REPORT

EFFECTS OF DEXAMETHASONE AND ATORVASTATIN ON ATRIAL SODIUM CURRENT AND ITS EARLY TACHYCARDIA-INDUCED ELECTRICAL REMODELING IN RABBITS

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ABSTRACT

Purpose: Atrial fibrillation (AF) results in tachycardia-induced ionic remodeling. Pharmacological prevention of tachycardia-induced ionic remodeling not only with "classical" antiarrhythmics but also with drugs which provide a basis for some of the pillars of the so-called "upstream" therapy of AF like corticosteroids or statins has been proposed as a therapeutic strategy. Amongst other ion currents, atrial sodium current I_{Na} and its tachycardia-induced alterations play an important role in AF pathophysiology. Thus, effects of a dexamethasone (DT) and atorvastatin treatment (AT) on atrial sodium current I_{Na} and its tachycardia-induced remodeling were studied in a rabbit model.

Methods: 9 groups with 4 animals were examined. Atrial pacemaker leads were implanted in all animals. No rapid atrial pacing (600/min) was performed in the control group but for 24 or 120 hours in the respective pacing groups. Instrumentation and pacing did not differ from the respective drug groups but an additional treatment with dexamethasone or atorvastatin (7 days) was performed.

Results: Rapid atrial pacing (RAP, 600/min) reduced I_{Na} after 24 hours (≈ -50%) with no further reduction after 120 hours. DT reduced I_{Na} (≈ -20%), current densities in consecutively tachypaced animals did not differ from those in untreated animals. AT reduced I_{Na} similar as RAP, subsequent RAP did not further diminish I_{Na}.

Conclusions: Impact of corticosteroids and statins on I_{Na} and its tachycardia-induced alterations also contribute to the mode of action of these substances in upstream treatment of atrial fibrillation.

Keywords: Atrial fibrillation, remodeling, sodium current, statin, corticosteroid, upstream.

INTRODUCTION

Atrial tachycardia in atrial fibrillation (AF) leads to multiple electrophysiological alterations of the atrium in terms of a tachycardia-induced "atrial remodeling" (Workman *et al.*, 2008) within a few hours after arrhythmia onset (Goette *et al.*, 1996). Amongst other things, typical electrophysiological features of atrial in-vivo remodeling are a progressive shortening, a reduced rate-adaption and an increased heterogeneity of atrial effective refractory period as well as anomalies of intraatrial conduction which finally result in a reduction of atrial wavelength (product of atrial effective refractory period and conduction velocity) and therefore promote atrial susceptibility to AF according to the "multiple wavelet hypothesis" (Workman *et al.*, 2008).

AF-induced alterations of atrial ion channels and their regulation mechanisms have been identified as one of the main molecular mechanisms of the above-mentioned in-vivo electrical remodeling (Workman *et al.*, 2008). Here, a marked reduction of the L-type calcium current ($I_{Ca,L}$) and altered transient outward potassium current (I_{to}) seem to play a prominent role (Workman *et al.*, 2008). Comparable to the time-course of the alterations of atrial in-vivo electrophysiology (Goette *et al.*, 1996), our group demonstrated in a rabbit model that ionic remodeling of these currents is also already initiated within a few hours after onset of atrial tachycardia (Laszlo *et al.*, 2008).

Not only as a direct consequence of the important scientific findings concerning the role of electrical atrial remodeling in AF pathophysiology, pharmacological prevention of the latter has been proposed as a therapeutic strategy for treatment of AF (Shinagawa *et al.*, 2003). Recently, not only "classical" antiarrhythmics for treatment of AF but also drugs without direct antiarrhythmic effects like steroids or, respectively, HMG-CoA reductase inhibitors (statins) which provide a basis for some of the pillars of the so-called "upstream" therapy of AF (Dobrev *et al.*, 2010) are discussed for this purpose: for example in dogs, it has been shown that both treatment with steroids (Shiroshita-Takeshita *et al.*, 2006) and accordingly statins (Shiroshita-Takeshita *et al.*, 2004) attenuate tachycardia-induced proarrhythmic alterations of the atrial effective refractory period with consecutively reduced atrial vulnerability. Again on the ionic level, former studies of our group with our rabbit model of tachycardia-induced electrical remodeling gave evidence that an affection of $I_{Ca,L}$ and I_{to} and their tachycardia-

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induced electrical remodeling by a steroid- and, respectively statin-treatment might be at least partly responsible for these in-vivo observations (Laszlo *et al.*, 2010a, Laszlo *et al.*, 2010b).

