Transient outward potassium current in rabbit atrium is depressed after short-time rapid atrial pacing but recovers after a longer pacing period

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Abstract. In rabbit, after short-time rapid atrial pacing (RAP), atrial ion currents are reduced similarly as in human chronic atrial fibrillation (AF). Using the rabbit model, time-course of transient outward potassium current (I_{to}) remodeling due to RAP was studied.

RAP (600 bpm) was applied *via* an atrial lead for 0 (control), 24 and 120 h, $n = 4$ animals/group. Using patch clamp technique in whole-cell mode, current densities and biophysical properties were measured in isolated atrial myocytes.

After 24 h of RAP, a reduction of peak I_{to} (mean ± SEM, test potential +50 mV, +37°C) was observed (60.3 ± 5.4 pA/pF (control, $n = 20$) vs. 28.0 ± 2.5 pA/pF (24 h, $n = 21$)). Inactivation of I_{to} was slower after 24 h, other biophysical properties were unaltered. However, I_{to} recovered after 120 h: 51.7 ± 4.5 pA/pF ($n = 26$, p = n.s. *vs.* control). Inactivation tended to also recover to initial values but was still different to control.

Early I_{to} remodeling due to RAP in rabbits seems to be more complex than previously thought: a time course of I_{to} remodeling with swayings has to be considered when using the rabbit model of RAP in order to study early remodeling or rather its therapeutic manipulation.

Key words: Atrial fibrillation — Electrical remodeling — Transient outward potassium current — Rapid atrial pacing — Patch clamp

Introduction

Atrial fibrillation (AF) leads to atrial remodeling including electrophysiological, mechanical and structural alterations (Wijffels et al. 1995; Allessie et al. 2002). A significant reduction of the transient outward potassium current (I_{to}) was seen in human chronic AF (Bosch et al. 1999; Dobrev and Ravens 2003) and in experimental models of AF (Yue et al. 1997; Bosch et al. 2003).

In rabbits, I_{to} current density is reduced to a similar extent as in human chronic AF within 24 h of rapid atrial pacing (RAP). Therefore, this animal model has been suggested as a small animal model for the early phase of AF-induced atrial electrical remodeling (Bosch et al. 2003). However, in the study from Bosch and co-workers, I_{to} current density tends – without reaching statistical significance – to reascend after 96 h of RAP. Not only because I_{to} tends to increase anew after 96 h instead of remaining reduced as expected but also as other groups suggested that persistent AF might involve a remodeling process different from that resulting from RAP (Dun et al. 2003), we further increased (120 h) the pacing interval in the present study.

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Materials and Methods

All animal care procedures were in accordance with the institutional guidelines of the University of Tübingen and the investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Right RAP (600 bpm) was applied *via* a transvenously implanted lead in rabbits for various periods: 0 (control), 24 and 120 h, $n = 4$ animals in each group. Single myocytes for patch clamp techniques were isolated from left atrium

by enzymatic dissociation using a Langendorff-setup. Instrumentation of the animals, RAP procedure and myocyte isolation were done as previously described in detail (Bosch et al. 2003; Laszlo et al. 2007).

Bath solution contained (in mmol/l): NaCl 136, KCl 5.4, $CaCl_2$ 2.0, $MgCl_2$ 1.0, NaH_2PO_4 0.33, HEPES 5 and glucose 10 to record I_{to}, pH was adjusted to 7.4 with NaOH; TEA-Cl 150, $CaCl_2$ 1.8, $MgCl_2$ 1.0, HEPES 10 and glucose 5 to record $I_{Ca,L,}$ pH was adjusted to 7.4 with TEA-OH. Pipette solutions contained (in mmol/l): KCl 20, K-aspartate 110, $MgCl_2$ 1.0, HEPES 10, EGTA 5, Mg_2ATP 5, GTP 0.1 and phosphocreatine 5 to record $I_{to,}$ pH was adjusted to 7.4 with KOH; CsCl 140, HEPES 10, EGTA 10, $MgCl_2$ 2.0, Mg_2ATP 3, GTP 0.4 and phosphocreatine 5 to record $I_{Ca,L}$, pH was adjusted to 7.2 with CsOH. Bath temperature was 37 ± 1°C. To record I_{to}, $I_{Ca,L}$ and $I_{K,r}$ were blocked with 1 µmol/l nisoldipine (Sigma, Deisenhofen, Germany) and 1 µmol/l dofetilide (Pfizer, Karlsruhe, Germany), respectively.

