Transthyretin amyloid fibrils affect viability and functional properties of cardiomyocytes

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Purpose:Senile systemic amyloidosis (SSA), is a sporadic disease whose main symptom is a severe cardiomyopathy associated with arrhythmias. SSA is characterized by the presence of extracellular amyloid fibrillar aggregates of transthyretin (TTR), a plasma protein carrying the thyroid hormone and the retinol binding protein. The aggregates are deposited in several tissues and are responsible for tissue functional impairment. To date, liver and heart transplantation are the only medical treatments; so, a thorough investigation of the molecular basis functional and viability impairment induced by TTR aggregates is expected to identify new pharmacological targets and to develop novel therapeutic strategies.

Material and Methods: Cardiomyocytes cell line (HL-1) and ventricular cardiac myocytes isolated from mouse heart were exposed to prefibrillar and fibrillar aggregates of TTR. The TTR aggregates internalization into HL-1 is assessed by immunofluorescence detection whereas cell citotoxicity is determined by JC-1 assay. Modifications of intracellular calcium levels were studied by fluorescence microscopy on HL-1 and the effect of TTR aggregates on action potential profile is determined by single cell patch-clamp technique on mouse ventricular cardiomyocytes.

Results: Only prefibrillar aggregates were able to interact with cell membrane and were internalized. This resulted in a moderate impairment of cell viability at concentration of 10 μM. In the same cells exposed to TTR prefibrillar aggregates, the cytosolic calcium content showed a progressive rise over time; it did not reach a steady state level and came back to its basal levels upon TTR removal from bath solution. By patch-clamp technique we investigated the effect of the enhanced intracellular calcium on the electrical properties of mouse ventricular myocytes. Action potential recordings were performed at increasing rate of stimulation (0.5, 1 and 2 Hz) before and after application of TTR. The results showed a progressive prolongation of the action potential that was associated with a marked increase of the duration of the plateau phase. These effects were seen at any frequency of stimulation.

Conclusions: TTR internalization is associated with rise of cytoplasmic calcium content and electrical abnormalities in exposed cells. These data demonstrate a proarrhythmic effect of TTR aggregates in cardiomyocytes, a possible cause of SSA cardiomyopathy.