At the latest by the introduction of atrial-selective sodium channel blockage as a new strategy for AF treatment (Burashnikov *et al.*, 2009), another atrial ion current which was somewhat scientifically unattended within the last years is (again) a matter of interest: As the magnitude of atrial sodium current I_{Na} also influences both atrial conduction velocity and atrial effective refractory period (Kneller *et al.*, 2005), impact of tachycardia-induced alterations of this current on atrial in-vivo electrical remodeling is also likely (Workman *et al.*, 2008). Therefore, first aim of our study was to describe the thitherto unknown time-course of early ionic remodeling of I_{Na} in our rabbit model. In addition, as a consequence of our previous studies with steroids and statins, we also hypothesized that a treatment with these drugs might affect I_{Na} and its tachycardia-induced electrical remodeling and therefore might be also partly responsible for the above-mentioned in-vivo observations of Shiroshita-Takeshita. Hence, second purpose of our study was to examine potential alterations of I_{Na} and its tachycardia-induced electrical remodeling by a steroid or statin treatment.

MATERIALS AND METHODS

Experiments were approved by the local ethics commission. Our rabbit model of early tachycardia-induced electrical remodeling including its methodical pros, cons and limitations was previously discussed in detail (Bosch *et al.*, 2003, Laszlo *et al.*, 2010a, Laszlo *et al.*, 2010b, Laszlo *et al.*, 2008). 9 groups of 4 rabbits each were examined in our study. Animals were instrumented with a modified atrial pacemaker as previously described in detail (Laszlo *et al.*, 2010a). No rapid atrial pacing (600/min) was performed in the control group (CO) but for 24 (P24) or 120 (P120) hours in the respective pacing groups before heart removal. "Dexamethasone only"-group (DO) and accordingly "Atorvastatin only"-group (AO) were treated like CO but with additional dexamethasone (0.5 mg/kg bodyweight dexamethasone intramuscular (Laszlo *et al.*, 2010b) every 24 hours for 7 days before removal of the heart) or atorvastatin treatment (2.5 mg/kg bodyweight atorvastatin p.o. every 24 hours (Laszlo *et al.*, 2010a) for 7 days before heart removal). Finally, "dexamethasone and paced 24 hours"-group (DP24), "dexamethasone and paced 120 hours" (DP120), "atorvastatin and paced 24 hours"-group (AP24) and accordingly "atorvastatin and paced 120 hours" (AP120) were treated like the corresponding "paced only"-groups but with additional dexamethasone or atorvastatin.

Atrial myocytes for patch clamp techniques were isolated as previously described (Bosch *et al.*, 2003, Laszlo *et al.*, 2010a, Laszlo *et al.*, 2010b, Laszlo *et al.*, 2008). To record I_{Na}, bath solution included (in mmol/l) NaCl 5, CsCl 132.5, $MgCl_2$ 1, HEPES 20, $CaCl_2$ 1.0, Glucose 11, pH was calibrated to 7.4 with CsOH. Pipette solution contained Cs-fluoride 135, NaCl 5, HEPES 5, Mg_2ATP 5, EGTA 10, pH was adjusted to 7.2 with CsOH. Bath temperature was 20±1°C. Currents were recorded using whole-cell configuration of the patch-clamp technique. To minimize errors of measurement due to run-down in whole-cell mode, I_{Na} was routinely recorded 5 minutes after rupture of cell membrane. To record I_{Na}, cells were depolarised (rate 0.1 Hz) from a holding potential of -140 mV to various test potentials between -70 mV and +20 mV. Current amplitudes were normalized to cell capacitance in each cell. Atrial sodium current can be separated into an early or peak and, respectively, a late component (Sossalla *et al.*, 2010). Only the peak component was registered in our current study and is termed as sodium current I_{Na} throughout the manuscript. Statistical comparison between groups was done by using two way repeated measures ANOVA/ Holm-Sidak t-test.