Currents were recorded using the whole-cell configuration of the patch-clamp technique. Pipettes with resistances between 2 and 5 MΩ, when filled with pipette solution, were connected to a patch clamp amplifier (Axopatch 200B; Axon Instruments, Forster City, USA). Recordings for current densities were started 5 min after cell membrane rupture. I_{to} was elicited by depolarising the cell from a holding potential of –80 mV to various test potentials between –40 and +70 mV for 200 ms after a 40 ms pre-pulse to –40 mV in order to inactivate I_{Na}. Current amplitudes were normalized to the cell capacitance in each cell to obtain current densities. Assuming a full activation of I_{to} at a test potential of +70 mV, voltage-dependent activation was obtained by normalizing the current amplitudes of various test potentials between –40 mV with the current amplitude at +70 mV. The obtained curve was fitted using a Boltzman relation. Voltage-dependent inactivation was determined with a 1 s pre-pulse from a holding potential of –80 mV to potentials between –100 and +70 mV followed by a 500 ms test pulse to +60 mV to record I_{to}. The obtained curve was also fitted by a Boltzman relation. Recovery from inactivation was assessed with a dual pulse protocol in which two identical 300 ms pulses, P1 and P2, were applied from a holding potential of 80 mV to +50 mV at an increasing P1–P2-interval. The amplitude of P2 was normalized to the amplitude of P1. The recovery curve was fitted by a biexponential function (Fermini et al. 1992). Finally, I_{to} inactivation kinetics were determined by obtaining the inactivation time constant τ from a monoexponential curve fitted of the current decay during a depolarization to +50 mV. $I_{Ca›L}$ was elicited by depolarizing the cell from a holding potential of –80 mV to various test potentials between –40 mV and +50 mV for 200 ms.

Data are expressed as mean ± SEM. Statistical comparison between groups was performed by Mann-Whitney U test and a two-tailed probability of 5% to indicate statistical significance.

Results

Effects of RAP on I_{to} current densities are illustrated in Figure 1 and Table 1, respectively. Panel 1A shows representative recordings of I_{to}, the current-voltage relations of peak I_{to} are given in panel 1B. As indicated in panel 1C, exemplarily at a test potential of +50 mV, after 24 h of RAP, a significant reduction of I_{to} current density was observed (60.3 ± 5.4 pA/pF (control, n = 20) vs. 28.0 ± 2.5 pA/pF pA/pF (24 h, n = 21)). However, current density returned to initial values after 120 h of RAP: 51.7 ± 4.5 pA/pF (120 h, n = 26, p = n.s. vs. control).

To exclude loss of atrial capture after prolonged atrial stimulation as an explanation for I_{to} recovery, we also measured $I_{Ca,L}$ in all animals. As expected (Bosch et al. 2003), RAP again led to a significant downregulation of $I_{Ca,L}$ after 24 h (–27.3 ± 1.7 pA/pF (control, n = 20, test potential 0 mV) vs.

Table 1. Overview of the effects of RAP on I_{to} current densities respectively on biophysical properties

	I_{to}		
	Control	24 h	120 h
Current densities			
n	20	21	26
current density (pA/pF)	60.3 ± 5.4	28.0 ± 2.5	51.7 ± 4.5
Voltage-dependent activation			
n	20	21	22
$V_{h,act}$ (mV)	20.2 ± 0.8	23.2 ± 1.2	19.8 ± 0.8
K_{act} (mV)	17.9 ± 0.7	19.9 ± 1.2	18.1 ± 0.7
Voltage-dependent inactivation			
n	11	13	13
$V_{h,inact}$ (mV)	–21.2 ± 2.3	–23.3 ± 2.4	–26.9 ± 1.0
K_{inact} (mV)	–19.1 ± 2.0	–19.9 ± 2.1	–12.8 ± 0.9
Time constants of recovery from inactivation			
n	8	7	7
$T_{rec,fast}$ (ms)	479 ± 45	318 ± 43	308 ± 29
$T_{rec,slow}$ (ms)	3586 ± 611	3289 ± 977	2728 ± 371
Time constant of inactivation			
n	20	22	22
T_{inact} (ms)	11.8 ± 0.8	18.5 ± 1.6	14.7 ± 0.8
test potential (mV)	50	50	50

n, numbers of cells used for analysis; $V_{h,act}$ and $V_{h,inact}$, half-activation and half-inactivation voltage; K_{act} and K_{inact}, slope factors for activation and inactivation; $T_{rec,fast/slow}$, time constants of recovery from inactivation; T_{inact}, time constant of inactivation.

