Intermittent Optogenetic Tachypacing of Atrial Engineered Heart Tissue Induces Only Limited Electrical Remodelling

Marc D. Lemoine, MD,*†‡ Marta Lemme, PhD,*† Bärbel M. Ulmer, PhD,*† Ingke Braren, PhD,*† Susanne Krasemann, PhD,§ Arne Hansen, MD,*† Paulus Kirchhof, MD,†¶¶ Christian Meyer, MD,†¶¶ Thomas Eschenhagen, MD,*† and Torsten Christ, MD,*†

Abstract: Atrial tachypacing is an accepted model for atrial fibrillation (AF) in large animals and in cellular models. Human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CM) provide a novel human source to model cardiovascular diseases. Here, we investigated whether optogenetic tachypacing of atrial-like hiPSC-CMs grown into engineered heart tissue (eEHT) can induce AF-remodelling. After differentiation of atrial-like cardiomyocytes from hiPSCs using retinoic acid, eEHTs were generated from ~1 million atrial-like hiPSC-CMs per eEHT. AEHTs were transduced with lentivirus expressing channelrhodopsin-2 to enable optogenetic stimulation by blue light pulses. AEHTs underwent optical tachypacing at 5 Hz for 15 seconds twice a minute over 3 weeks and compared with transduced spontaneously beating isogenic AEHTs (1.95 ± 0.07 Hz). Force and action potential duration did not differ between spontaneously beating and tachypaced AEHTs. Action potentials in tachypaced AEHTs showed higher upstroke velocity (138 ± 15 vs. 87 ± 11 V/s, n = 15–13/3; P = 0.018), possibly corresponding to a tendency for more negative diastolic potentials (73.0 ± 1.8 vs. 68.0 ± 1.9 mV; P = 0.07). Tachypaced AEHTs exhibited a more irregular spontaneous beating pattern (beat-to-beat scatter: 0.07 ± 0.01 vs. 0.03 ± 0.004 seconds, n = 15–13/3; P = 0.008). Targeted expression analysis showed higher RNA levels of KCNJ12 [Kir2.2, inward rectifier (k_i)]; 69 ± 7 vs. 44 ± 4, P = 0.014] and NPPB (NT-proBNP; 39,690 ± 4834 vs. 23,671 ± 3691; P = 0.024). Intermittent tachypacing in AEHTs induces some electrical alterations found in AF and induces an arhythmogenic spontaneous beating pattern, but does not affect resting force. Further studies using longer, continuous, or more aggressive stimulation may clarify the contribution of different rate patterns on the changes in AEHT mimicking the remodeling process from paroxysmal to persistent atrial fibrillation.

Key Words: optogenetics, tachypacing, retinoic acid, atrial hiPSC-CMs, channelrhodopsin-2, tissue engineering, action potential, remodelling

(J Cardiovasc Pharmacol™ 2021;77:291–299)

INTRODUCTION

Twenty-five years after the seminal description of atrial fibrillation (AF)-induced electrical and contractile changes in the atria of goats (“AF begets AF”),¹ we have still not fully understood the changes in the atrial action potential (AP), diastolic potential and contractile function in atrial myocardium. A better understanding of the processes leading to “electrical remodeling” and “structural remodeling” because of intermittent high atrial rates is needed to identify novel therapeutic opportunities to prevent AF. Tachypacing has been used to study the changes leading to atrial and ventricular tachyarrhythmias in large animals.¹⁻⁵ Human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CM) and their integration into engineered heart tissues (EHTs) provide an opportunity to study these processes in human cells and myocardial tissue composed of human cells.⁶⁻⁸ There is an interest to know whether and to what extent hiPSC-CMs could be used as a model to study AF.⁹ However, hiPSC-CMs generated with standard differentiation protocols and cultured in standard 2D or 3D EHT represent predominantly
ventricular-like myocytes. We showed previously that tachypacing of ventricular-like EHTs (vEHT) by optogenetic means can evoke distinct electrophysiological changes including downregulation of calcium currents, shortening of refractoriness and inducibility of sustained arrhythmias.

Recently, protocols have been established that allow cardiac differentiation of hiPSCs towards an atrial-like phenotype by adding retinoic acid directly after the mesodermal induction phase. The present study characterized the electrical and molecular changes induced by intermittent optogenetic tachypacing of atrial-like EHTs (aEHT).

