

Abstract n° P-21-16**Characterization of novel voltage-sensitive dyes for cardiac applications**

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The field of cardiac electrophysiology has greatly benefited from the development of voltage-sensitive dyes (VSD) allowing for high resolution imaging of membrane potentials from the heart surface. Conventional dyes have excitation spectra in the blue/green range [1], which limits the depth of penetration due to high absorbance and photon scattering properties. Applications in the near-infrared, which has better depth penetration, remain relatively limited. However, ventricular myocardium may be up to 2 cm thick in larger mammalian species. Furthermore, VSDs are limited by the signal:background ratio and signal stability.

Our study sought to develop and assess novel families of VSDs with a focus on large Stoke shifts and near-infrared spectral properties. Three VSDs were synthesized from two heterocyclic fluorophores and their properties assessed in intact rat hearts. Spectral properties were assessed for each dye. Signal:background ratios and signal kinetics where measured in epifluorescence imaging experiments.

Optimal peak emission wavelengths for all dyes was found between 700 nm and 750 nm. The largest observed Stoke shift was found with fluorophore 1 family ($\lambda_{exc} = 476$ nm and $\lambda_{em} = 730$ nm). Signal background ratios (Fig.1) ranged from 3% (fluorophore 2) to 7% (fluorophore 3), compared to 5.5% for the conventional dye, di-4-ANEPPS [2].

	DF/F (%)	APD ₅₀ (ms)
DI-4-ANEPPS	5.5	45 ± 1.8
Fluorophore 1	6.5	45 ± 1.8
Fluorophore 2	< 3	48 ± 2.4
Fluorophore 3	7	55 ± 3.7

Figure 2. Signal background ratios of novel VSDs compared to the conventional dye, di-4-ANEPPS and representative experimental optical APD80 values.

Rapid signal decay indicative of internalization was observed for one VSD. However, in other two, signal stability was improved compared to di-4-ANEPPS.

Novel families of VSDs were characterized with emission wavelengths shifted to the red. Overall, these dyes show improved voltage sensitivity and show potential for application in cardiac optical mapping.

[1] Matiukas, A., et al., Heart Rhythm, 2007. 4(11): p. 1441-1451.

[2] Walton, R.D., et al., Heart Rhythm, 2010. 7(12): p. 1843-1849.

Abstract n° P-22-02**Gender Differences in the Reaction of Autonomic Nervous System to Remote Ischaemic Preconditioning in Young Healthy Subjects**

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Remote ischemic preconditioning (RIPC) has been shown to be cardioprotective in clinical settings. However, the mechanisms underlying this effect, including neural input, are not presently known. Here we investigate the response of autonomic nervous system (ANS) to RIPC in men and women using the heart rate variability (HRV) assay.

RIPC was performed on healthy male and female individuals (20-30 years old) by 3 cycles of 4 min inflation to 200 mmHg and 5 min deflation of the cuff fastened on the upper limb. A mental load (the Serial Sevens Test) and hyperventilation were carried out prior to and after the RIPC protocol. HRV analysis included frequency measurements ranged from 0.01 to 0.35 Hz. Electroencephalography (EEG) and laser Doppler flowmetry were also recorded before and after RIPC prior to the mental load and hyperventilation.

Reactive hyperemia associated with RIPC was more pronounced in women. RIPC significantly reduced HR and augmented alpha and theta activity of EEG both in men and women indicating parasympathictonia. RIPC itself had no effect on the spectral characteristics of HRV in women but significantly increased the indices of low and very low frequency in men. RIPC increased the values of HRV indices of very low frequency during the mental load and hyperventilation both in men and women. However, these parameters were still maintained at higher level in men. These results reveal for the first time considerable gender differences in the reaction of ANS to RIPC. RIPC was characterized by activation of both sympathetic and parasympathetic systems in men with predominant role of the central mechanisms of autonomic regulation. In contrast, the activity of parasympathetic system prevailed in women with relatively low influence of the central mechanisms.

Abstract n° P-22-03**Gender differences in morphological and functional aspects of exercise-induced left ventricular hypertrophy in a rat model**

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Background: Long-term exercise training is associated with characteristic alterations of the myocardium (athlete's heart). Referring to the latest studies, sex hormones may be involved in the regulation of exercise-induced left ventricular (LV) hypertrophy. We aimed at understanding the gender-specific functional and morphological alterations in the LV and the underlying molecular changes in a rat model of athlete's heart.

Methods and results: We divided our young, adult male and female rats into control and exercised groups. Athlete's heart was induced by swim training (200 min/day swimming for 12 weeks). Following the training period we assessed LV hypertrophy with echocardiography. LV pressure-volume (P-V) analysis was performed to investigate in vivo LV function. Molecular biological studies were performed. Interaction between gender and training was tested by two-way ANOVA. LV hypertrophy estimated by echocardiography and post-mortem heart weight data was more pronounced in females. The induction of Akt signaling was more significant in females (p-Akt/Akt ratio: +57.7% female vs. +21.4% male, $p < 0.05$). Suppressed phosphorylation of Erk was observed in female exercised rats, but not in male ones. α -myosin heavy chain (MHC)/ β -MHC ratio did not differ in males, but increased markedly in females