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Original Paper

Inhibition of Histone Deacetylases Induces K^+ Channel Remodeling and Action Potential Prolongation in HL-1 Atrial Cardiomyocytes

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Key Words

Atrial fibrillation • Cardiac action potential • Epigenetic regulation • Histone deacetylase • Ion channel • Potassium channel • Tachypacing • Trichostatin A

Abstract

Background/Aims: Cardiac arrhythmias are triggered by environmental stimuli that may modulate expression of cardiac ion channels. Underlying epigenetic regulation of cardiac electrophysiology remains incompletely understood. Histone deacetylases (HDACs) control gene expression and cardiac integrity. We hypothesized that class I/II HDACs transcriptionally regulate ion channel expression and determine action potential duration (APD) in cardiac myocytes. ***Methods:*** Global class I/II HDAC inhibition was achieved by administration of trichostatin A (TSA). HDAC-mediated effects on K^+ channel expression and electrophysiological function were evaluated in murine atrial cardiomyocytes (HL-1 cells) using real-time PCR, Western blot, and patch clamp analyses. Electrical tachypacing was employed to recapitulate arrhythmia-related effects on ion channel remodeling in the absence and presence of HDAC inhibition. ***Results:*** Global HDAC inhibition increased histone acetylation and prolonged APD_{90} in atrial cardiomyocytes compared to untreated control cells. Transcript levels of voltage-gated or inwardly rectifying K^+ channels *Kcnq1*, *Kcnj3* and *Kcnj5* were significantly reduced, whereas *Kcnk2*, *Kcnj2* and *Kcnd3* mRNAs were upregulated. Ion channel remodeling was similarly observed at protein level. Short-term tachypacing did not induce significant transcriptional K^+ channel remodeling. ***Conclusion:*** The present findings link class I/II HDAC activity to regulation of ion channel expression and action potential duration in atrial cardiomyocytes. Clinical implications for HDAC-based antiarrhythmic therapy and cardiac safety of HDAC inhibitors require further investigation.

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Introduction

Electrophysiological properties of cardiomyocytes are governed by ion channels, pumps and exchangers. Ion channel dysfunction represents a key factor in the pathophysiology of heart rhythm disorders [1-5]. Specifically, alterations of ion channel expression contribute to the initiation and maintenance of cardiac arrhythmias [6, 7]. Current pharmacological antiarrhythmic therapy is limited by reduced effectiveness and side effects in a significant subgroup of patients [8-10]. Targeted therapeutic approaches based on underlying molecular mechanisms may improve future antiarrhythmic management. However, specific mechanisms contributing to defective regulation of ion channel expression during cardiac arrhythmia are poorly understood.

We hypothesized that regulation of gene expression programs through epigenetic modulation may affect cardiac electrophysiology and promote arrhythmogenesis. DNA within the nucleus of eukaryotic cells is packaged together with histones and non-histone proteins in a dynamic manner as chromatin [11]. Histones are subject to modification, including acetylation and deacetylation mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively [12]. Deacetylation of histone lysine residues results in chromatin condensation and modification of gene transcription. In addition, HDACs interact with non-histone proteins such as transcription factors, co-activators and co-repressors [13]. Recent reports revealed that HDACs are involved in cardiac hypertrophy [14-16], fibrosis [17-19] and metabolism [20]. Furthermore, QT-interval prolongation and ventricular arrhythmias have been observed during small molecule HDAC inhibition for anticancer therapy in humans, suggesting cardiac electrophysiological effects of HDAC inhibition [21-24].

The mechanisms underlying electrophysiological actions of HDAC modulation are not known. This study was designed to investigate transcriptional and functional effects of HDAC inhibition on repolarizing cardiac K^+ channels. To this end, the hydroxamic acid trichostatin A (TSA), an established and highly specific small molecule HDAC inhibitor with antitumor activity [25-28], was employed. At the molecular level, TSA serves as pan-HDAC inhibitor of class I (HDACs 1, 2, 3, and 8) and II HDACs (HDACs 4, 5, 6, 7, 9, and 10) at nanomolar concentrations by reversibly binding to the catalytic site of the enzyme [29]. TSA is a zinc-dependent HDAC inhibitor that harbors three distinct domains (Fig. 1 A) [30]. First, the surface recognition site is formed by an aromatic group and interacts with the binding pocket of HDAC molecules. Second, a zinc binding domain coordinates to the active site of Zn^{2+} ion. Third, the linker region connects the surface recognition site to the zinc binding domain. Specifically, structural studies using TSA bound to HDAC revealed that the