METHODS

Differentiation of hiPSC and Generation of Atrial-like EHTs

This investigation conforms to the principles outlined by the Declaration of Helsinki. Skin fibroblasts were taken with informed consent of the donors. All procedures involving the generation and analysis of hiPSC lines were approved by the local ethics committee in Hamburg (Az PV4798, 28.10.2014). Differentiation and generation of EHTs was performed as described before. In short, expansion of 2 undifferentiated hiPSC control cell lines (UKEi001-A/ERC001 and UKEi003-C/ERC018) was performed in FTDA medium. Embryoid body (EBs) formation was induced in stirred suspension cultures (spinner flasks). Mesodermal induction was achieved with BMP-4 (10 ng/mL), activin A (3 ng/mL), and basic fibroblast growth factor (5 ng/mL) in the absence of insulin in Roswell Park Memorial Institute medium. Specification of cardiac differentiation of mesodermal progenitors was performed by wingless-less integration site signal inhibition (XAV939, 1 μmol/L). Based on previous reports, differentiation of atrial-like myocytes was achieved by retinoic acid treatment (1 μmol/L) for the first 3 days of wingless-related integration site signaling inhibition. Retinoic acid (Sigma Aldrich R2625) was prepared as described previously. All experiments were performed with 3 individual differentiation batches (1xERC001 and 2xERC018), culture, and measurements were performed in parallel for unpaced and tachypaced EHT to minimize confounding factors. One-third of the aEHT was from ERC001, two-thirds from ERC018.

ChR2 Transduction

To implement an optogenetic sensor, aEHTs were transduced with EF1a-hChR2 (H134R) lentivirus during casting. Specifically, a multiplicity of infection equal to 0.2 was used during EHT casting. ChR2 expression was performed and verified as described before.

Force and AP Recording

Contraction analysis was performed by a video optical analysis system as previously described. APs were measured with standard sharp microelectrodes in intact aEHTs as previously described during spontaneous activity and field-stimulation at a fixed rate (pulse duration of 0.5 milliseconds, 50% above voltage threshold). A detailed description how to isolate hiPSC-CM from EHT for patch-clamp measurements is given elsewhere. APs were recorded from optically paced and unpaced ChR2 transduced retinoid acid-treated aEHTs 35–42 days after casting. AP parameter (APD, maximum upstroke velocity) and effective refractory period were calculated with the Lab-Chart software (ADInstruments, Spechbach, Germany) and analyzed offline by the CAPA software (Thieleczek and Wettwer. CAPA, Version 3.12 [Software]. 2019. Available from https://scce-capa.eu).

Molecular Biology

Expression analysis was performed as described before. In short, we used RNAeasy mini Kit (Qiagen, CA) to extract RNA. RNA concentration was determined per fluorometric quantitation with QubitTM (Thermo Fisher Scientific; Waltham, MA) according to the manufacturer’s instructions. We used 50 ng of RNA for expression analysis by nanoString nCounter SPRINT Profiler according to the manufacturer’s instructions. Raw data were analyzed with nSolverTM Data Analysis Software including background subtraction using negative controls and normalization to housekeeping genes (ABCF1, ACTB, CLTC and TUBB).

Immunohistochemistry

A-EHTs were fixed with 4% buffered formalin and processed for paraffin embedding. After embedding in paraffin using a Leica ASP300S tissue processor with a Leica EG1160 embedding station, sections (4 μm) were processed for immunohistochemistry as follows: After dewaxing and inactivation of endogenous peroxidases (PBS/3% hydrogen peroxide), antibody-specific antigen retrieval was performed using the Ventana Benchmark XT machine (Ventana, Tuscon, Arizona). Sections were blocked and after-ward incubated with the primary antibody against Collagen I (Col1A1; sc-8783; Santa Cruz). Sections were incubated with primary antibody for 1 hour, then, the antigen Histofine Simple Stain MAX PO immune-enzyme polymer (#414161F, medac GmbH, Wedel, Germany) was used as secondary antibody. Detection of secondary antibodies and counter staining was performed with an ultraview universal 3,3’-Diaminobenzidine detection kit from (Ventana, Tuscon, Arizona). Staining was evaluated in a blinded fashion. Representative pictures were taken with a digital Leica DMD109 microscope.

Statistics

Statistical analyses were performed with Prism software 6.0 (GraphPad, San Diego, CA). Data are expressed as mean ± SEM in bar graphs and scatterplots. Poincaré index was calculated with the following equation: SD1 = (2/2 × SD(1-CL)) and SD2 = (2 × (SD(1-CL))2 – 0.5 × (SD(1-CL) – CL + 1)). Differences between groups were analyzed by unpaired and paired t test when appropriate. The incidences of tachycardia were compared using Fisher’s exact probability test. Results were considered statistically significant if the P value was less than 0.05. Replicates were expressed as n/number of EHT/number of differentiation batches. Power calculation was performed following general methods as described in...
RESULTS

Optical Tachypacing in Atrial-like EHT

ChR2 transduced, retinoic acid-treated hiPSC-CMs with a percentage of cTnT-positive cells equal to 92.3 ± 3.1% (n = 3 batches) were used to cast aEHTs. AEHT started synchronous beating after 10.5 ± 0.3 days of culture (n = 29). Optical tachypacing was started at day 14 (Fig. 1). For this purpose, aEHTs were optically stimulated using an optogenetic platform described recently. AEHTs could be paced up to 5 Hz. However, because chronic continuous tachypacing led to quick desensitization of ChR2 with a subsequent lack of capture, we used an interval tachypacing protocol as described before that allowed long-term pacing with complete capture over 3 weeks.