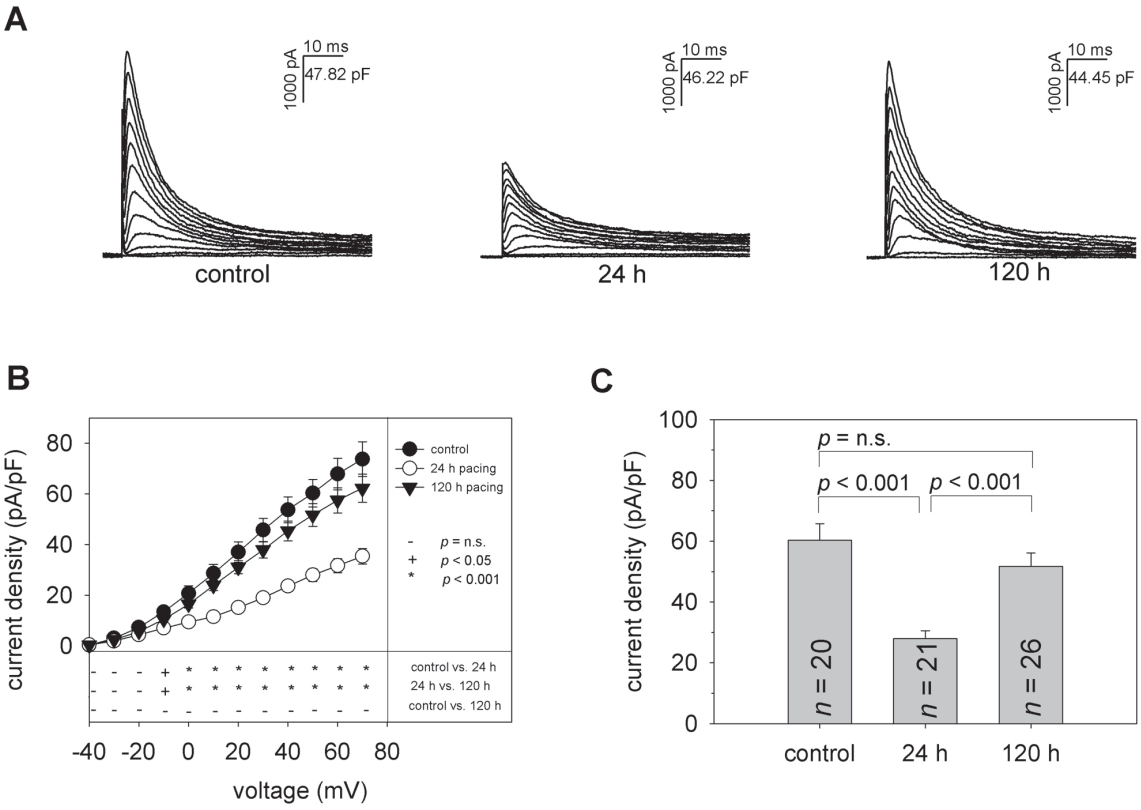

Figure 1. Effects of rapid atrial pacing (RAP) on I_{to} current densities: **A.** Representative recordings of I_{to} at control, after 24 and 120 h of RAP, respectively. I_{to} was elicited by depolarising the cell from a holding potential of –80 mV to various test potentials between –40 and +70 mV for 200 ms after a 40 ms pre-pulse to –40 mV in order to inactivate I_{Na}. Only 70 ms of the whole pulses are shown, capacitive artifact at the beginning of each pulse was subsequently removed. Cell capacity is shown in the legend. Note slower inactivation of I_{to} after 24 h. **B.** Current-voltage relations of peak I_{to} current densities. **C.** I_{to} current densities exemplarily at a test potential to +50 mV.

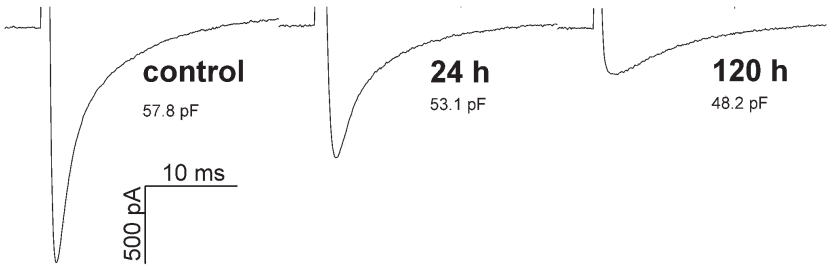

Figure 2. Effects of RAP on L-type Ca channel. From left to right typical recordings of each group (test potential 0 mV, cell capacity is indicated below group name). Similar current reduction as in previous studies (Bosch et al. 2003) can be used as an indirect evidence against loss of atrial capture after longer pacing intervals as a possible explanation of I_{to} recovery.

