Neuronal control of human cardiac system: modulation by bioactive lipids (omega-3 and PGE_2)

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1) Background:

Arrhythmia is defined by an abnormal rhythm of the heart. In fact, the physiological heart rhythm is initiated by the sinus node that sends the electrical impulses to the atrioventricular (AV) node. From this node extend the bundle of His that carries the electrical signals to ventricles via the Purkinje fibers. This cardiac cycle leads to the ventricles contraction involving trabeculae and papillary muscles[1,2].

The production of the action potentials is influenced by (a balance between) two sets of nerves: the parasympathetic (vagus nerve) and sympathetic nervous system releasing acetylcholine (ACh) and noradrenaline (NA), respectively[3,4]. The change in the production of the action potentials produces abnormal rhythms (bradycardia / tachycardia)[5,6]. Despite different therapies, various side effects exist and arrhythmias associated with heart failure (HF) remain the main cause of sudden cardiac death[7,8]. Another therapeutic approach could be linked to recent observations on bioactive lipids: Electrical vagus stimulation increased omega-3 production and decreased prostaglandins and leukotrienes[9]. Moreover, paradoxical and protective effects of Prostaglandin (PG) E_2 in cardiac remodelling are described in HF.

The omega-3 such as eicosapentaenoic (EPA), docosahexaenoic (DHA) and docosapentaenoic (DPA) acids and their metabolites called SPM [Specialized Pro-resolving Mediators: maresins (MaR), resolvins (Rv) and protectins D (PD)] are mostly involved in the resolution of inflammation[10]. However, there are few studies on human cardiovascular[11–13] and even less on the neuronal systems[9]. In our preliminary and published studies we found that (RvD5 and MaR1) are highly produced by human left ventricle[14] and they reduce the PGE_2 induced contractions in human coronary arteries[13]. Clinical studies have shown that DHA or EPA lower heart rate[15]. While omega-6, like PGE_2 produced by cyclooxygenase-2 under inflammatory conditions, are enhanced in cardiac myocytes and other cell types of patients with HF[16].

However, to our knowledge, the *ex-vivo* effects of omega-3 metabolites and PGE_2 in the human heart neuromodulation have not yet been investigated.

2) Aims of the project:

First we will measure the omega-3 released by human heart neurons and node cells with or without PGE_2, then we will select 2-3 SPM of interest and we would like to **test the effect of**:

a) omega-3, selected SPM or PGE_2 on the neurotransmitter release (ACh, NA) in human hearts innervated tissues (sinus and AV nodes cells/neurons, purkinje fibers, trabeculae and papillary muscles) under normal or inflammatory conditions (preparations derived from HF patients or treated with TNF-α).

b) EPA, DHA, DPA, and selected SPM on cardiac muscles contractions. The effect of the omega-3 on human cardiac muscles has not been investigated. We will extend this aim by studying the effect of SPM on cardiomyocytes and fibroblasts under inflammatory conditions.

c) omega-3 and selected SPM with or without PGE_2 on the physiological response of isolated rat heart. The effect of the selected SPM will be investigated in Langendorff system on perfused rat hearts after a pharmacological induction of arrhythmia. The heart electrical activity will be monitored using an electrocardiogram (ECG)[17]. We will also compare the effect of the omega-3 on the rat heart contractions.

3) Methods:

The experiment will be done by using human heart samples obtained at Bichat, Saint-Louis, Saint-Denis and Rothschild hospitals (with informed consent of the patient and agreement from the ethic advisory boards).

Ex-vivo pharmacological studies (organ bath system), the heart samples (sinus node, AV node, trabeculae and papillary muscles) used for electrical stimulation (EFS: Electric Field Stimulation) will be dissected within one

hour following surgery and set-up in our organ bath system[18–20]. In addition, fibroblast cell culture and isolation of cardiomyocytes will be performed.

In-vitro experiments: Western blot, Immunohistochemistry and/or Real-time PCR, (enzymes, receptors), ELISA (NE, PGE_2), mass spectrometry LC-MS (SPM), Varioskan/chemiluminescence (ACh).

In addition, the *ex-vivo* animal model (Langendorff technique) with perfused rat hearts will be used (collaboration with the Centre Chirurgicale Marie Lannnelongue or UMRS1180).

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