Chronic Optical Tachypacing Does Not Induce Contractile Dysfunction in Atrial-like EHTs

The spontaneous beating rate did not differ between tachypaced and unpaced EHTs (114 ± 4 vs. 117 ± 4 bpm, P = 0.603, Fig. 2C). Force and contraction kinetics were measured under 2.5 Hz field stimulation to minimize frequency-dependent alterations. Reduction of force is a hallmark of AF remodeling. Force and relaxation time were not affected in tachypaced aEHTs, but time to peak of force was significantly shorter than in unpaced aEHTs (94.5 ± 2.5 vs. 105 ± 4 milliseconds; n = 15–13/3; P = 0.03, unpaired t test, Fig. 2). In parallel, fibrosis marker such as collagen1A1 did not show a difference in EHT by immunohistochemical analysis (see Fig. 1, Supplemental Digital Content 1, http://links.lww.com/JCVP/A547).

Tachypacing in Atrial-like EHTs Tends to Hyperpolarize Membrane Potential But Does Not Reduce Early Repolarization Fraction

Electrical remodeling in AF in humans leads to lower resting membrane potential (RMP) and shorter APD. Therefore, we measured APs after 3 weeks of optical tachypacing. To allow measurements under constant rate, we used field stimulation at 3 Hz to override spontaneous beating. TOP tended to be lower in tachypaced versus unpaced EHTs (71.0 ± 1.8 vs. 68.0 ± 1.9 mV, P = 0.070, unpaired t test), but the difference did not reach the level of significance. However, tachypaced aEHTs showed faster upstroke velocity than unpaced EHTs (138 ± 15 vs. 87 ± 11 V/s; P = 0.018, unpaired t test, Fig. 2C) and a nonsignificant trend toward higher AP amplitude (APA: 102 ± 3 vs. 93 ± 3 mV, P = 0.068). APD20 (80.1 ± 5.0 vs. 80.4 ± 6.1 milliseconds; P = 0.791), APD50 (110 ± 5 vs. 117 ± 4 milliseconds; P = 0.372), APD90 (172 ± 6 vs. 177 ± 8 milliseconds; P = 0.604, Fig. 3) and effective refractory period (effective refractory period: 176 ± 7 vs. 191 ± 9 milliseconds; P = 0.222) were not significantly different between tachypaced and unpaced aEHTs. Atrial-like AP shape of aEHT was confirmed by a high repolarization fraction (APD90/ APD30). This parameter was not different in tachypaced (0.35 ± 0.01) compared with unpaced aEHTs (0.39 ± 0.02) and was almost identical compared with the untransduced aEHT reported previously by us (0.41 ± 0.01). Because AF diminishes very early repolarization, we calculated a repolarization fraction based on APD30 (APD90 – APD30)/ APD90. However, this parameter also did not differ between paced (0.66 ± 0.03, n = 9) and unpaced aEHTs (0.67 ± 0.03) (0.67 ± 0.03, n = 8).

Tachypacing in Atrial-like EHT Increased Beating Irregularity

Video optically recorded spontaneous contractions of aEHTs showed that tachypacing did not affect the spontaneous beating frequency (Fig. 2D), but led to a higher beating irregularity calculated by beat-to-beat scatter (0.07 ± 0.01 seconds, n = 15/3 vs. 0.03 ± 0.004 seconds, n = 13/3; P = 0.008, unpaired t test, Fig. 2E). Higher irregularity of beating could be confirmed in AP recordings in aEHT superfused with Tyrode’s solution (Fig. 4). Rate variability was assessed with Poincaré plots of cycle length of spontaneous APs (Fig. 5). Tachypaced aEHTs showed higher dispersion than unpaced EHTs (Fig. 5A), as quantified by calculation of Poincaré plot indexes (SD1 and SD2, Fig. 5B). Both parameters were higher in tachypaced than in unpaced aEHTs (SD1 0.11 ± 0.01 vs. 0.06 ± 0.01 seconds and SD2 0.15 ± 0.01, n = 12/3 vs. 0.08 ± 0.01 seconds, n = 13/3; P < 0.05, unpaired t test). During AP recordings, we tried to induce tachycardia by burst pacing as performed previously in vEHT. However, we could not induce any sustained tachycardia neither in tachypaced nor in unpaced aEHT.

