

## Alterations in Cardiac Cell-to-Cell Coupling Can Facilitate AF in Old Guinea Pig.

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**Abstract.** *We hypothesize that in addition to atrial structural remodeling the alterations in cardiac cell-to-cell coupling due to the ageing may facilitate induction and persistence of AF. Experiments were conducted on male and female 4-weeks-old and 24-weeks-old guinea pigs (GP). Heart atrial tissue was processed for ultrastructure examination using electron microscopy. Gap junction connexin-43 distribution was detected by in situ immunostaining while the level of Cx43 mRNA and expression of Cx43 protein were determined by real time PCR and western blotting. Expression of mRNA of extracellular matrix metalloproteinase-2 (MMP2) that is involved in myocardial remodeling was also analyzed. Subcellular examination revealed interstitial fibrosis, flattened adhesive junctions with widened extracellular spaces and lower number of gap junctions in old versus young GP heart atria. The density of Cx43-positive gap junctions was lower in atria of old comparing to young GPs. In parallel, the atrial tissue levels of Cx43 mRNA and Cx43 protein were decreased in old versus young GP. In contrast, mRNA expression of MMP2 was significantly higher in old versus young GP. The changes were more pronounced in old males comparing to females GP. Findings indicate that age-related down-regulation of atrial Cx43, dehiscence of adhesive junctions and up-regulation of MMP-2 can facilitate development of AF in old heart.*

*Key words:* cardiac connexin-43; MMP2, atrial fibrillation, old guinea pig

### 1. Introduction

Atrial fibrillation (AF) affects approximately 1 % of the general population and up to 8 % of subjects over the age of 80 [1]. AF is associated with decreased quality of life, increased morbidity and a 30 % higher risk of death [2], and thus is a major contributor to cardiovascular mortality. Indeed, AF has long been recognized as a powerful risk factor for stroke (up to 15 % of all strokes are attributable to this disorder), heart failure and mortality. Advancing age amplifies the risk of all of these sequels of AF. The prevalence of this arrhythmia rises sharply with aging [3, 4, 5]. High incidence of AF creates a huge burden on the health care system, both in terms of morbidity, mortality, and cost. Despite recent advances in AF therapy, including the broader use of anticoagulant therapy, adequate rate control, and newer, safer techniques to maintain sinus rhythm, which may prospectively help to prevent adverse outcomes in AF patients, mortality and morbidity in AF patients remains unacceptably high [5]. Because of demographic changes, the prevalence of AF will increase in the next decades, requiring better primary prevention strategies and better treatment options. Despite the progress in pathophysiology of atrial fibrillation (AF) the molecular mechanisms of this most common cardiac arrhythmia in aged population are still not fully elucidated. We have previously shown that unlike to young the aged guinea pig heart is much prone to develop AF upon repetitive burst stimulation. We hypothesize that in addition to

atrial structural remodeling the alterations in cardiac cell-to-cell coupling due to the ageing may facilitate induction and persistence of AF.

## 2. Subject and Methods

Experiments were conducted on male and female 4-weeks-old and 24-weeks-old guinea pigs (GP). Heart atrial tissue was processed for ultrastructure examination using electron microscopy. Gap junction connexin-43 distribution was detected by in situ immunostaining while the level of Cx43 mRNA and expression of Cx43 protein were determined by real time PCR and western blotting. Expression of mRNA of extracellular matrix metalloproteinase-2 (MMP2) that is involved in myocardial structural remodeling was also analyzed.

## 3. Results

Burst pacing induced only very brief (1 to 6 s) poststimulus arrhythmias in young while prolonged (5 to 15 minutes) atrial fibrilloflutter in old guinea pig heart (Fig. 1). Subcellular examination revealed interstitial fibrosis, flattened adhesive junctions with widened extracellular spaces and lower number of gap junctions in old versus young GP heart atria (Fig. 2). The density of Cx43-positive gap junctions was lower in atria of old comparing to young GPs. In parallel, the atrial tissue levels of Cx43 mRNA and Cx43 protein were decreased in old versus young GP. In contrast, mRNA expression of MMP2 was significantly higher in old versus young GP. The changes were more pronounced in old males comparing to females GP.