RESULTS

Pacing-induced alterations of sodium current I_{Na}

Sodium current (I_{Na}) was reduced after 24 h of rapid atrial pacing (≈ -50%) and was not further decreased after 120 h (fig. 1) for example at a test potential of -40 mV, I_{Na} was decreased from -75.4±6.1 pA/pF (CO, n=17) to -36.1±3.5 pA/pF (P24, n=22, $p<0.001$ CO vs. P24) respectively -35.8±5.3 pA/pF (P120, n=10, $p<0.001$ CO vs. P120) as also shown in fig. 4.

Effects of dexamethasone on sodium current I_{Na} and its tachycardia-induced alterations

Fig. 2 and also fig. 4 (overview of the study) illustrate the effects of dexamethasone on sodium current I_{Na} and its tachycardia-induced alterations. Dexamethasone alone slightly (≈ -20%) reduced I_{Na} (DO: -61.7±5.1 pA/pF, n=15, $p<0.05$ CO vs. DO, test-potential -40 mV). However, I_{Na} current densities after consecutive rapid atrial pacing did not differ significantly from those in untreated animals (DP24: -41.6±6.9 pA/pF, n=14, $p<0.05$ DO vs. DP24; DP120: -36.8±3.8 pA/pF, n=24, $p<0.001$ DO vs. DP 120).

Effects of atorvastatin on sodium current I_{Na} and its tachycardia-induced alterations

As shown in figure 3 and 4, atorvastatin treatment alone remarkably reduced I_{Na} (AO: -36.9±3.4 pA/pF, n=18, $p<0.001$ CO vs. AO). Atrial tachypacing (24h and 120 h) did not further decrease I_{Na} (AP24: -41.7±5.8 pA/pF, n=19; AP120: -37.6±5.0 pA/pF, n=19, test-potential -40 mV).

DISCUSSION

In our study, rapid atrial pacing remarkably reduced I_{Na} already after 24 h with no further reduction after 120 h. Regarding tachycardia-induced down-regulation of atrial I_{Na}, findings are discrepant: two groups described a decrease of I_{Na} in dogs after several weeks of atrial tachypacing (Gaspo *et al.*, 1997, Yagi *et al.*, 2002) and the reduction of the current seems to come along with a decrease of the mRNA encoding the sodium channel's α-subunit (Yue *et al.*, 1999). In patients with atrial fibrillation, reduced I_{Na} current density (-16 %) was also reported by Sossalla et al. whereas Bosch et al. did not find any statistical significant alterations of this current although there was a trend towards an approximately 10% reduction (Sossalla *et al.*, 2010, Bosch *et al.*, 1999). mRNA encoding sodium channel α-subunit was not changed in goats with electrically induced atrial fibrillation (van der Velden *et al.*, 2000) and also in AF patients with valve diseases (Gaborit *et al.*, 2005) but in exchange, Navβ2 mRNA (another subunit of the channel (Balser, 2001)) was reduced in these patients. Most important, alterations of I_{Na} in our study are yet comparable to those in tachypaced dogs and together with alterations of other ion currents (Bosch *et al.*, 2003, Yue *et al.*, 1997, Yue *et al.*, 1999), this is indicative for a good comparability of our and, respectively, Shiroshita-Takeshita's animal model.

Dexamethasone treatment alone slightly reduced I_{Na} but current densities in consecutively tachypaced animals did not differ from those in untreated animals. In contrast, atorvastatin treatment alone reduced I_{Na} similar as rapid pacing alone whereupon subsequent rapid atrial pacing did not induced further downregulation. To the best of our knowledge, effects of both a dexamethasone and an atorvastatin treatment on atrial sodium current were not yet described. In the initially mentioned studies of Shiroshita-Takeshita et al., both prednisone treatment and simvastatin treatment attenuated pacing-induced shortening of atrial effective refractory period and accordingly AF-inducibility (Shiroshita-Takeshita *et al.*, 2004, Shiroshita-Takeshita *et al.*, 2006). As changes in I_{Na} current density both alter atrial effective refractory period and atrial wavelength (Kneller *et al.*, 2005), dexamethasone- and, respectively, atorvastatin-induced modulation of I_{Na} have to be also taken into accounts as another potential mechanism for the these in-vivo observations besides the influence of $I_{Ca,L}$ and I_{to} and their