Optical Tachypacing Increases Higher Abundance of Inward Rectifier Transcripts

The transcript abundance of KCNJ12 but not of KCNJ1, which both code for the inward rectifier channels,
was higher in tachypaced aEHTs compared with unpaced EHTs (see Table 1, Supplemental Digital Content 2, http://links.lww.com/JCVP/A548). Interestingly, mRNA expression of KCNJ3 and KCNJ5 was not changed. The same holds true for KCNA5 (coding for Ikur), KCND3, and KCNJ2 (coding for Kv4.3 and the accessory subunit KCaP2). POSTN, which codes for peristin, a marker of extracellular matrix and fibrosis, was even lower in tachypaced aEHTs (426 ± 54 vs. 819 ± 151, P = 0.034), whereas NPPB mRNA levels, coding for natriuretic peptide B, were higher (39,690 ± 4834 vs. 23,671 ± 3691; P = 0.024, Fig. 6). Other genes coding for ion channels and markers for fetal gene program, hypertrophy, fibrosis, and apoptosis did not differ among the 2 groups (Fig. 6, see Table 1, Supplemental Digital Content 2, http://links.lww.com/JCVP/A548).

FIGURE 2. Effect of chronic optical tachypacing on contractility of atrial-like EHTs. A, Representative video-based registration of force over time of unpaced and optically tachypaced atrial-like EHTs. B, Mean values for paced EHTs at 2.5 Hz for absolute force, time-to-peak to 80% (TTP 80%), and relaxation time to 80% (RT 80%). C, Mean values for spontaneously beating EHTs for absolute force, frequency, and beat-to-beat scatter. All measurements were performed at day 35. Data are expressed as mean ± SEM (unpaired t test; unpaced n/n = 13/3 and tachypaced 16/3 with n/n = number of EHT/number of differentiation batches).

FIGURE 3. Effect of chronic optical tachypacing on APs of atrial-like EHTs. Representative APs in optically tachypaced (blue) and unpaced (black) atrial-like EHT (A). Mean values (B) for TOP, APA, maximum upstroke velocity (dV/dtmax), APD20, APD50 and APD90 were measured from day 35 to day 42 at 37°C under electrical field stimulation at 3 Hz. Data are expressed as mean ± SEM (unpaired t test; unpaced n/n = 13/3 vs. tachypaced 16/3; n/n = number of EHT/number of differentiation batches).
FIGURE 4. Effect of chronic intermittent optical tachypacing on beating regularity in atrial-like EHTs. Representative AP traces of unpaced (A) and tachypaced (B) atrial-like EHTs measured with sharp microelectrode during spontaneous beating.

DISCUSSION

Here, we have investigated whether optogenetic tachypacing in aEHT induces AF-like remodeling. Main findings are:

1. Similarly to vEHTs, optogenetic tachypacing is feasible in aEHTs.
2. Tachypacing in aEHT induces some of the expected changes of AF remodeling (a trend to more negative take-off potential and to increased AP amplitude), whereas other key features reported in tachypaced animal models were not found, eg, shortening of APD and drop in early repolarization fraction.
3. Tachypaced aEHTs showed an arrhythmic spontaneous beating pattern.

Tachypaced Atrial-like EHTs—a Useful Addition to Experimental Models for AF

EHTs with atrial-like hiPSC-CMs should enable a reasonably reproducible comparison of the response to the electrical situation in AF, resembling the AP shape of human cardiomyocytes. It may open the opportunity to compare genetic forms of AF and thereby complement the existing large animal models and murine genetically modified models. The model would allow to test eg, candidate drug therapies at relatively low cost and is compatible with the 3R principles (replacement, reduction, and refinement of experiments with animals in research).

Electrical Remodeling of Atrial-like EHTs upon Chronic Optical Tachypacing

In contrast to adult human CM, hiPSC-CMs do not have a stable RMP. Therefore, we have taken TOP as a surrogate. There was a trend to a more negative TOP in tachypaced aEHTs. Early, rather small studies showed a more negative RMP in persistent AF, raising the possibility of further adjustments in the differentiation protocol of aEHTs. From the largest available dataset based on AP measured in human atrial tissue (from 238 patients in sinus rhythm and 214 in persistent AF), we performed a power calculation. This calculation predicts that around 30 experiments in each group in human preparations would be needed to reach 80% power to detect a statistically significant difference in RMP. As far as we know from human right atrial samples of persistent AF, the slightly more negative RMP has important indirect effects. Upstroke velocity and APA are increased in AF, although sodium channel conductivity is reduced by 20%. This can be explained by more negative RMP, which causes a higher sodium channel availability. Similar to these data, tachypaced aEHTs showed a trend to more negative TOP, a larger take-off and APA (Fig. 3), whereas expression of SCN5A did not differ from unpaced aEHTs (Fig. 6). This pattern supports our assumption that the slightly more negative TOP in tachypaced aEHT seems to be a true finding.