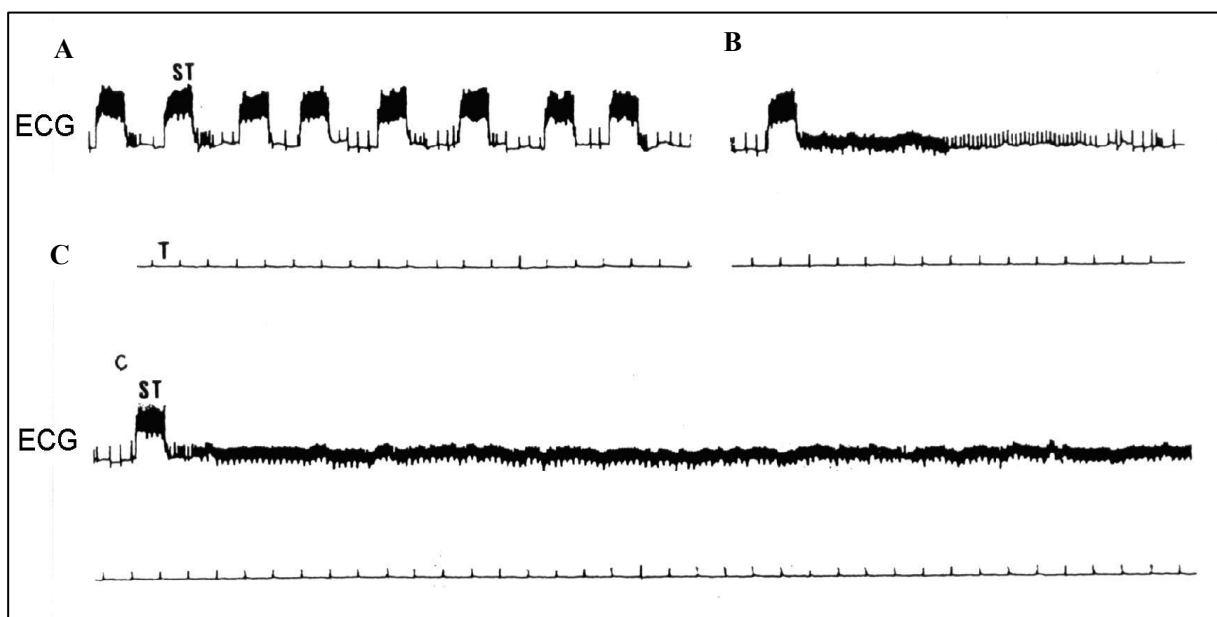


Fig.1. ECG of the atria during burst stimulation (A) and post-stimuli arrhythmias in young (B) and old (C) guinea pig heart.