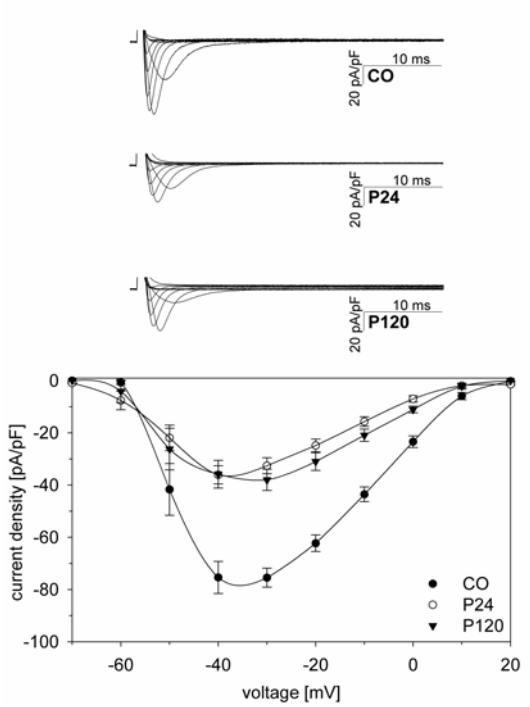

Fig. 1: Effects of rapid atrial pacing on atrial sodium current I_{Na}. (Top) Typical recordings, I_{Na} was elicited by depolarizing (30 ms) the cell from a holding potential of -140 mV to test potentials between -70 mV and +20 mV. A capacitive artifact at the beginning and the end of the test pulse was subsequently removed. (Bottom) IV-relation of the currents.

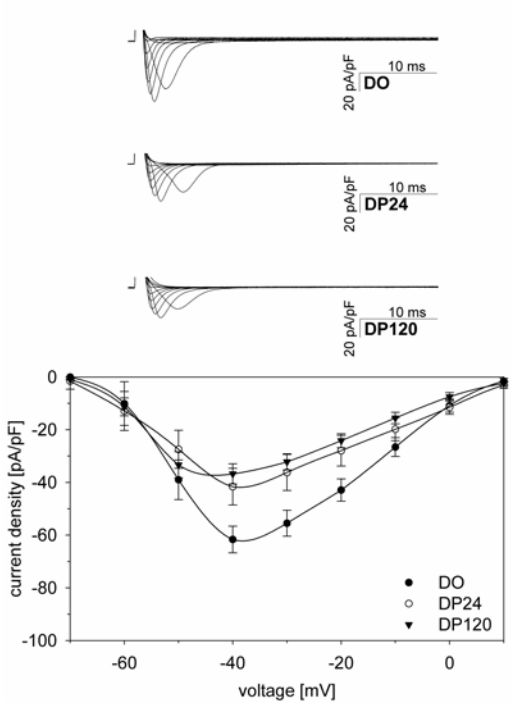

Fig. 2: Effects of dexamethasone on atrial sodium current I_{Na} and its tachycardia-induced alterations. (Top) Typical recordings, I_{Na} was elicited by depolarizing (30 ms) the cell from a holding potential of -140 mV to test potentials between -70 mV and +20 mV. A capacitive artifact at the beginning and the end of the test pulse was subsequently removed. (Bottom) IV-relation of the currents.