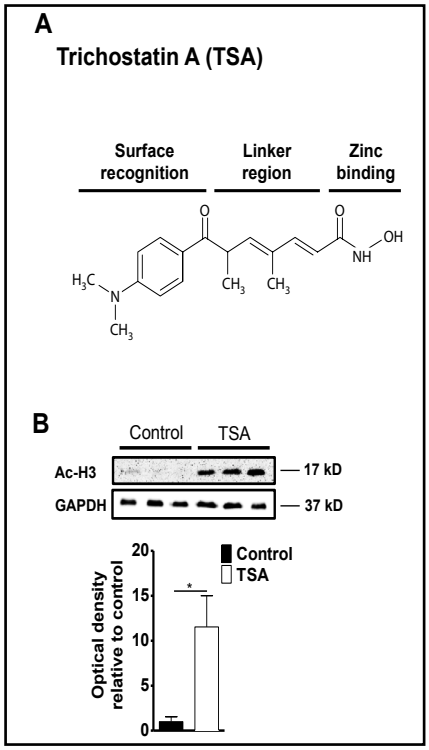

Fig. 1. Increased histone acetylation after pan-HDAC inhibition by trichostatin A (TSA). A. Chemical structure ($C_{17}H_{22}N_2O_3$) of TSA. Functional domains of the compound are indicated. B. Protein levels of acetylated histone H3 (Ac-H3) were analyzed by Western blot in HL-1 cells incubated with 100 nM TSA for 12 hours, followed by quantification of optical density normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Immunoblots (upper panel) and mean values (±SEM) obtained following TSA application (n=3) or control treatment (n=3) (lower panel) are shown; *p<0.05.

surface-recognition element interacts with the entrance rim of the active site, whereas the zinc binding domain forms complexes with the metal ion at the bottom of the site pocket located inside the HDAC molecule [31-33].

The results of this work revealed epigenetic, HDAC-mediated regulation of action potential duration and K^+ channel expression in atrial cardiomyocytes.

Materials and Methods

HL-1 cell culture

HL-1 cells (kindly provided by Dr. William Claycomb, Louisiana State University Health Science Center, New Orleans, LA, USA) were derived from atrial tumor (AT-1) cells of transgenic mice expressing the SV40 large T antigen under the control of the atrial natriuretic factor (ANF) promoter and exhibit cardiomyocyte-specific ion channel expression [34, 35]. Cells were cultured in supplemented Claycomb medium (JRH Biosciences, Lenexa, KS, USA) as previously described [36, 37].

Drug administration

Trichostatin A (Sigma-Aldrich, Steinheim, Germany) was prepared as 5 mM stock solution in DMSO. Aliquots of the stock solutions were diluted freshly to the desired concentrations with culture media on the day of experiments. TSA treatment of HL-1 cell cultures was performed in supplemented Claycomb medium (Sigma-Aldrich, St. Louis, MO, USA) for 12 h when cells were 60-80% confluent, unless indicated otherwise.

Action potential recordings

Cardiac action potentials were recorded from HL-1 cells 12 h after application of 100 nM TSA using the whole-cell patch-clamp technique. Fire-polished capillary glass pipettes (World Precision Instruments, New Haven, CT, USA) were back-filled with 140 mM KCl, 1.0 mM $MgCl_2$, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5 mM ethyleneglycol tetraacetic acid (EGTA), 5 mM adenosine triphosphate potassium salt (KATP), 0.1 mM guanosine triphosphate sodium salt (NaGTP) and 3 mM phosphocreatine sodium salt (Na_2CP) (pH adjusted to 7.2 with KOH). The extracellular solution was Tyrode's solution (140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose and 10 mM HEPES, pH adjusted 7.4 with NaOH). Pipettes had resistances between 1.5 and 5 MΩ. Data were low-pass filtered at 1 to 2 kHz (–3 dB, four-pole Bessel filter) before digitalization at 5 to 10 kHz. Recordings were performed using a commercially available amplifier (Warner OC-725A, Harvard Apparatus, Holliston, MA, USA) and pCLAMP software (Molecular Devices, Sunnyvale, CA, USA) for data acquisition and analysis. Action potentials were elicited in current clamp mode by injection of brief current pulses (2 ms, 1 nA) at a stimulation frequency of 1 Hz and recorded at near physiological temperatures of 30 - 32°C.

