NOD-1 activation increases the spontaneous activity and the I(f) current of murine sinoatrial node cells and alters their response to sympathetic stimulation.

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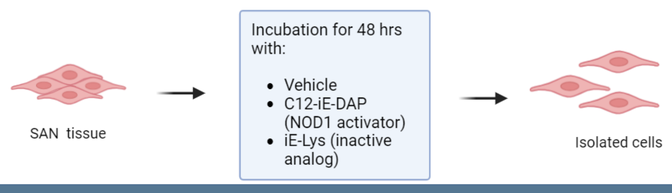
1 Background and aim

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 molecules expressed by pathogens and damaged 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?

2 Materials & Methods

Patch-clamp experiments were performed on single SAN cells isolated from the mouse heart after 48hrs incubation with vehicle, C12-iE-DAP 20 µg/ml (NOD1 activator) and iE-Lys (inactive analog of NOD1).



3 Results

3.1 SAN cells express NOD1 and its activation increases the spontaneous activity of SAN cells. The DDR is the only AP parameter altered by NOD1 activation

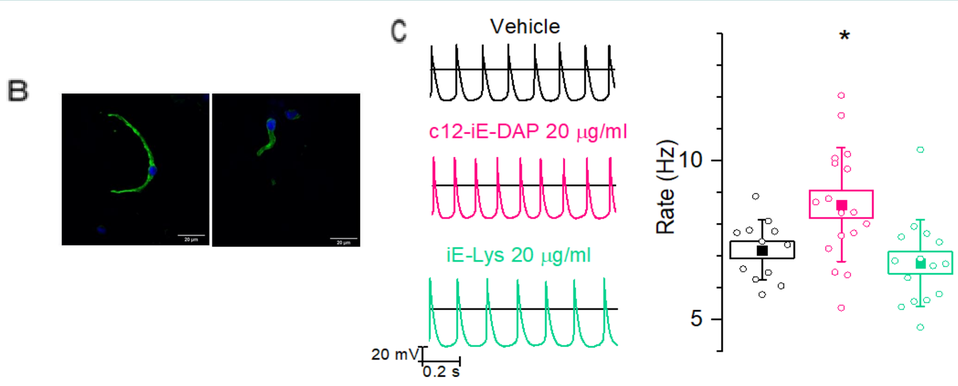


*Fig. 1 A. SAN cells labeled with anti-NOD1 antibody diluted 1:100 (green); nuclei are marked with Hoechst. B, left. Representative SAN action potentials (APs) recorded after 48 hours of incubation with vehicle, C12-iE-DAP, and iE-Lys B, right: Boxplot showing individual and mean rate data in the three conditions; empty circles represent the spontaneous AP frequency of each cell tested, filled squares the mean value, bars the standard deviation, and box the SEM. *, P<0.05 (One-way ANOVA, post-hoc test: Fisher). C APs parameters analyzed with ParamAP; *, p < 0.05; (one-way ANOVA, post-hoc test: Fisher). DDR: slow diastolic depolarization; APD: action potential duration; THR: threshold potential; MDP: maximum diastolic potential; APA: action potential amplitude; MUV: maximum upstroke velocity.*

3.2 NOD1 activation increases I_f

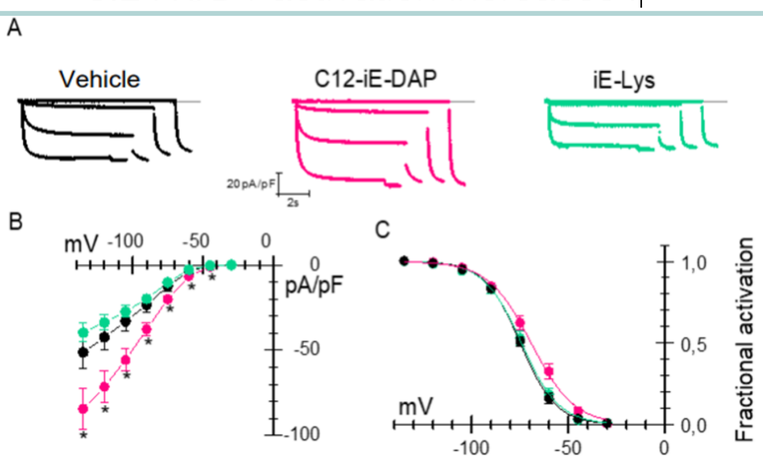


*Fig. 2 A. Representative traces of I_f current recorded in SAN cells after 48 hours of incubation with vehicle, C12-iE-DAP 20 µg/ml and iE-Lys 20 µg/ml. B. Mean current-voltage curves obtained in the three conditions (vehicle n=15, C12-iE-DAP n=14, iE-Lys n=12). C. Mean activation curves obtained in 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).*

