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Dear members of the ESC Council,

Thank you very much for awarding me with the *First Contact Initiative Grant*, which enabled me to make an extraordinary scientific experience at the University of California San Francisco, UCSF. My host PI, MD Marco Conti, and his co-workers possess exceptional knowledge in the field of phosphodiesterase biochemistry and the laboratories are well equipped. I am thankful to Professor Conti and his co-workers, especially Dr. Delphine Mika, for their incredibly great hospitality and their willingness to let me participate in their day-to-day lab routine.
During my time at the UCSF, I furthermore was able to attend several seminars to achieve an impression of the high profile scientific community clustered at the Bay Area.
The *First Contact Initiative Grant* allowed me to tap this highly stimulating scientific environment to enrich my own knowledge, establish new contacts for future collaborations but importantly to improve some of my methods that were initially established in the Conti Labs.
To grant me this multipurpose opportunity I express my honest gratitude to the ESC Council members.

Sincerely yours,

Ruwan Perera

Report on the Outcome

Background:

3',5'-cyclic adenosine monophosphate (cAMP) is a highly versatile ubiquitous second messenger. In the heart, beta-adrenergic receptor (β-AR) mediated cAMP signaling requires complex yet rigorous control mechanisms in order to elicit beneficial prompt increases in cardiac output, at the same time preventing cardiac dysfunction evoked by excessive stimulation. Over the last 30 years of groundbreaking research, the paradigm of cAMP compartmentation, according to which cAMP shall be confined in subcellular microdomains, has increasingly gained acceptance. Unlike cell organelles, cAMP microdomains are not confined by physical barriers. Uncontrolled diffusion of local cAMP pools is rather prevented through directed and localized degradation by cAMP-hydrolyzing enzymes phosphodiesterases (PDEs). Therefore, cAMP microdomains may change more dynamically than rigid cellular structures in response to cardiac stress and disease.

Out of the five cAMP-hydrolyzing PDE families being expressed in rodent cardiomyocytes (PDE1, 2, 3, 4, 8), PDE4, encoded in three individual genes (*pde4a, -b and -d*), by far contributes most isoforms and splice variants to total cAMP hydrolytic activity. Importantly, these genes are highly conserved, also in human myocardium, underscoring the importance of PDE4 for normal β-AR-cAMP signal regulation. Cardiac PDE4 activity has repeatedly been reported to prevent stress-induced arrhythmias in both rodent and human myocardium[1-3]. Especially PDE4B and PDE4D have been linked to multiple functionally relevant cAMP microdomains. For instance, PDE4B is implicated in regulation of the L-type calcium channel (LTCC) current and feedback regulation of β_1-ARs[2,4], whereas PDE4D isoforms might functionally associate with cellular calcium release and re-uptake units[1,5]. Moreover, different PDE4D isozymes have been shown to regulate β_1- and β_2-AR cAMP signals, to build up distinct receptor subtype-specific cAMP microdomains[6,7]. Only recently, b-AR blockers such as metoprolol have been shown to disrupt these β-AR/PDE4 microdomains, causing inadvertently sustained cAMP signaling by uncontrolled diffusion[8]. In this regard, the high abundance of differentially localized PDE4-regulated cAMP microdomains makes PDE4 isoforms interesting candidates for alternative, perhaps even isoform-specific therapeutic approaches.

Host institution:

I used the *First Contact Initiative Grant* to visit MD Marco Conti (University of California San Francisco, UCSF), who is a world-known expert in the field of cyclic nucleotide phosphodiesterases. His knowledge and that of his co-workers regarding cellular and subcellular compartmentalized cAMP regulation by phosphodiesterases (PDEs), especially in cardiomyocytes and the underlying biochemistry is exceptional. Furthermore, the Conti Labs possess precious PDE subfamily-specific knockout mouse models as well as PDE isoform-specific antibodies for immunoblotting (often enough being the single ones that convey high degrees of specificity). Using such outstanding tools, over the past years his group published numerous studies that set grounds for a better understanding of how certain PDE families regulate especially cardiomyocyte function and how disease-associated changes in PDE expression and activity in fact contribute to cardiomyopathy and heart failure[1-6,8-12].

Purpose and outcome:

My visit to the Conti Labs aimed at setting up a future collaboration between both of our laboratories and as a preparation therefore to deepen my knowledge especially about the biochemistry, underlying PDE regulation. Importantly his group has developed further the 2-step PDE activity assay invented by Thompson & Appleman[13] making it readily applicable on myocardial tissue and cells. As I recently established this method in our own laboratories, analogously to that published by Richter et al.[11], it was highly beneficial to learn how this assay is actually performed by the first author himself. Furthermore, I could do some effective trouble-shooting by addressing methodological issues we experienced when executing this technique.