RNA isolation and RT-qPCR

Total RNA was isolated from HL-1 cells with TRIzol-Reagent (Invitrogen, Karlsruhe, Germany), followed by chloroform extraction and isopropanol precipitation. RNA was quantified by assessing optical density at 260 and 280 nm (NanoDrop 2000, Thermo Fisher Scientific, Wilmington, DE, USA). Complementary DNA synthesis was carried out with the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific, Waltham, MA, USA) using 2-3 µg (10 µl) of total RNA, 4 µl of 5-fold reaction mixture, and 2 µl Maxima enzyme mix in 20 µl final volume. Quantitative real time PCR (RT-qPCR) was performed using the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. 96 well optical detection plates (Applied Biosystems) were loaded to a total volume of 10 µl per well, consisting of 0.5 µl cDNA, 5 µl TaqMan Fast Universal Master Mix (Applied Biosystems), and 6-carboxyfluorescein (FAM)-labeled TaqMan probes and primers (TaqMan Gene Expression Assays; Applied Biosystems) detecting *Kcnq1* (Mm00434640_m1), *Kcnk2* (Mm01323942_m1), *Kcnk3* (Mm04213388_s1), *Kcnj2* (Mm00434616_m1), *Kcnj3* (Mm00434618_m1), *Kcnj5* (Mm01175829_m1), *Kcnj12* (Mm00440058_s1), *Kcnj4* (Mm02027786_s1), *Kcnd3* (Mm01302126_m1), *Kcne1* (Mm01215533_m1), *Kcna4* (Mm00445241_s1), *Kcna5* (Mm00524346_s1), and *Kcnh2* (Mm00465377_mH). Primers and probes detecting glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used for normalization. All RT-qPCR reactions were performed in triplicate or higher replicates, and non-template control (NTC) and dilution series were included on each

plate for quantification. Data are expressed as an average of triplicates. Data analyses were performed with the Applied System using the second derivative method. All measurements were adjusted using a standard probe, and quantification was corrected for efficiency calculated with the standard curves.

Protein extraction and Western blot analysis

Protein immunodetection was performed by sodium dodecyl sulfate (SDS) gel electrophoresis and Western blotting [38, 39]. HL-1 cells were lysed in radioimmunoprecipitation (RIPA) buffer containing 20 mM Tris-HCl, 0.5% NP-40, 0.5% sodium-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, 1 mM NaF, and inhibitors of proteases (Complete) and phosphatases (PhosStop) (Roche Applied Science, Indianapolis, IN, USA). Homogenates were centrifuged at 4°C for 30 min at 14000 g. Supernatants were collected, and the protein concentration was determined using the bicinchoninic acid (BCA) protein assay (Thermo Scientific, Rockford, IL, USA). Equal amounts of protein were separated on 6–20% SDS polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and developed using primary antibodies directed against acetyl-histone H3 (Ac-H3) (sc-8655; Santa Cruz Biotechnology, Heidelberg, Germany), KCND3 (sc-11686; Santa Cruz Biotechnology), KCNJ2 (sc-18708; Santa Cruz Biotechnology), KCNJ3 (APC-005; Alomone Labs; Jerusalem, Israel), KCNJ5 (sc-23635; Santa Cruz Biotechnology), KCNK2 (sc-11556; Santa Cruz Biotechnology), KCNQ1 (sc-10646; Santa Cruz Biotechnology). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit (ab6721; Abcam, Cambridge, UK), mouse anti-goat (sc-2354; Santa Cruz Biotechnology), and goat anti-mouse (sc-2005; Santa Cruz Biotechnology) secondary antibodies were used where appropriate. Signals were developed using the enhanced chemiluminescence assay (ECL Western Blotting Reagents, GE Healthcare, Buckinghamshire, UK) and quantified with ImageJ 1.41 Software (National Institutes of Health, Bethesda, MD, USA). After removal of primary and secondary antibodies (ReBlot Strong Stripping Solution, Merck, Germany), the membranes were re-probed with anti-GAPDH antibodies (G8140-11; US Biological, Swampscott, MA, USA) and corresponding secondary antibodies (sc-2005; Santa Cruz Biotechnology). Protein content was normalized to GAPDH for quantification of optical density.

Rapid electrical stimulation of HL-1 cells

Gelatin-/fibronectin-coated 6-well dishes were seeded with $2x10^6$ HL-1 cells. After 24h incubation the cells were ≥90% confluent and subjected to electrical stimulation using the C-Pace EP system (IonOptix, Milton, MA, USA), a multi-channel stimulator designed for chronic stimulation of bulk quantities of cultured cells in incubators, providing continual uniform electric field stimulation of cardiac myocytes. The cells were stimulated with 10 V / 10 ms pulses at 5 Hz stimulation rate. Following rapid electrical stimulation for 12 h, cell-viability was visually assessed by microscopic examination, before cells were harvested and subjected to RNA isolation.