–18.3 ± 2.1 pA/pF (24 h, $n = 26$, $p < 0.001$) and current was further decreased after 120 h (–12.57 ± 1.5 pA/pF, $n = 20$, $p < 0.001$)) (Figure 2).

Voltage-dependent activation, voltage-dependent inactivation and recovery from inactivation of I_{to} did not differ significantly between the different groups (Table 1).

However, RAP altered the inactivation kinetics: after 24 h, I_{to} inactivation was slower compared to control (determined by an increased inactivation time constant τ exemplarily at a test potential of +50 mV: 11.8 ± 0.8 ms (control) vs. 18.5 ± 1.6 ms (24 h), $p < 0.001$). After 120 h of RAP, inactivation time constant tends to return to initial values (14.7 ± 0.8 ms)

but is nevertheless significantly increased compared to control ($p < 0.001$) and not significantly different compared to 24 h of RAP (Figure 3 and Table 1).

Discussion

In tachypaced animals as well as in patients with chronic AF, a reduction of I_{to} can be observed (Van Wagoner et al. 1997; Yue et al. 1997; Bosch et al. 1999). Multiple reports suggest a transcriptional downregulation of channel-forming proteins as a mechanism which is at least jointly responsible for this observation (Yue et al. 1997; Bosch et al. 1999; Grammer et al. 2000). As shown previously, in rabbit current decline is associated with a decrease in Kv4.3 mRNA already in the very early phases of atrial remodeling suggesting a transcriptional downregulation as a mechanism underlying reduced I_{to} (Bosch et al. 2003). However, current reduction was much more pronounced than the decrease in mRNA expression so effects of RAP on other regulatory mechanisms (e.g. modulating β-subunits (Deschenes and Tomaselli 2002; Radicke et al. 2006)) are likely.

Studies on human atrial remodeling are limited as only myocytes from patients with chronic AF (undergoing cardiac surgery) are available. The rabbit pacing model used in our current study has been suggested as a model especially for the early phases of AF-induced atrial remodeling as after short-time RAP, current densities of I_{to} (and $I_{Ca,L}$) are reduced similarly to human chronic AF (Bosch et al. 1999; Bosch et al. 2003).

In our present study, we confirmed that RAP in rabbit results in a remarkable downregulation of I_{to} within 24 h as already shown by Bosch et al. (2003). Otherwise than expected – after a longer pacing interval, I_{to} recovers and is not significantly different to control values. However, as I_{to} is reduced in chronic AF, further experiments with even longer pacing periods (possibly months to check whether I_{to} decreases anew) are needed.

Dun et al. (2003) showed an increase in I_{to} (compared to RAP) in dogs after long periods of AF. Comparison (chronic AF vs. RAP) in rabbits would be desirable, too. Unfortunately this is hardly possible as normally it is not feasible to induce permanent AF in rabbits as atrial size does not reach "critical atrial mass" according to Moe's/Allessies's multiple wavelet theory (Moe 1962; Allessie et al. 1985).

As a conclusion, early I_{to} remodeling due to RAP in rabbits seems to be more complex than previously thought: a time course of I_{to} remodeling with swayings has to be considered. Additionally, potential differences between AF-induced electrical remodeling respectively electrical remodeling induced by RAP have to be taken into account when using the rabbit model in order to study early remodeling or rather its therapeutic manipulation.

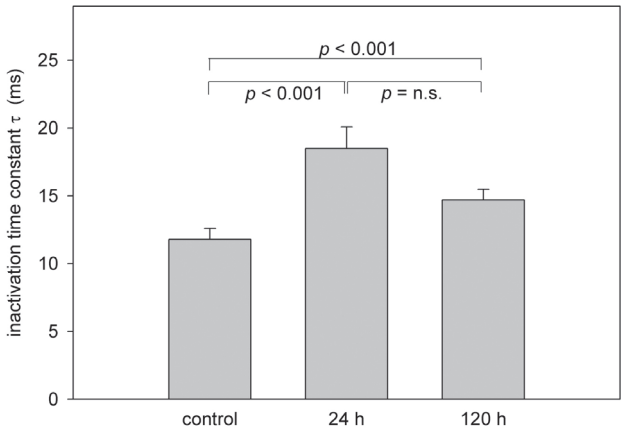

Figure 3. Effect of RAP on I_{to} inactivation kinetics: after 24 h, time constants τ of current inactivation is significantly increased and tends to return to initial values after a longer pacing interval.

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