans la cellule, susceptible de moduler l'activité électrique de la cellule.

Nous avons mis en évidence chez la Drosophile, l'implication de PAINLESS, canal TRPA, dans le couplage mécano-électrique de la cellule cardiaque. Au cours de la dépolarisation membranaire, PAINLESS est responsable d'une entrée de charges calciques qui prolonge la dépolarisation et régule la fréquence cardiaque. En plus de son rôle dans le couplage excitation-contraction, PAINLESS semble également impliqué dans la régulation de l'activité cardiaque lors des mouvements de la larve. Le cœur de la drosophile a la particularité de s'arrêter quand la larve se contracte pour avancer. Ceci est sans doute dû à une augmentation de la pression autour du cœur ou à un étirement direct des cellules cardiaques par les muscles d'attachement. La perte de cette réponse chez les mutants painless, et les différentes expériences mimant ces stimuli (étirement membranaire, variation de pression) menées sur le cœur in situ, confirment l'implication de PAINLESS dans la transduction du signal mécanique en signal électrique.

En conclusion, il apparait que PAINLESS est impliqué dans la régulation de l'activité cardiaque au cours de stimuli mécaniques et qu'il est un élément majeur de la voie de transduction du signal mécanique.