Action Potential Shape

In aEHT, tachypacing did not shorten APD90. This may be explained by the fact that the classic finding observed any cycle length.24,27,28 It remains unclear whether APD90 would have been consistently shorter in tachypaced aEHTs at low rate. Of note, in contrast to tachypacing models other animal models of AF show a prolongation of the atrial APD, eg, hypertensive sheep,30,31 and special groups of patients with AF, eg, AF in long QT syndrome.30,31 The inability to slow down beating rate in aEHT currently represents an interesting feature of this model, but also a limitation of the use of hiPSC-CMs for studying electrical remodeling. Better understanding of pacemaker mechanisms in EHT is necessary and potentially efforts to create left atrial EHTs to develop strategies to overcome that issue.

AF affects not only late but also early repolarization. Contribution of transient potassium outward currents is less in AF, resulting in a prolongation of APD90 despite reduction of I_{Ca,L}. In contrast to the shortening of APD90, the prolongation of APD90 in AF is a consistent finding observed any cycle lengths. The absence of a smaller early repolarization fraction at 3 Hz in tachypaced aEHT suggests that the remodeling of early repolarization is lacking in this model. In this context, it should be noted that AP shape in aEHT is closer to AP in persistent AF than in SR because the typical notch dome shape is missing. This suggests that in our aEHT even under control conditions transient outward currents are as small as in human AF. Further patch clamp and molecular biology studies characterizing different repolarizing currents in aEHTs may help in this respect.

In addition, further adjustments in the differentiation protocol of atrial-like hiPSC-CM may be necessary to improve similarity of atrial AP shape.
Multiple pathomechanisms are described to initiate AF: focal mechanisms (automaticity/rotors/triggered activity), micro-reentry and macroreentry. In intact human atrial trabecula, arrhythmias can be evoked by catecholamines, probably of focal origin as a result of Ca²⁺-overload. In vEHTs, EADs can be evoked by potassium channel block. We did not observe EADs in aEHT. Intermittent tachypacing did not evoke inducibility by burst pacing in aEHT, which contrasts with tachypacing-remodeled vEHT. The reasons are unknown, but likely lie in the different electrophysiology of vEHT and aEHT. Regardless, our findings in vEHT suggest that the small size of EHT per se does not preclude the occurrence of tachycardia. We would expect that larger EHT (“carpet-like, several cm edge length”) should allow generation even of macro-reentries.

Irregularity of Spontaneous Beating of Atrial-like EHT

Irregular spontaneous beating of tachypaced aEHTs could be seen as a sign of electrical instability, potentially contributing to arrhythmia episodes in early AF but not in persistent AF. Spontaneous ectopy arising from the pulmonary vein–left atrial junction is now recognized as a major driver of AF, and ablation studies have identified increased ectopy in other parts of the left atrium. Whether the irregular rate observed in aEHTs after tachypacing is driven by similar changes in electrical function remains unknown. It is also unclear whether irregular beating in aEHT mimics electrical instability. Although the inhibitor of the funny current (I₅) ivabradine markedly slows down beating rate in vEHT and stops them at high concentrations, beating rate in aEHT (both tachypaced and unpaced) was ivabradine-insensitive (up to 1 μM, data not shown), suggesting that the I₅, an important driver for spontaneous diastolic depolarization in vEHT, is not the main driver of electrical instability in tachypaced aEHTs.

Absence of Contractile Dysfunction in Tachypaced Atrial EHT

Unexpectedly, force was not smaller in tachypaced versus unpaced aEHT. This is different to force measurements in persistent AF preparations compared with sinus rhythm, where force is smaller at any rate between 0.5 and 3 Hz. Possible reasons for lack of reduction of force include the intermittent manner of pacing (instead of continuous tachypacing), the relatively small difference of tachypacing rate compared with the intrinsic rate frequency (aEHT: 5 vs. 2 Hz, human: 6 vs. 1 Hz) or the short duration (3 weeks) of pacing. As the electrical dysfunction can precede contractile dysfunction in models of AF, longer periods of tachypacing seem reasonable to further investigate this.
FIGURE 6. Effect of chronic optical tachypacing on mRNA expression in atrial-like EHTs. Data of mRNA levels of optically paced and unpaced atrial-like EHTs. All values were normalized to the housekeeping genes (GAPDH and PGK1) and expressed as fold difference over unpaced atrial-like EHTs. Data are expressed as mean ± SEM (P < 0.05, unpaired t test; n/n = 6/3, n/n = number of EHT/number of differentiation batches). Each differentiation batch (1xERC001, 2xERC018) were prepared in parallel for unpaced and paced aEHTs.
CONCLUSIONS

Atrial-like EHTs are a promising tool to study AF, but tachypacing under the current conditions induced only some of the established signs of human AF remodeling. Reﬁnement of differentiation as well of pacing protocols should help to overcome the present limitations, including the high basal beating rate of aEHT that impedes the analysis of changes in refractoriness.