An important asset to our future cooperation is the Conti Labs' experience in co-immunoprecipitation (co-IP) of functionally active subcellular cAMP signal complexes containing certain PDE isoforms[6] to assess how functionally relevant cardiomyocyte cAMP microdomains are build up in detail and regulated by certain PDE isoforms. Learning how the highly talented postdocs in the Conti Labs perform immunoprecipitation (IP) and co-IP, I got familiar with the pitfalls of IPing and co-IPing functionally active PDE subfamilies and isoforms to subsequently measure their activities.

As one of our own strengths lies in Förster Resonance Energy Transfer (FRET) based live-cell imaging of cAMP dynamics, I myself could contribute by improving the acquisition of the FRET setup in the Conti Lab. Since we mostly analyze cAMP dynamics in freshly isolated cardiomyocytes of transgenic mouse models expressing FRET-based cyclic nucleotide biosensors[14,15], I furthermore established the isolation of adult mouse cardiomyocytes in the Conti Labs. Initially intended as a preparation for our future joint projects the cell isolation setup is already well appreciated by the Conti Labs co-workers.

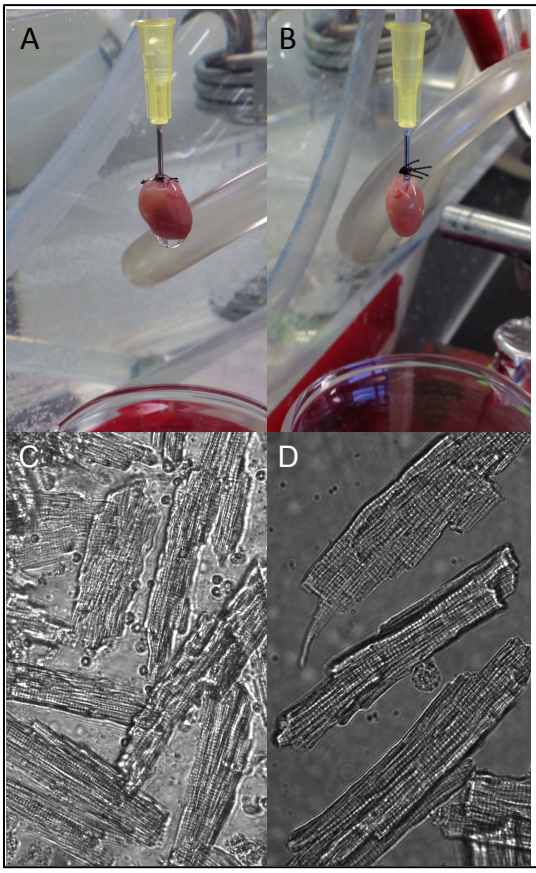

Figure 1. Adult cardiomyocyte isolation by modified Langendorff perfusion. Hearts of mice aged 8-12 weeks were quickly excised and immediately subjected to retrograde perfusion on a modified Langendorff setup (A). Upon perfusion with digestion enzymes hearts become translucent and loose their rigidity, a hint for proper digestion of the cardiac connective tissue (B). The digested heart was quickly removed from the perfusion apparatus und carefully homogenized. One drop of homogenate was briefly analyzed by microscopy to asses the initial cell quality (C). Cells were step-wise accustomed to increasing concentrations of Ca^{2+} to achieve a final concentration of 1mM. Thereafter cell quality was again analyzed by microscopy (D).

To gain access to facilities for functional readouts such as Ca^{2+} imaging, ion current and contractility measurements, during my stay in San Francisco I contacted PhD Yiang K. Xiang at UC Davis (UCD) and visited their laboratories to evaluate a possible collaboration with him and PhD Donald M. Bers, head of the Pharmacology Department, UCD. The meeting was very fruitful and gave perspectives to a joint project between UCSF, UCD and HRCG. However details still need to be discussed.

In summary, my visit to San Francisco and Davis, enabled by the *First Contact Initiative Grant* of the ESC, was a great success that set grounds for a highly vivid multi-site collaboration in order to investigate molecular cardiac pathology by benefiting from world-leading expertise at each location, including PDE regulation and biochemistry (UCSF), electrophysiology and Ca^{2+} imaging (UCD), FRET imaging of cyclic nucleotide dynamics and experimental cardiac disease models (HRCG).

Appendix:

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