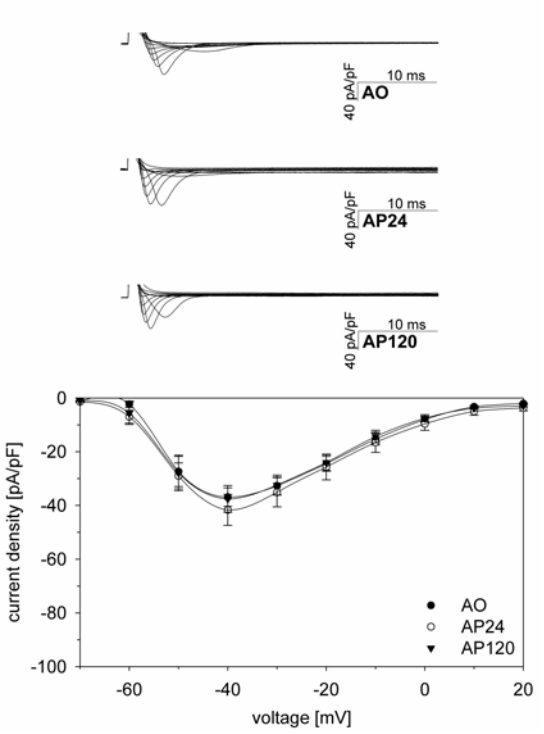

Fig. 3: Effects of atorvastatin on atrial sodium current I_{Na} and its tachycardia-induced alterations. (Top) Typical recordings, I_{Na} was elicited by depolarizing (30 ms) the cell from a holding potential of -140 mV to test potentials between -70 mV and +20 mV. A capacitive artifact at the beginning and the end of the test pulse was subsequently removed. (Bottom) IV-relation of the currents.

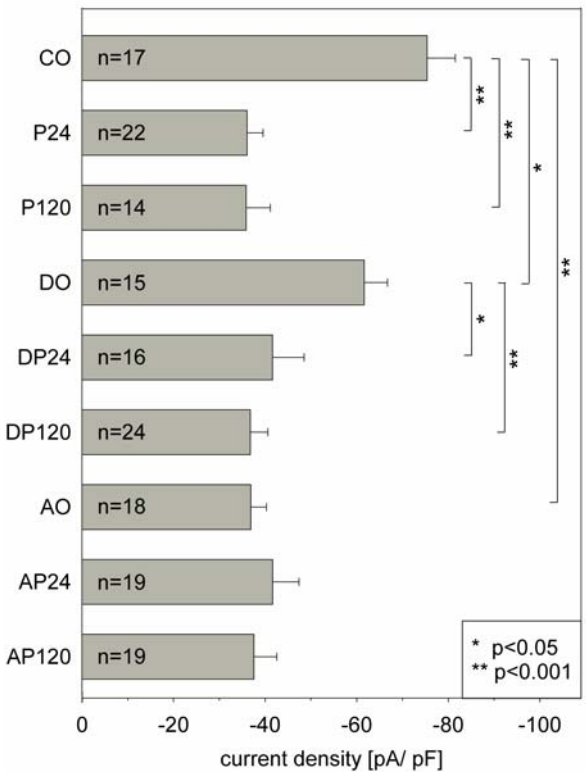

Fig. 4: Overview on the results of our study. The box plots illustrate the alterations of I_{Na} current density (pA/ pF, exemplarily at a test potential of – 40 mV) by rapid atrial pacing/ and or pharmacological treatment. CO = control group, P24 = paced 24 hours, P120 = paced 120 hours, DO = Dexamethasone only, AP24 = Dexamethasone and paced 24 hours, AP120 = Dexamethasone and paced 120 hours, AO = Atorvastatin only, AP24 = Atorvastatin and paced 24 hours, AP120 = Atorvastatin and paced 120 hours. N=number of cells patched in each group.

tachycardia-induced alterations by these drugs as described in our previous experiments (Laszlo *et al.*, 2010a, Laszlo *et al.*, 2010b).

As the basal current density of an ion channel is mediated by a complex interaction between various signalling cascades which are capable of being influenced by both rapid atrial pacing and dexamethasone/ atorvastatin treatment, it is difficult to define the exact mechanisms upon which the net current density in each group are based. Regulation of cardiac sodium channel is discussed in an excellent review by H. Abriel (Abriel, 2007). Most probably, the registered changes are likely to be the final common pathway of multiple alterations of multiple signalling cascades by rapid atrial pacing and/ or dexamethasone/ atorvastatin treatment whereas the exact mechanisms have to be examined in further experiments.

In summary, our experiments give evidence that the impact of corticosteroids and HMG-CoA reductase inhibitors on I_{Na} and its tachycardia-induced alterations also contributes to the mode of action of these substances in AF treatment. Functional relevance of these observations in conjunction with other aspects of atrial electrophysiology as well as potential molecular mechanisms of the drug- or tachycardia-induced alterations of I_{Na} net current have to be further evaluated.

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