ACKNOWLEDGMENTS

Authors thank the members of the hiPSC-CM working group at the Department of Experimental Pharmacology and Toxicology, UKE-Hamburg for their support with stem cell culture and CM differentiation and Dr. Bastian Geelhoed (Department of Cardiology, University Heart and Vascular Center, Hamburg, Germany) for help with power calculations. Authors are grateful to Anna Steenpaß for excellent technical assistance.

REFERENCES

1. Wijffels MC, Kirchhof CJ, Dorland R, et al. Atrial ﬁbrillation begets atrial ﬁbrillation: a study in awake chronically instrumented goats. Circulation. 1995;92:1954–1968.
2. Ehrlich JR, Cha TJ, Zhang L, et al. Characterization of a hyperpolarization-activated time-dependent potassium current in canine cardiomyocytes from pulmonary vein myocardial sleeves and left atrium. J Physiol. 2004;557:583–597.
3. Qi XY, Yeh YH, Xiao L, et al. Cellular signaling underlying atrial tachycardia remodeling of L-type calcium current. Circ Res. 2008;103:845–854.
4. Morillo CA, Klein GJ, Jones DL, et al. Chronic rapid atrial pacing: structural, functional, and electrophysiological characteristics of a new model of sustained atrial ﬁbrillation. Circulation. 1995;91:1588–1595.
5. Jayachandran JV, Sih HJ, Winkle W, et al. Atrial ﬁbrillation produced by prolonged rapid atrial pacing is associated with heterogeneous changes in atrial sympathetic innervation. Circulation. 2000;101:1185–1191.
6. Lemoine MD, Mannhardt I, Breckwoldt K, et al. Human iPS-derived cardiomyocytes cultured in 3D engineered heart tissue show physiological upstroke velocity and sodium current density. Sci Rep. 2017;7:10.
7. Lemoine MD, Krause T, Koivumäki JT, et al. Human induced pluripotent stem cell-derived engineered heart tissue as a sensitive test system for QT prolongation and arrhythmic triggers. Circ Arrhythmia Electrophysiol. 2018;11:e006035.
8. Prondzynski M, Lemoine MD, Zech AT, et al. Disease modeling of a mutation in α-actinin 2 guides clinical therapy in hypertrophic cardiomyopathy. EMBO Mol Med. 2019;11:e11115.
9. Boyle PM, Trayanova NA. Leave the light on: chronic optogenetic tachypacing of human engineered cardiac tissue constructs. Cardiovasc Res. 2020;116:1405–1406.
10. Horváth A, Lemoine MD, Löser A, et al. Low resting membrane potential and low inward rectiﬁer potassium currents are not inherent features of hiPSC-derived cardiomyocytes. Stem Cell Rep. 2018;10:822–833.
11. Lemme M, Ulmer BM, Lemoine MD, et al. Atrial-like engineered heart tissue: an in vitro model of the human atrium. Stem Cell Reports. 2018;11:1378–1390.
12. Lemme M, Braren I, Prondzynski M, et al. Chronic intermittent tachypacing by an optogenetic approach induces arrhythmia vulnerability in human engineered heart tissue. Circulation. 2019;116:1487–1499.
13. Devalla HD, Schwach V, Ford JW, et al. Atrial-like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial-selective pharmacology. EMBO Mol Med. 2015;7:394–410.
14. Breckwoldt K, Letufte-Brenière D, Mannhardt I, et al. Differentiation of cardiomyocytes and generation of human engineered heart tissue. Nat Protoc. 2017;12:1177–1197.

