NOD-1 activation increases the spontaneous activity and the I_f current of murine sinoatrial node cells

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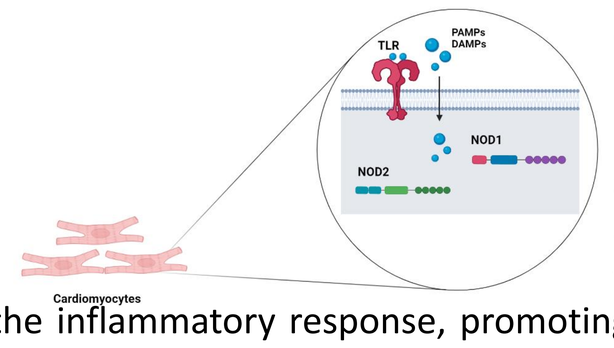
SIRC Società Italiana di Ricerche Cardiovascolari

DBS Dipartimento di Bioscienze

Background and Aims



Cardiomyocytes are capable of triggering a local inflammatory response since they express the Pattern Recognition Receptors (PRRs) which are a class of receptors that can directly recognize specific molecular structures on the surface of pathogens, apoptotic host cells, and damaged senescent cells. In particular, the activation of **NOD1**, a cytosolic PRR, is capable of increasing the inflammatory response, promoting cardiac remodeling and inducing arrhytmogenesis leading to the alteration of the cardiac function. Linscheid et al. (PMID: 31253831) previously showed that NOD1 transcript is abundantly present in SAN cells. Taken together these indications lead to the hypothesis of a close relation between inflammation and Sinoatrial Node Dysfunctions (SNDs).

Does the activation of NOD1 alter the activity of SAN?

Materials & Methods

Patch-clamp experiments were performed on single SAN cells isolated from the mouse heart. In order to detect changes in the electrical activity of the cells, a physiological Tyrode's solution was used externally, while a high K^+, low Ca^{2+} was used in the recording pipette.

48hrs incubation with:

SAN murine tissue → • **Vehicle** • **C12iEDAP** (NOD1 activator) • **iELys** (inactive analog) → Cells isolation

Results

1. SAN cells express NOD1 and its activation increases the spontaneous activity of SAN cells

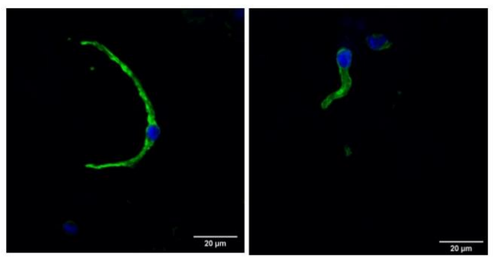


Fig 1

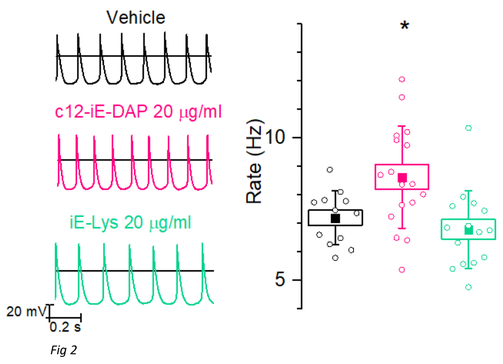


Fig 2

Fig. 1 SAN cells labeled with anti-NOD1 antibody diluted 1:100 (green); nuclei are marked with Hoechst.
Fig 2,left: *Representative SAN action potentials (APs) recorded after 48 hours of incubation with vehicle, C12-iE-DAP (NOD1 agonist), and iE-Lys (inactive analog). Fig.2, right: Boxplot showing individual and mean rate data in the three conditions; empty circles represent the spontaneous AP frequency of each cell tested, filled squares the mean value, bars the standard deviation, and the box the SEM (One-way ANOVA, post-hoc test: Fisher)*

2. The DDR is the only AP parameter altered by NOD1 activation

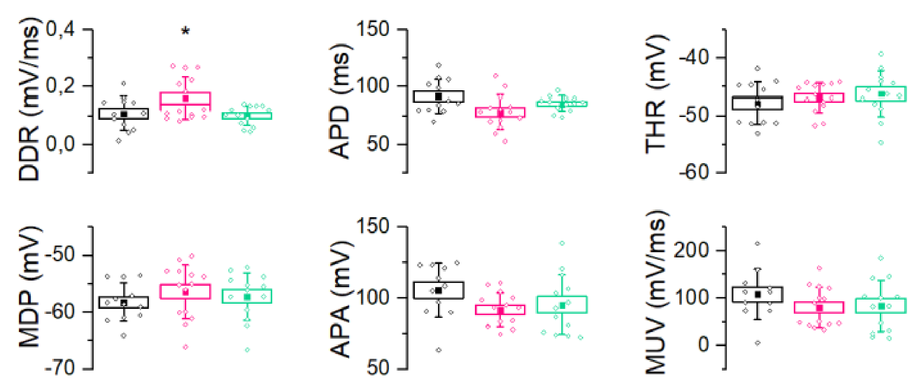


Fig 3

*Fig 3-APs parameters analyzed with ParamAP; *, p < 0.05; (one-way ANOVA, post-hoc test: Fisher). In each box plot, empty circles represent the value of a single cell, filled squares the mean value, bars the standard deviation, and the box the SEM. DDR: slow diastolic depolarization; APD: action potential duration; THR: threshold potential; MDP: minimum membrane potential value; APA: action potential amplitude; MUV: maximum upstroke velocity.*