Statistics

Data are expressed as mean ±SEM of n experiments. Multiple comparisons were performed using one-way ANOVA, as no interaction between independent nominal variables (i.e., TSA concentrations) could be expected. If the hypothesis of equal means could be rejected at the

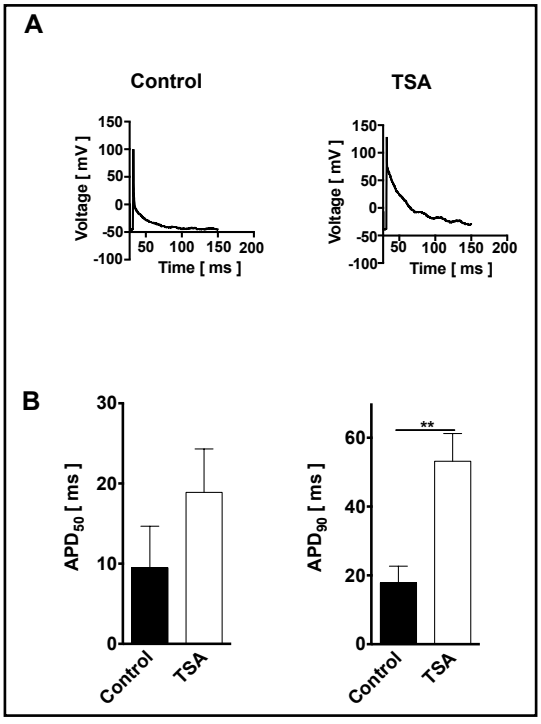

Fig. 2. Action potential prolongation induced by HDAC inhibition. A. Representative action potentials recorded from HL-1 cells under untreated control conditions and after administration of 100 nM trichostatin A (TSA) for 12 h. B. Corresponding mean (±SEM) action potential durations at 50% (APD_{50}) and 90% (APD_{90}) repolarization for controls (n=5) and following HDAC inhibition (n=9). **p<0.01 versus control conditions.

0.05-level, pairwise comparisons of groups were made and the probability values were adjusted for multiple comparisons using the Holm-Sidak correction. Statistical analyses were performed with GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA, USA). Statistical differences of continuous variables were determined using unpaired Student's t tests (two-sided tests). Mann-Whitney U tests were applied if F tests of two samples indicated unequal variance. P<0.05 was considered statistically significant.

Results

HDAC inhibition causes action potential prolongation in HL-1 cardiomyocytes

HL-1 cells were employed to elucidate transcriptional and functional actions of HDAC-inhibition. The cells exhibit multiple features of adult atrial cardiomyocytes including typical ionic currents [34, 40]. First, TSA (Fig. 1 A) was applied to assess the effects of broad spectrum class I/II HDAC inhibition on action potential duration (APD). Inhibition of histone deacetylation by application of 100 nM TSA for 12 h significantly increased the level of acetyl-histone H3 by 11.5-fold (n=3, p=0.04) compared with untreated controls (n=3), indicating effective target modulation by the intervention (Fig. 1 B). TSA treatment prolonged APD at 90% repolarization (APD_{90}) by 2.9-fold to 53±8 ms (n=9, p=0.005) compared to untreated HL-1 cells (18±5 ms; n=5) (Fig. 2 A and B). APD at 50% repolarization (APD_{50}) was not significantly affected by TSA (control, 10±5 ms, n=5; TSA, 19±5 ms, n=9; p=0.25).

TSA induces transcriptional remodeling of potassium channel expression

Prolongation of the action potential duration may be caused by reduced expression of repolarizing K^+ channels. To explore the molecular basis of AP modulation induced by HDAC inhibition, mRNA levels of 13 potassium channel subunits relevant to cardiac electrophysiology were analyzed by real-time qPCR in HL-1 cells (Fig. 3A – 3C). TSA application (100 nM, 12 h) was associated with significant transcriptional changes affecting multiple K^+ channels (Fig. 3 B and C; n=3 independent assays) compared with untreated control cells (Fig. 3 A). HDAC inhibition was characterized by reduced mRNA expression of the $I_{K,Ach}$ channel subunits *Kcnj3* ($K_{ir}3.1$; -36%, p=0.049) and *Kcnj5* (-93%, p=0.0006) involved in repolarization of atrial cardiomyocytes, as well as the pore-forming