Molecular Changes in Tachypaced Atrial-like EHTs

One of the major contributors to maintaining (and initiating) AF is structural changes. Tachypacing did not induce ﬁbrosis in aEHT. One of the limitations of the current system is the lack of ﬁbroblasts in hiPSC-EHTs, which may explain the lack of formation of ﬁbrovascular extracellular matrix in tachypaced aEHTs. It remains unclear at present if adding ﬁbroblasts is necessary to allow induction of ﬁbrosis in our aEHT. Nevertheless, a preselected set of genes revealed a signiﬁcantly decreased peristin (POSTN) expression (Fig. 6). This is unexpected because peristin, a secreted ECM protein associated with ﬁbrosis development, is upregulated in atria of patients with heart failure and AF patients, suggesting that peristin plays an active role in atrial ﬁbrosis and structural remodeling in AF. In addition, tachypacing resulted in upregulation of brain natriuretic peptide (NPPB; Fig. 6), similar to rapid activation of atrial human cells and human AF, where elevated BNP concentrations are a good biomarker for AF. We saw KCNJ12 but not KCNJ1 mRNA abundance to be higher in tachypaced aEHT. In mouse ventricular cardiomyocytes, a genetic knock-out of KCNJ1 had a larger impact on inward rectiﬁer current than the knock-out of KCNJ12. Effects of over expression of KCNJ1 versus KCNJ12 are not known. The situation in (human) atrium is unclear. Interestingly, both KCNJ1 and KCNJ12 are upregulated in human atrial tissues from patients with AF, with a larger increase in KCNJ12 than in KCNJ1. An increase in inward rectiﬁer currents is a consistent ﬁnding in AF. However, it remains unclear whether this ﬁnding results from an increase in KCNJ1 and/or KCNJ12. The situation may be even more complex, because a substantial proportion of the native IK1 may result from heteromultimer formation among diverse Kir2 family subunits. The increase in one of the genes encoding for inward rectiﬁer currents is in line with the trend in more negative RMP in tachypaced aEHT and with a more negative RMP in human samples of atrial ﬁbrillation. In stark contrast to human AF, we found no decrease in mRNA abundance of KCNJ3 and KCNJ5 encoding for IK1,ACH. Data about mRNA encoding for transient potassium outward current are complicated in interpretation. As described before for human AF, we found the abundance of KCNA5, KCND3, and KCNH2 transcripts encoding for Kv1.5, Kv4.3, and KChIP2, respectively, unchanged. However, reduced transient outward currents are a consistent finding in human AF, suggesting post-translational modiﬁcation and/or channel tracking must take place. This may limit the value of mRNA expression studies for KCNA5, KCND3, and KCNH2. The same holds true for Ca2+ currents. Smaller Ica is a consistent ﬁnding in human AF, whereas mRNA expression for the pore forming channel subunit CACNA1C was found decreased, not changed or even increased (for recent review). It should be noted that we used for mRNA expression analysis, a targeted approach to characterize a small number of genes expressed in human cardiomyocytes. RNA sequencing would provide an unbiased and a more complete characterization.
15. Zhang Q, Jiang J, Han P, et al. Direct differentiation of atrial and ventricular myocytes from human embryonic stem cells by alternating retinoid signals. Cell Res. 2011;21:579–587.

16. Lee JH, Protze I, Laksman Z, et al. Human pluripotent stem cell-derived atrial and ventricular cardiomyocytes develop from distinct mesoderm populations. Cell Stem Cell. 2017;21:179–194.

17. Mannhardt I, Breckwoldt K, Letufle-Brenière D, et al. Human engineered heart tissue: analysis of contractile force. Stem Cell Rep. 2016; 7:29–42.

18. Schotten U, Greiser M, Benke D, et al. Atrial fibrillation-induced atrial contractile dysfunction: a tachycardiomypathy of a different sort. Cardiovasc Res. 2002;53:192–201.

19. Wettwer E, Hála O, Christ T, et al. Role of I Kur in controlling action potential shape and contractility in the human atrium: influence of chronic atrial fibrillation. Circulation. 2004;110:2299–2306.

20. Christ T, Wettwer E, Voigt N, et al. Pathology-specific effects of the Ikur/ I to/I ACS blocker AVE0118 on ion channels in human chronic atrial fibrillation. Br J Pharmacol. 2008;154:1619–1630.

21. Dobrev D, Graf E, Wettwer E, et al. Molecular basis of downregulation of G-protein -coupled inward rectifying K’ current (IKur) in chronic human atrial fibrillation decrease in GIRK4 mxa correlates with reduced Ikur and muscarinic receptor-mediated shortening. Circulation. 2001;104: 2551–2557.

22. Wettwer E, Christ T, Endig S, et al. The new antiarrhythmic drug veramakalant: ex vivo study of human atrial tissue from sinus rhythm and chronic atrial fibrillation. Cardiovasc Res. 2013;98:145–154.

23. Ford J, Milnes J, Wettwer E, et al. Human electrophysiological and pharmacological properties of XEN-D0101: a novel atrial-selective K/V1.5/Kur inhibitor. J Cardiovasc Pharmacol. 2013;61:408–415.

24. Ford J, Milnes J, Haou SE, et al. The positive frequency-dependent electrophysiological effects of the IKur inhibitor XEN-D0103 are desirable for the treatment of atrial fibrillation. Heart Rhythm. 2016;13:555–564.

25. Ravens U, Katircioglu-Öztürk D, Wettwer E, et al. Application of the RIMARC algorithm to a large data set of action potentials and clinical parameters for risk stratification of atrial fibrillation. Med Biol Eng Comput. 2015;53:263–273.