3. NOD1 activation increases I_f

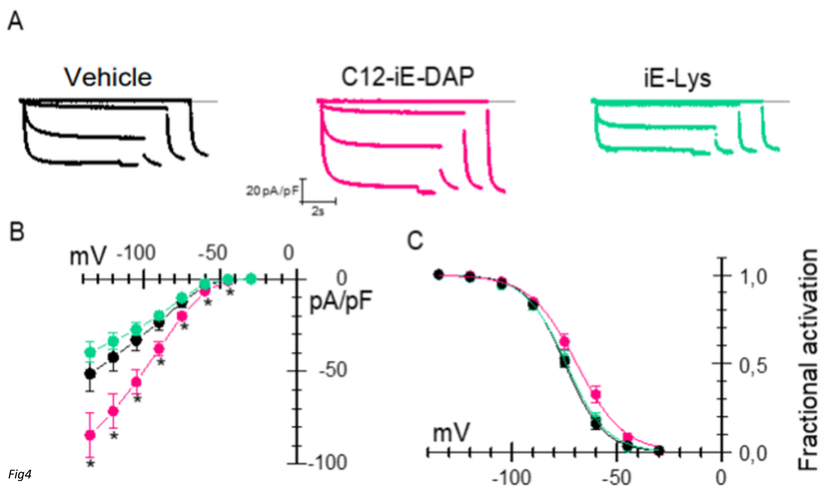


Fig4

*Fig4- A. Representative traces of I_f current recorded in SAN cells after 48 hours of incubation with vehicle, C12-iE-DAP 20 µg/ml (NOD1 agonist), and iE-Lys 20 µg/ml (inactive analog). B. Mean current-voltage curves obtained in the three conditions (vehicle n=15, C12-iE-DAP n=14, iE-Lys n=12). C. Mean activation curves obtained in the three conditions. Mean V1/2 values (from Boltzmann fitting): -74.9 ± 0.2 mV for vehicle (n=15); -69.3 ± 0.6 mV for C12-iE-DAP (n=14); -74.3 ± 0.4 mV for iE-Lys (n=12). *, p < 0.05 vs control and iE-Lys (one-way ANOVA, post-hoc test: Fisher).*

4. NOD1 reduces the response of nodal cells to β-adrenergic stimulation cells

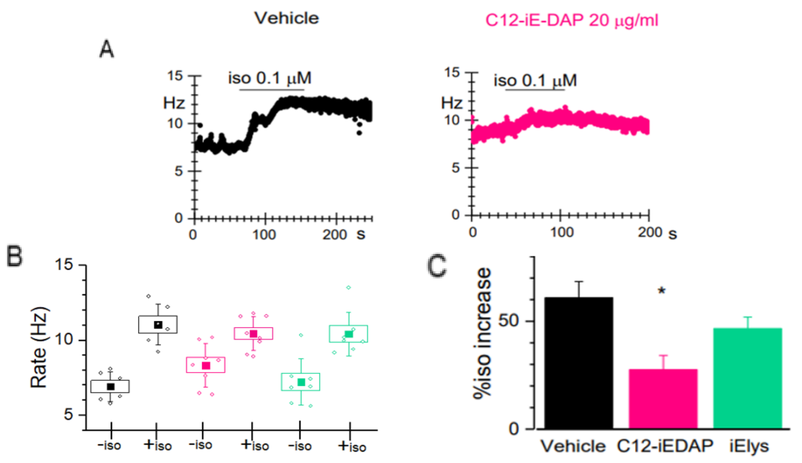


Fig5

*Fig. 5- A. Time course of the APs of SAN cells treated with vehicle or C12iEDAP (NOD1 agonist)for 48 hours in the absence and presence of Iso 0.1 µM. B. Summary data of the AP frequency of cells in the three experimental conditions before and during isoprenaline perfusion C. Percentage increase in the frequency of Aps following perfusion with Iso: vehicle 60.9 ± 7.5% (n=6), C12iEDAP 27.5 ± 6.7% (n=8), iE-Lys 46.6 ± 5.2% (n=8); *, p < 0.05 vs control and iE-Lys (one-way ANOVA, post-hoc test: Fisher).*

Conclusions

- NOD1 is expressed in SAN cells
- NOD1 activation increases the APs frequency and the I_f current
- NOD1 activation reduces the response of SAN cells to sympathetic stimulation

→ **NOD1 is a new player in SNDs**