3.3 NOD1 does not alter calcium currents

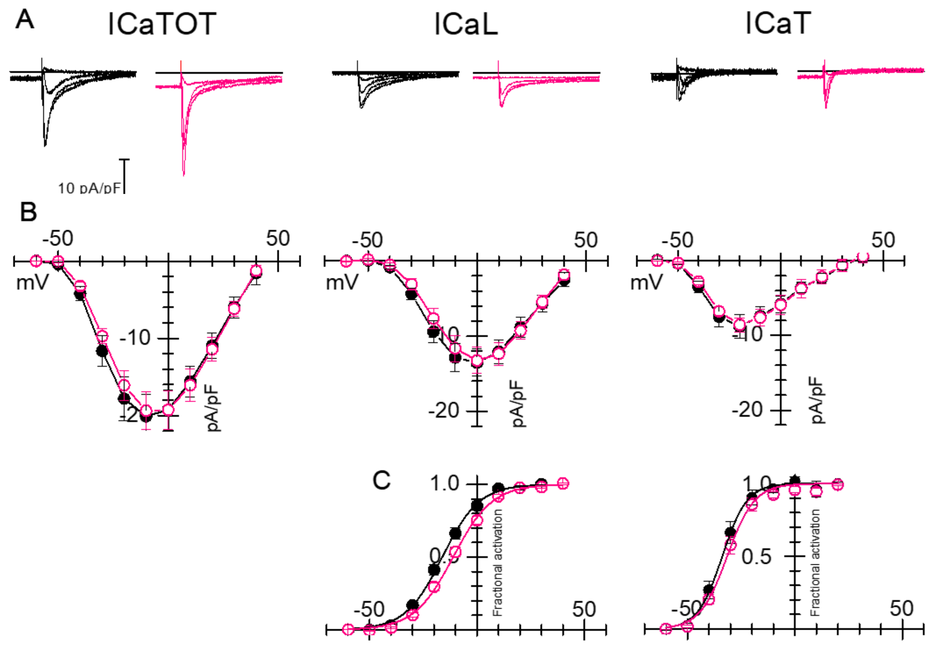


*Fig 3. A. Representative traces of ICaTOT, ICaL, and ICaT Currents after 48 hours of incubation with vehicle and C12-iE-DAP 20 µg/ml. B. Average Current/Voltage Curves of ICaTOT, ICaL, and ICaT currents; No significant differences between the curves were observed. C. Mean activation curves obtained in the two conditions. A shift of 5 mV towards more negative values was observed in the activation curve of ICaL in C12-iE-DAP treated cells compared to the control * , p < 0,05 vs Control (F-test).*

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

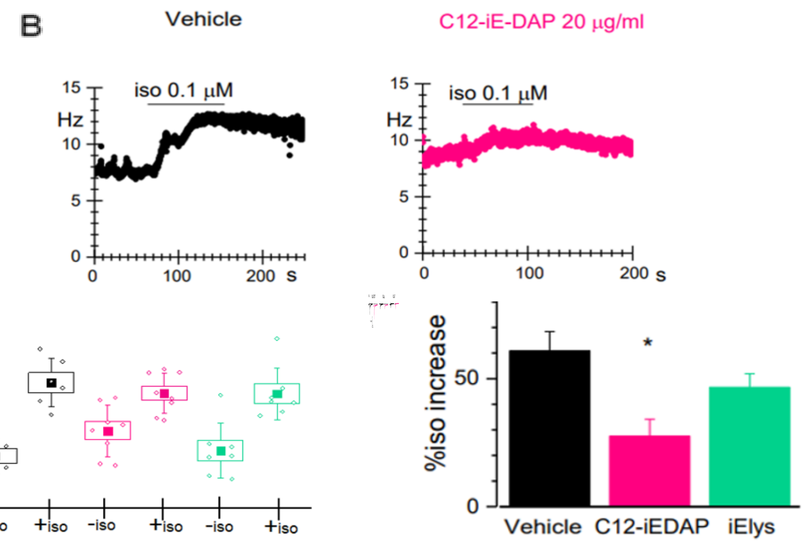


*Fig. 4 A. Time course of the APs rate of SAN cells treated with vehicle or C12iEDAP for 48 hours in the absence and presence of Iso 0.1 µM. B. Boxplot showing individual and mean AP frequency of cells in the three experimental conditions before and during isoprenaline perfusion C. Percentage increase in the frequency of APs induced by 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).*

3.5 C12-iE-DAP treatment reduces isoprenaline effect in SAN murine tissue

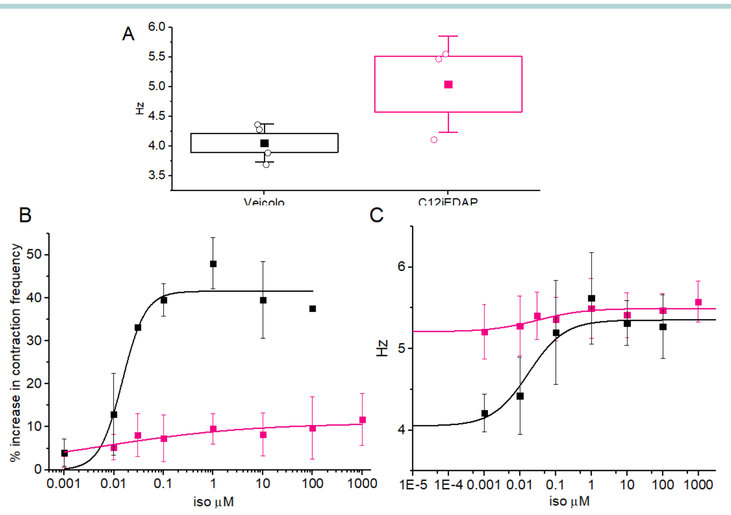


Fig. 5 A. Box Plot of Basal Frequency of Tissues following 48-hour incubation with Vehicle (n=4) and C12-iE-DAP (n=3). B. Dose-Response relationships showing the percentage increase in contraction frequency induced by isoproterenol. Each point represents the average of n=2/4 doses. Fitting the experimental data to the Hill equation yielded the following value Vehicle: Maximum effect = 41.56%, EC50 = 0.01 µM, n = 1.87, C12-iE-DAP: Maximum effect = 10.93%, EC50 = 0.005 µM, n = 0.28. The two curves are statistically different (F-test). C. Dose-Response Curves of Contraction Frequency; each point represents the average of n=2/4 doses of isoproterenol.

4 Conclusions

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

NOD1 is a new player in SNDs