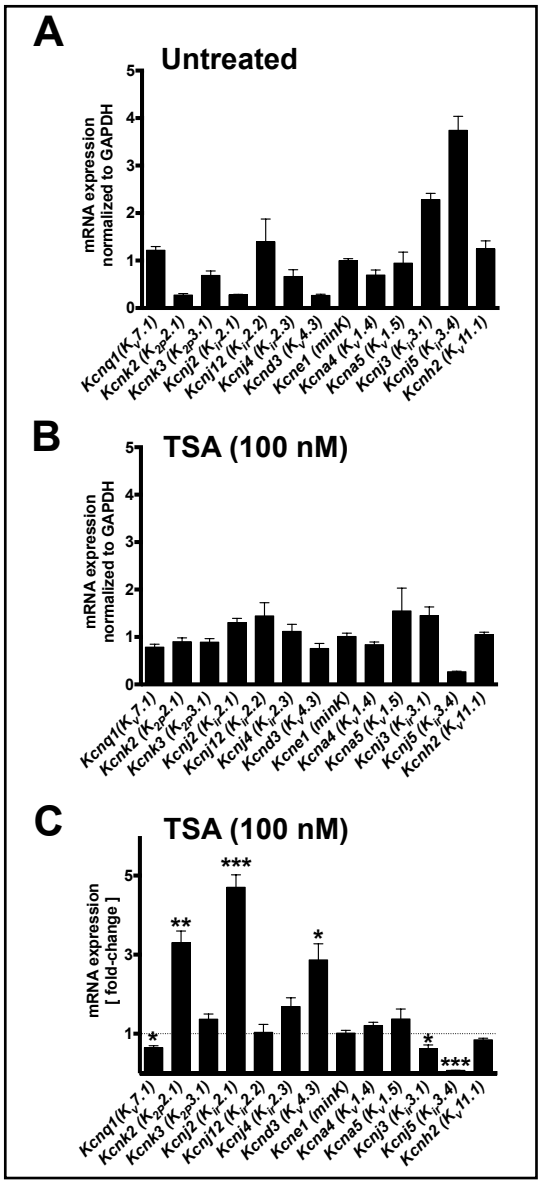

Fig. 3. HDAC-related remodeling of K^+ channel mRNA. Transcription of cardiac ion channel DNA was assessed in HL-1 cells under baseline conditions (Untreated; n=3; A) and after HDAC inhibition using 100 nM trichostatin A (TSA; n=3) (B). C. TSA-induced changes in mRNA expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data are expressed as mean±SEM; *p<0.05; **p<0.01; ***p<0.001.

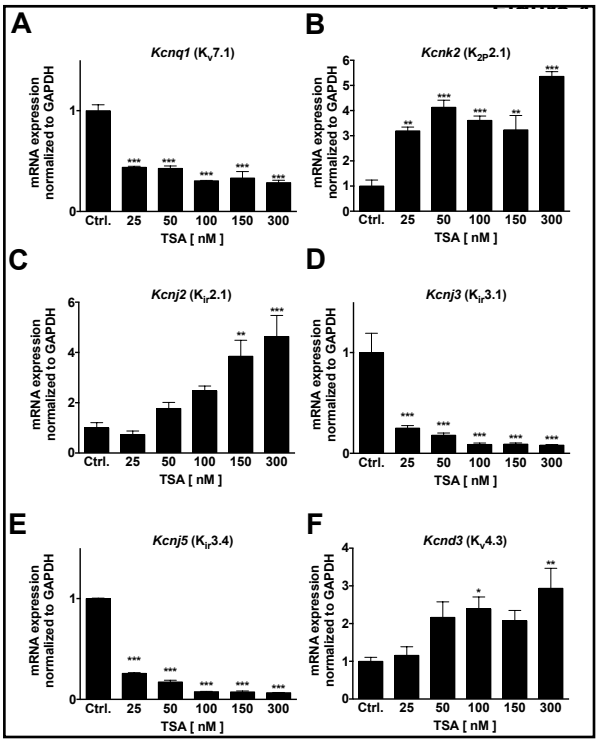

Fig. 4. Concentration-dependent effects of trichostatin A (TSA) on K^+ channel mRNA transcription. A.-F. Changes in mRNA expression of indicated channels were determined after administration of increasing concentrations of TSA relative to untreated controls. Data are given as mean (±SEM) normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Levels of significance (*p<0.05; **p<0.01; ***p<0.001) include adjustments for multiple comparisons using ANOVA followed by Holm-Sidak post hoc testing.

slowly-activating delayed-rectifier K^+ current (I_{Ks}) channel subunit *Kcnq1* (K_v7.1; -35%, p=0.013), a major contributor to atrial and ventricular action potential repolarization. These changes are consistent with delayed repolarization and prolonged APD of cardiomyocytes. By contrast, significantly increased mRNA levels were observed for *Kcnk2* (K_{2P}2.1, TREK-1; +330%, p=0.002), *Kcnj2* (K_{ir}2.1; +470%, p=0.0003), and *Kcnd3* (K_v4.3; +286%, p=0.01) channels.