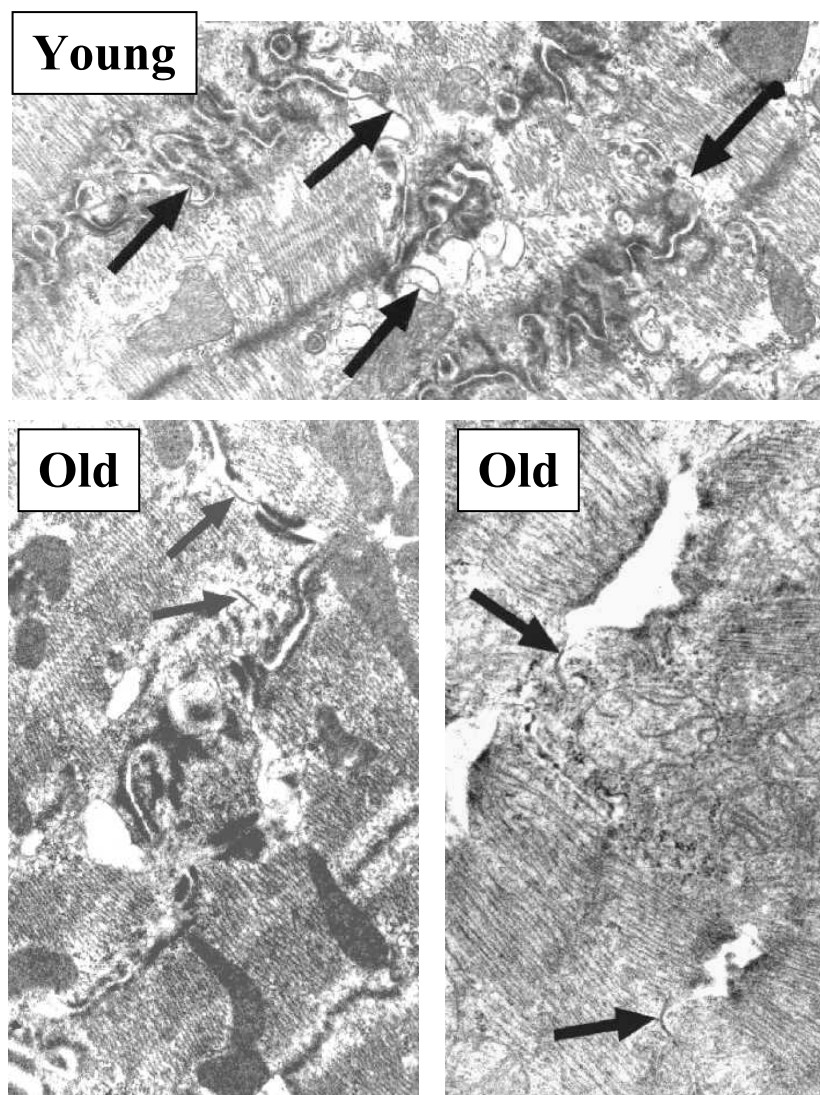


Fig. 2. Representative electron microscopic pictures showing numerous gap junctions (arrows) in young guinea pig atria. While lower number of gap junctions and dehiscence of adhesive junctions is seen in atrial tissue of old guinea pig heart. Magnification 25 000x.

#### 4. Discussion

Atrial fibrillation (AF) is the most common cardiac arrhythmia in clinical practice. However, precise mechanisms that lead to the onset and persistence of AF have not completely been elucidated. Numerous risk factors have been established for incident AF and its progression, such as cardiovascular diseases (hypertension, diabetes, coronary artery disease, valvular heart disease, failing heart), subclinical hyperthyroidism, genetic components as well as obesity and aging. These pathophysiological conditions are associated with acute and/or chronic inflammation and oxidative stress. Consequently, it results in activation of endogenous mechanisms of adaptation to preserve cardiovascular function. However, the process of adaptation is paradoxically linked with mal-adaptation. Its adverse effects contribute, besides others, to the increased propensity of the heart to life-threatening arrhythmias including AF that increases a risk of stroke. Our findings revealed pronounced extracellular matrix remodelling, i.e. fibrosis and increased expression of MMP2 in old guinea pig atria compared to atrial tissue of young guinea pig heart. It was accompanied by alterations in size and number of gap junctions as well as by down-regulation of gap junction protein, Cx43. Impairment of cell-to-cell communication has been established to contribute to

arrhythmogenesis and occurrence of re-entry arrhythmias, such as AF or VT/VF [7]. Thus, age-related alterations in Cx43-mediated electrical coupling may promote development of AF.

## 5. Conclusions

Our findings indicate that age-related down-regulation of atrial Cx43, dehiscence of adhesive junctions and up-regulation of MMP-2 can facilitate development of AF in old heart.

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