26. Skibsybe L, Jespersen T, Christ T, et al. Refractoriness in human atria: time and voltage dependence of sodium channel availability. J Mol Cell Cardiol. 2016;101:26–34.

27. Franz MR, Karaisk PL, Li C, et al. Electrical remodeling of the human atrium: similar effects in patients with chronic atrial fibrillation and atrial flutter. J Am Coll Cardiol. 1997;30:1785–1792.

28. Bosch RF, Zeng X, Grammer JB, et al. Ionic mechanisms of electrical remodeling in human atrial fibrillation. Cardiovasc Res. 1999;44:121–131.

29. Kistler PM, Sanden P, Dodic M, et al. Atrial electrical and structural abnormalities in an ovine model of chronic blood pressure elevation after prenatal corticosteroid exposure: implications for development of atrial fibrillation. Eur Heart J Engl. 2006;27:3045–3056.

30. Zellerhoff S, Pistulli R, Mönnig G, et al. Atrial Arrhythmias in long-QT syndrome under daily life conditions: a nested case control study. J Cardiovasc Electrophysiol. 2009;20:401–407.

31. Lemoine MD, Duverger JE, Naud P, et al. Arrhythmogenic left atrial cellular electrophysiology in a murine genetic long QT syndrome model. Cardiovasc Res. 2011;92:67–74.

32. Nattles D, Dobrev D. Electrophysiological and molecular mechanisms of paroxysmal atrial fibrillation. Nat Rev Cardiol. 2016;13:575–590.

33. Greiser M, Kerfant BG, Williams GS, et al. Tachycardia-induced silencing of subcellular Ca2+ signaling in atrial myocytes. J Clin Invest. 2014; 124:4759–4772.

34. Christ T, Rozmarina N, Engel A, et al. Arrhythmias, elicited by catecholamines and serotonin, vanish in human chronic atrial fibrillation. Proc Natl Acad Sci. 2014;111:11913–11918.

35. Voigt N, Hejman J, Wang Q, et al. Cellular and molecular mechanisms of atrial arrhythmogenesis in patients with paroxysmal atrial fibrillation. Circulation. 2014;129:145–156.

36. Snider P, Standley KN, Wang J, et al. Origin of cardiac fibroblasts and the role of perisin. Circ Res. 2009;105:934–947.

37. Molina CE, Abu-Taha IH, Wang Q, et al. Profibrotic, electrical, and calcium-handling remodeling of the atria in heart failure patients with atrial fibrillation. Circulation. 2019;8:1383.

38. Wu H, Xie J, Li GN, et al. Possible involvement of TGF-β/Periostin in fibrosis of right atrial appendages in patients with atrial fibrillation. Int J Clin Exp Pathol. 2015;8:6859–6869.

39. Mace LC, Yermalitskaya LV, Yi Y, et al. Transcriptional remodeling of rapidly stimulated HL-1 atrial myocytes exhibits concordance with human atrial fibrillation. J Mol Cell Cardiol. 2009;47:485–492.

40. Sidorova TN, Yermalitskaya LV, Mace LC, et al. Reactive γ-ketoaldehydes promote protein misfolding and preamyloid oligomer formation in rapidly-activated atrial cells. J Mol Cell Cardiol. 2015; 295–302.

41. Lamirault G, Gaborit N, Meur NL, et al. Gene expression profile associated with chronic atrial fibrillation and underlying valvular heart disease in man. J Mol Cell Cardiol. 2006;40:173–184.

42. Chua W, Purmiah Y, Cardoso VR, et al. Data-driven discovery and validation of circulating blood-based biomarkers associated with prevalent atrial fibrillation. Eur Heart J. 2019;40:1268–1276.

43. Engdahl J, Svennberg E, Friberg L, et al. Stepwise mass screening for fibrosis of right atrial appendages in patients with atrial fibrillation. J Mol Cell Cardiol. 2009;47:299–302.

44. Lamirault G, Gaborit N, Meur NL, et al. Gene expression profile associated with chronic atrial fibrillation and underlying valvular heart disease in man. J Mol Cell Cardiol. 2006;40:173–184.

45. Chua W, Purmiah Y, Cardoso VR, et al. Data-driven discovery and validation of circulating blood-based biomarkers associated with prevalent atrial fibrillation. Eur Heart J. 2019;40:1268–1276.

46. Engdahl J, Svennberg E, Friberg L, et al. Stepwise mass screening for fibrosis of right atrial appendages in patients with atrial fibrillation. J Mol Cell Cardiol. 2009;47:299–302.

47. Lamirault G, Gaborit N, Meur NL, et al. Gene expression profile associated with chronic atrial fibrillation and underlying valvular heart disease in man. J Mol Cell Cardiol. 2006;40:173–184.