HDAC inhibitors such as TSA induce biological effects at nanomolar concentrations in a dose-dependent manner. To further characterize pharmacological regulation of K^+ channel transcription by HDAC inhibition, concentration-dependent effects of TSA were assessed. Messenger RNA expression of channels exhibiting significant modulation by 100 nM TSA (Fig. 3) was quantified in one (*Kcnk2*, *Kcnj2*, *Kcnj3*, *Kcnj5*) or two (*Kcnq1*, *Kcnd3*) independent assays after application of increasing concentrations of TSA (25 nM, 50 nM, 100 nM, 150 nM, and 300 nM) for 12 h relative to control conditions in the absence of TSA (Fig. 4A – 4F). *Kcnj3* (25 nM: -75%, p<0.001; 300nM: -92%, p<0.001) and *Kcnj5* transcript levels (25 nM: -74%, p<0.001; 300 nM: -93%, p<0.001) underlying atrial $I_{K,Ach}$ exhibited concentration-dependent downregulation (Fig. 4 D and E). In addition, we observed reduced *Kcnq1* mRNA expression (25 nM: -56%, p<0.001; 300 nM: -71%, p<0.001) (Fig. 4 A). Corresponding to our screening data presented above, TSA induced concentration-dependent enhancement of *Kcnk2* (5.2-fold increase at 300 nM; p<0.0001) and *Kcnd3* (2.9-fold increase at 300 nM; p=0.003) transcript levels (Fig. 4 B and F). Furthermore, *Kcnj2* mRNA levels normalized to GAPDH were increased by 4.6-fold (300 nM TSA, p=0.0006) (Fig. 4C).

HDAC-related transcriptional remodeling alters cardiac K^+ channel protein levels

Translational effects of transcriptionally regulated K^+ channels were assessed by Western blot analysis in HL-1 cells incubated with 100 nM TSA for 12 h (Fig. 5A – 5F). Suppressed mRNA expression of $I_{K,Ach}$ channel subunits resulted in KCNJ3 and KCNJ5 protein reduction by 49% (p=0.012; n=6) and 31% (p=0.041; n=6), respectively (Fig. 5 D and E). Similarly, reduced protein expression was observed for KCNQ1 after HDAC inhibition (-43%; p=0.004; n=6) (Fig. 5 A). In addition, enhanced mRNA transcription after HDAC inhibition resulted in upregulated KCNK2 (+93%; p=0.009; n=6) and KCNJ2 protein levels (+87%; p=0.037; n=6),

