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The neuro-cardiac interaction defines an extracellular microdomain required for neurotrophic signaling

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Purpose: Sympathetic neurons (SNs) innervate the myocardium with a defined topology that allows physiological modulation of cardiac activity. Limiting amounts of neurotrophins released by cardiac cells control SN viability and myocardial distribution, whose impairment has been described in a number of heart diseases (e.g. myocardial infarction, heart failure). Therefore, the fine control of cardiac innervation is crucial to ensure the physiological sympathetic function. It has been demonstrated that SNs directly interact with cardiomyocytes (CMs). Although it has been proposed that these contact sites may have a role in adrenergic stimulation of the CMs, whether direct interaction is needed for efficient neurotrophic signalling and correct innervation pattern is not known.

Methods and Results: Electron microscopy and immunofluorescence on mouse heart slices and SN/CM co-cultures showed close association between neurons and CMs and enrichment of the NGF receptor (TrkA) at the contact site, suggesting that specialized and locally organized signalling domains exist (neuro-cardiac junction, NCJ). In addition, silencing of NGF expression by CMs in co-cultures led to 66% decrease of neuronal density, supporting that NGF released by CMs sustains SN viability.

We tested whether SN/CM interactions are required for NGF-mediated pro-survival signalling to the neuron and correct myocardial innervation. Cultured neurons in contact with CMs showed fast TrkA activation, NGF uptake, bigger synaptic boutons and survived NGF withdrawal, whereas CM-conditioned medium did not sustain neuronal viability because of the very low NGF concentration (1.61 pg/mL). Altogether, these results support that the NCJ is essential for intercellular neurotrophic signalling. Consistently, NGF concentration at the contact site was estimated by using the TrkA inhibitor K252a and resulted about 1000-fold higher (1.75 ng/mL) when compared to that in CM conditioned medium.

Dystrophin accumulation on CM membrane contacted by SNs was observed in mouse cardiac slices, CM-conditioned medium.

Conclusion: Our results support that the NCJ is essential for intercellular neurotrophic signaling to the neuron and correct myocardial innervation.

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Role of the mitochondrial protein Opa1 in the regulation of the cardiac sympathetic neuron physiology

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Background: Cardiac sympathetic neurons (cSNs) are essential for cardiac homeostasis, as they mediate heart adaptation to stress conditions, and their basal activity is required to modulate cardiac myocyte tropism (1). cSN viability and activity depend on the correct mitochondrial function. The Optic Atrophy-1 protein (Opa1) controls mitochondrial fusion, ATP production and apoptosis, and its mutation is associated to Autosomal Dominant Optic Atrophy (ADOA), a neurodegenerative disease, characterized by ‘retinal ganglion cell’ death, myopathy and peripheral neuropathy. While the key role of Opa1 in the physiology of central neurons has been provided, less is known on its role in peripheral neurons, including cSNs. The aim of this study is thus to assess the role of Opa1 in cSN physiology and viability.

Methods: If: morphometry, ECHO and telemetry-based ECG recordings, both at baseline and during moderate exercise, were performed to characterize the effects of Opa1 haploinsufficiency on cSN physiology, during aging.

Results: We generated a mouse model haploinsufficient for Opa1, in cSNs, by crossing mice expressing Cre-recombinase, under the control of tyrosine hydroxylase (THO) promoter, with Ioxop-Opa1 transgenic mice (TOH-Hoe-Opa1-i/i). These mice were analysed at different ages from 3 to 18 mo. Reduction in the transcriptional level of Opa1 (about 50%) causes, already in the adulthood (3 mo.), a 25% reduction in the density of cSNs, which display fragmentation, reduction in the size of active releasing sites, and alterations in the innervation pattern, as compared to sex- and age-matched controls. Such alterations progresses during aging, a condition associated to dysfunctional sympathetic function and innervation. The reduction in cSNs density, in TOH-Opa1−/− mice, resulted in a dysfunctional extrinsic control of the cardiac rhythm. Indeed, the heart rate variability was reduced already in adult TOH-Opa1−/− mice (SDNN: Control: 6.17 ± 0.36 vs TOH-Opa1−/−: 3.63 ± 0.46, in msec) and the reduction was more pronounced in older mice (SDNN: Control: 3.86 ± 0.60 vs TOH-Opa1−/−: 1.17 ± 0.19, in msec). Consistently, TOH-Opa1−/− displayed reduced adaption to exercise.

Conclusions: Our data demonstrate that the Opa1 is essential for cSN homeostasis, and indicate that Opa1 haploinsufficiency leads to precarious cSN aging. The mechanisms responsible for Opa1 haploinsufficiency-dependent cSN degeneration will be assessed in vitro, with a focus on the NGF signalling. To translate our findings to the human pathology, we will analyse SN phenotype in skin biopsies from ADOA patients.

(1) Zaglia, et al Cardiovacs Res 2013

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