First Contact Initiative Grant

Report of visit to host institution (March 4-7 2012)

Awardee: Dr John O'Sullivan

Mater Misericordiae University Hospital

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Host: Dr Christoph Maack

Klinik für Innere Medizin III (Kardiologie, Angiologie, Internistische Intensivmedizin),

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This institutional visit involved an introduction to the research team and the facilities, a comprehensive review of the research goals of the laboratory, attendance at the laboratory weekly meeting, practical "hands-on" learning of the techniques employed, and presentation of my own work to the laboratory staff and the entire medical faculty.

Introduction to host research

My visit commenced with a review of published work and work presented at conferences, by reviewing poster presentations in the laboratory.

This was a very useful start to proceedings, as it clarified the complex research by use of schematics and illustrations, and I was given the opportunity to ask questions throughout the process.

In particular, recent work on mitochondrial biogenesis in this laboratory was discussed. An abtract presented at the ESC 2011 entitled "Mitochondrial transhydrogenase is a key regulator of antioxidative capacity in cardiac myocytes" was discussed in detail. By illustrative presentation, I was able to understand how Dr Maack took advantage of a loss-of-function mutation in the nicotinamide nucleotide transhydrogenase (Nnt) gene in C57BL/6J (J-), but not C57BL/6N (N-) mice. Using these mice, he was able to demonstrate that oxidative stress, cardiac fibrosis and LV dysfunction in response to increased cardiac workload are mediated by NADPH-consumption by the Nnt to support NADH-coupled respiration, resulting in increased mitochondrial H2O2 formation. Lack of Nnt in C57BL/6J (but not BL6/N) mice substantially ameliorated oxidative stress and cardiac remodelling after pressure overload.

Next we discussed a study which investigated the energetic consequences of the role of the myocyte Na+-Ca^2+ exchanger (NCX) in heart failure, using a patch-clamping technique in guinea pig myocytes. Cytosolic Ca^2+[Ca^2+][m] and mitochondrial [Ca^2+][c] was determined within the same cell after varying Ca^2+ influx via L-type Ca^2+ channels (l(Ca^2+)) or the NCX. Despite comparable comparable [Ca^2+](c), sarcoplasmic reticulum Ca^2+ release, but not NCX-mediated Ca^2+ influx, led to stimulation of Ca^2+-sensitive dehydrogenases of

the Krebs cycle. It could therefore be concluded that increased contribution of the NCX to cytosolic Ca^{2+} transients, which occurs in cardiac myocytes from failing hearts, impairs mitochondrial Ca^{2+} uptake and the bioenergetic feedback response. This mechanism could contribute to energy starvation of failing hearts.

In a related study, taking into account that the regeneration of antioxidative enzymes requires NADPH, which is indirectly regenerated by the Krebs cycle, and Krebs cycle dehydrogenases are activated by [Ca(2+)]m, that in failing myocytes, elevated [Na(+)]i promotes oxidative stress. Once again, a patch-clamp-based approach was used to simultaneously monitor cytosolic and mitochondrial Ca(2+) and, alternatively, mitochondrial H2O2 together with NAD(P)H in guinea pig cardiac myocytes. Cells were depolarized in a voltage-clamp mode (3 Hz), and a transition of workload was induced by beta-adrenergic stimulation. This study concluded that besides matching energy supply and demand, mitochondrial Ca2+ uptake critically regulates mitochondrial reactive oxygen species production. In heart failure, elevated [Na(+)]i promotes reactive oxygen species formation by reducing mitochondrial Ca2+ uptake.

Practical demonstration of research methods

After a comprehensive introduction to research in the field of mitochondrial biogenesis, I was next allowed to witess, and to partake in, the practical methodology of the research. First, I was shown the isolation of cardiomyocytes from guinea pig heart. I then transported

the myocytes to the IonOPTIX™ imaging equipment, and allowed to use the equipment to image the cardiomyocyte at different workloads in order to calculate varying Ca2+uptake. To monitor $[Ca^{2+}]_c$ together with $[Ca^{2+}]_m$, myocytes were loaded with the cell-permeable Ca^{2+} indicator rhod-2 acetoxymethyl esther (rhod-2 AM, 3 μmol/L; Invitrogen), which locates primarily to mitochondria, and then dialyzed with a pipette solution that contained indo-1 salt to monitor $[Ca^{2+}]_c$ Using different filters, I was able to image NADH/FADH concentrations.

I then transported isolated cardiomyocytes to the patch-clamping equipment, and was inducted into the procedures required for performing the procedure. I learned how to direct the fine-tipped micro-pipette inside the cellular membrane, recognize the voltage change associated with this manoeuvre, and how to apply different currents. I proceeded to voltage clamp the myocytes in the whole-cell configuration (37°C, pipette resistance 2 to 4 MΩ) and equilibrated with a physiological K^+-glutamate–based pipette solution. Myocytes were depolarized from −80 to 10 mV at 3 Hz for 80 ms, and isoproterenol (10 and 100 nmol/L) was used to increase workload via β-adrenergic stimulation. To inhibit the MCU, Ru360 (1 μmol/L) was added to the pipette solution. Alternatively, $[Na^+]$in the pipette solution was raised from 5 to 15 mmol/L to accelerate mitochondrial Ca^{2+} efflux via the mNCE. To vary trans-sarcolemmal Ca^{2+} influx, cellular membrane potential was varied in families of pulses from −40 to +80 mV after conditioning pulses to +100 mV to load the SR with Ca^{2+}. To maximize or

minimize SR Ca^{2+} load, isoproterenol (30 nmol/L) or thapsigargin (1 µmol/L) was applied, respectively.

Relevance for my research

The reason for my application for the ESC First Contact Initiative Grant (FCIG) was to learn necessary skills to further investigate the mechanism of cardiomycyte protection post ischaemia-reperfusion, which I had demonstrated in previous research. I identified the upregulation of apoptotic and necrotic pro-survival kinases post ischaemic-reperfusion, and these pathways implicated maintenance of mitochondrial membrane permeability as an essential step in the cardioprotective mechanism. I discovered Dr Christoph Maack's laboratory, which was performing pioneering work in mitochondrial biogenesis and its role in heart failure, and so I applied via the ESC FCIG to visit his laboratory.

A crucial part of my visit was not only to learn techniques, but also to obtain advice and mentorship. To this end, I presented my work to the laboratory members and the medical faculty including Dr Maack and Prof. Dr Michael Böhm. I received excellent feedback regarding the possible interaction of the pathways identified in my research and the potential effect on mitochondria. In addition, innumerable insightful suggestions were made as to how I could proceed to look in a more mechanistic fashion the effects on mitochondria, particularly the mitochondrial permeability transition pore.

In conclusion, my visit to Dr Maack's laboratory as a recipient of the ESC FCIG provided me with the resources to address critical questions which arose from my research. I am indebted to Dr Maack and everyone at the Universitätsklinikum des Saarlandes who helped me, and also the ESC for providing me with the means to undertake this visit. I hope to continue the link with Dr Maack's laboratory, and collaborate in the future.

John O'Sullivan

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