Molecular adaptations in human atrial fibrillation

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Summary

Clinical and experimental studies showed that electrical and contractile remodeling occurred early after onset of atrial fibrillation. Both processes could be reduced by blocking the L-type Ca^{2+} channel suggesting the notion that changes in the calcium homeostasis triggered by tachycardia induced intracellular calcium overload, play a pivotal role in the induction of these remodeling processes. To obtain insight in the underlying molecular mechanisms we first studied the molecular remodeling of proteins, which influence the calcium homeostasis in a heterogeneous group of AF patients (Chapter 2 and 3). We found that reductions in mRNA and protein expression of the L-type Ca^{2+} channel and sarcoplasmic reticulum Ca^{2+} ATPase occurred predominantly in patients with persistent AF. Furthermore, mRNA expression was found to be dependent on the duration of persistent AF. Patients with >6 months duration of AF revealed reductions in mRNA in contrast to patients with <6 months duration of AF. In these patients no changes in mRNA expression were found.

Secondly, we investigated molecular changes in ion-channels that contribute importantly to the action potential duration. Apart from the L-type Ca^{2+} channel, we investigated the contribution of potassium channel gene expression in right atrial appendages in paroxysmal and persistent AF, since an increase in potassium channel amount or activity could explain the electrical remodeling (Chapter 4). Reductions in mRNA and protein levels were found for several K+ channels in patients with persistent AF. In patients with paroxysmal AF these reductions were observed predominantly at the protein level and not at the mRNA level. In addition, the regulation of L-type Ca2+ channel and several K+ channels and its relation to AERP in patients with persistent and paroxysmal AF was studied (Chapter 5). We demonstrated a positive correlation between the ion-channel protein expression of L-type Ca2+ channel, Kv4.3, Kv1.5, HERG, minK and Kir3.1 and the AERP but also with the rate adaptation of AERP in patients with persistent and paroxysmal AF. The correlation between ion-channel protein amounts and AERP indicate that ion-channel protein remodeling, beside the electrical remodeling plays an important role in the vulnerability to AF. The reductions in L-type Ca2+ channel could represent a possible explanation for the electrophysiological changes during AF. Furthermore, the data indicate that reduced ion-channel protein expression occurres due to high atrial rate. We called this phenomenon ion-channel remodeling to describe the AF induced changes in ion-channel protein expression.

The impact of other compounds such as the natriuretic peptide system (Chapter 6) and the endothelin system (Chapter 7) were also studied. These studies revealed the influence of concomitant valvular disease in patients with persistent AF on mRNA expression of neurohumones. The right atrial appendage of these patients showed increased levels of ANP, BNP and endothelin-1 in combination with reduced expression of their receptors. Possibly the increased ANP and BNP mRNA enhance the myocyte relaxation properties by combatting the calcium overload. An increase in endothelin-1 might generate an additional increase in intracellular calcium concentrations by activation of the L-type

Ca^{2+} channel.

A remarkable finding during the study of mRNA and ion-channel protein remodeling was the discrepancy between changes in mRNA and protein levels in patients with paroxysmal AF (Chapter 4 and 5). Whereas ion-channel protein levels of the examined L-type Ca2+ channel, Kv1.5, Kir3.1 and minK were substantially decreased, the mRNA levels were essentially unaffected in paroxysmal AF. This discrepancy prompted us to the role of an adaptive mechanism which influence in AF was previously unknown, i.e. the activation of a proteolytic system. Different proteolytic pathways could be involved in AF. Since cytosolic calcium is increased during AF, proteolysis may be invoked by calcium dependent neutral proteases, calpain I and II. In Chapter 8 the increased proteolytic activity during paroxysmal and persistent lone AF due to activation of the calpain pathway was described. This increase seemed to be predominantly due to elevated activation and expression of calpain I.

In Chapter 9 we examined a variety of molecular changes in atrial tissue from patients with paroxysmal and persistent lone AF and related them to the level of calpain activity. Immunohistochemical detection of calpain I demonstrated increased staining at the intercalated disk and in the nucleus of atrial myocytes of AF patients. Accordingly, calpain activity was increased in patients with AF. Furthermore, an increased number of degenerative myocytes was observed in both patient groups with AF. Hibernating myocytes were only present in persistent AF and numbers increased with the duration of AF. Finally, calpain activity correlated inversely with the expression levels of ion-channel proteins, the degree of structural changes and the rate adaptation coefficient of AERP. These results strongly suggest that induction of calpain activation represents the missing link between the calcium overload observed in AF and remodeling of atrial myocytes during AF.

The incidence of AF increases due to ageing of the population and AF has the tendency to promote itself. Currently the arrhythmogenic electrophysiological changes (electrical remodeling) are well known, but can not explain by themselves that 'AF begets AF'. This thesis shows significant molecular and ultrastructural changes related to cellular hibernation. The latter support the notion of a second factor in AF promotion, since these - in part - form the basis for conduction slowing and dispersion of conduction and refractoriness. This thesis also shows a novel mechanism of protein remodeling taking place very early after AF onset. This mechanism is driven by calpain I activation. Future studies on pharmacological interventention in AF induced protein remodeling, like calpain activation, may prove effective in preventing atrial damage after AF onset. Such interventions may also break the chain of events by which AF tends to beget AF.