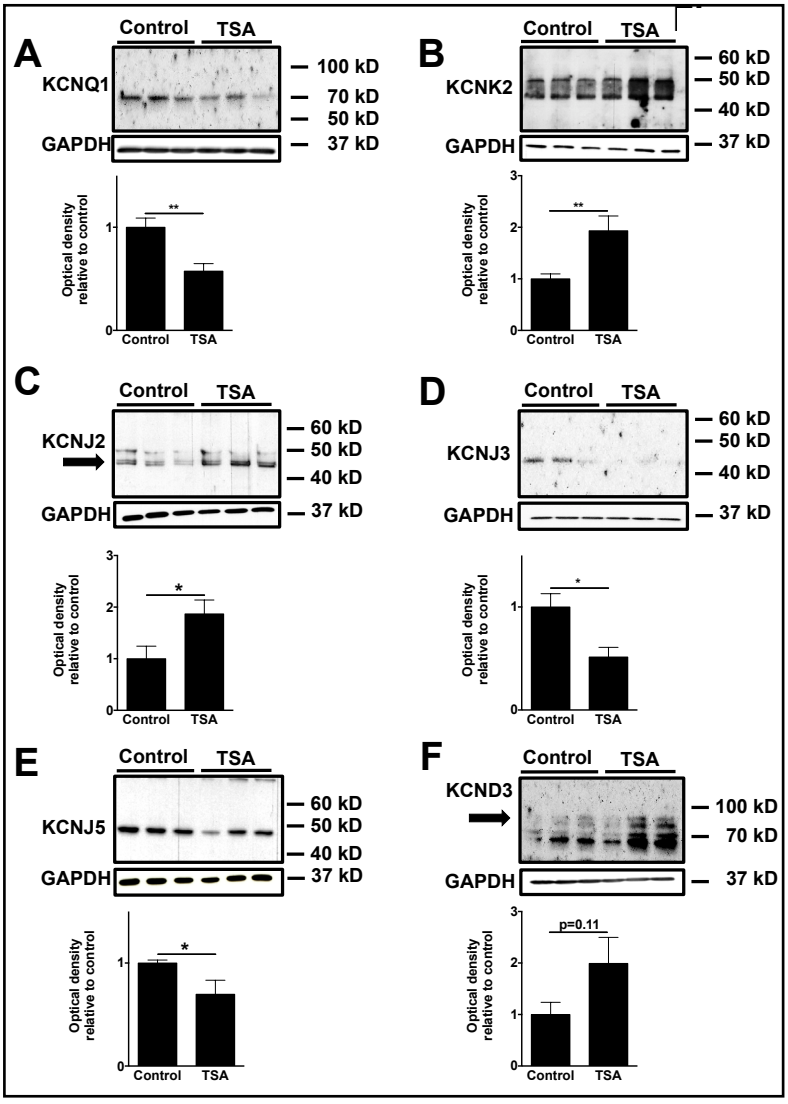

Fig. 5. Effects of HDAC inhibition on K^+ channel protein expression. Representative immunoblots (upper panels) and mean optical density values normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) obtained from six independent experiments (lower panels) are shown in the absence (Control) and presence of 100 nM trichostatin A (TSA) for subunits exhibiting significant transcriptional regulation (see Fig. 3): KCNQ1 (A), KCNK2 (B), KCNJ2 (C), KCNJ3 (D), KCNJ5 (E), and KCND3 (F). *p<0.05; **p<0.01 for indicated pairwise comparisons.

respectively (Fig. 5 B and C). Finally, KCND3 protein levels were not significantly altered by TSA application (n=6), indicating low relevance of HDAC-dependent modulation of pore-forming $K_v4.3$ subunits under the given experimental conditions (Fig. 5 F).

Tachypacing-associated upregulation of KCNJ5 potassium channels is reversed by broad-spectrum HDAC inhibition

Rapid electrical pacing of cultured cells *in vitro* or tissue *in vivo* is an established model of cardiac tachyarrhythmia that may induce electrophysiological remodeling similar to human findings [1, 3, 5, 35, 41, 42]. In particular, tachypacing of HL-1 cells has been shown to cause action potential shortening [35]. To test the hypotheses that rapid pacing induces AP shortening by K^+ channel upregulation, and that reduced APD may be reversed by HDAC inhibition and associated K^+ current suppression, experiments with HL-1 subjected to tachypacing (TP) were performed (Fig. 6A – 6F). Resulting mRNA levels were compared to non-paced control cells. Rapid electrical pacing for 12 h did not induce significant transcriptional regulation of *Kcnj3* or *Kcnj5* $I_{K,Ach}$ subunits, inward-rectifier potassium channel subunits *Kcnj2*, or *Kcnq1*, *Kcnk2*, and *Kcnd3* mRNA abundance, respectively (Fig. 6A – 6F; n=3 each). By contrast, HDAC inhibition (100 nM) during electrical tachypacing reduced mRNA levels of $I_{K,Ach}$ channels (*Kcnj3*: -33%, p<0.001, n=6; *Kcnj5*: -81%, p=0.003, n=3) (Fig. 6

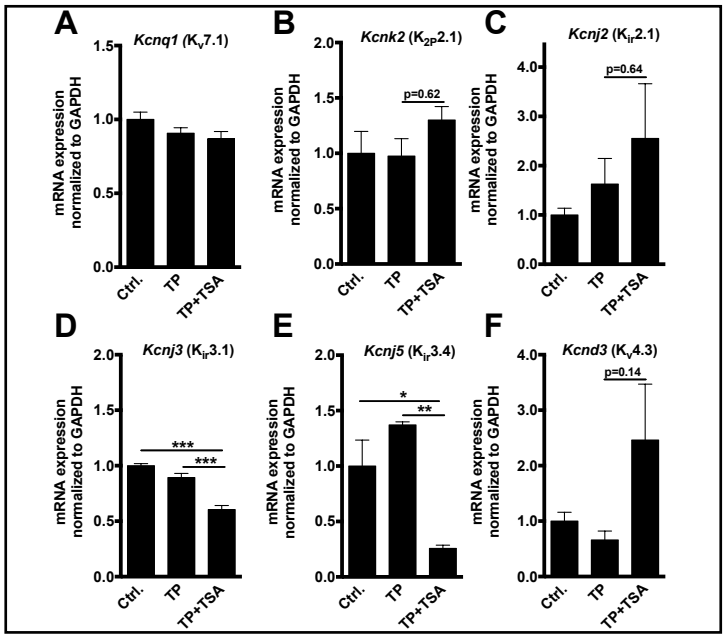

Fig. 6. Differential effects of electrical tachypacing (TP) and HDAC inhibition using 100 nM trichostatin A (TSA) on potassium channel mRNA levels. A.-F. Kcnq1 (A), Kcnk2 (B), Kcnj2 (C), Kcnj3 (D), Kcnj5 (E), and Kcnd3 (F) mRNA expression under indicated experimental conditions was calculated relative to untreated controls (Ctrl.) as mean (±SEM) normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (n=3 each). *p<0.05; **p<0.01; ***p<0.001. P values were calculated by ANOVA followed by Holm-Sidak post hoc testing.

D and E). Apparent increases of *Kcnk2* and *Kcnd3* mRNA abundance, and reduction of *Kcnq1* mRNA levels during TP in the presence of TSA were not significantly different from untreated controls or from cells subjected to TP alone, respectively, following correction for multiple statistical comparisons (Fig. 6 A, B and F).

Discussion

Electrophysiological effects of HDAC inhibition in cardiomyocytes

This study reveals action potential prolongation by broad spectrum HDAC inhibition in HL-1 atrial cardiomyocytes. Changes of cardiomyocyte electrophysiology were associated with reduced mRNA and protein levels of repolarizing KCNQ1, KCNJ3 and KCNJ5 potassium channels. Inhibition of the $I_{K,Ach}$ channel subunits KCNJ3 and KCNJ5 by tertiapin or by lentiviral knock-down was previously shown to prolong action potential duration in neonatal rat atrial cardiomyocytes and in intact atria [43]. Furthermore, reduction of repolarizing I_{Ks} conducted by KCNQ1/KCNE1 channels caused by genetic mutation or pharmacological inhibition accounts for APD prolongation and long QT syndrome [44-46]. Thus, decreased KCNJ3, KCNJ5 and KCNQ1 levels are likely to contribute mechanistically to prolonged cardiac repolarization after HDAC inhibition. Of note, regulation of additional (non-potassium) ion channels that were not investigated here may have contributed to APD regulation as well. Other K^+ channels (i.e., KCNK2, KCNJ2 and KCND3) were upregulated in response to pan-HDAC inhibition. Enhanced levels of voltage-gated potassium channels in isolation are expected to shorten action potential duration. Here, K^+ channel upregulation may have attenuated the prolongation of repolarization by K^+ channel suppression in HL-1 cells. However, it is important to note that overall expression levels of *Kcnk2*, *Kcnj2* and *Kcnd3* mRNAs were relatively low compared to *Kcnq1*, *Kcnj3* and *Kcnj5* (Fig. 3 A). Thus, low abundance of *Kcnk2*, *Kcnj2* and *Kcnd3* likely accounts for the lack of action potential shortening despite increased channel mRNA expression. Both shortening and prolongation of cardiomyocyte APD are clinically relevant, as they might either promote heart rhythm disorders or could be exploited for antiarrhythmic therapy, depending on the type of arrhythmia and on individual electrophysiological conditions.

The emerging role of HDACs in atrial fibrillation pathophysiology and therapy

Effective and safe management of AF still poses a major clinical challenge in cardiovascular medicine. Antiarrhythmic drugs frequently rely on non-selective inhibition of potassium or sodium channels, resulting in prolongation of APD, refractory periods, or electrical conduction velocity [47]. To date there is no clinical antiarrhythmic concept targeting arrhythmia-induced electrical remodeling. HDACs represent potential novel targets as they may act as key regulators of proarrhythmic gene programs. Shortening of atrial effective refractory periods and APD are observed in chronic AF patients, promoting electrical re-entry and perpetuation of the arrhythmia [7]. In the present work, HDAC inhibition prolonged atrial APD, suggesting that HDAC inhibition may be utilized in antiarrhythmic interventions in patients with chronic (i.e., persistent or long-standing persistent) AF. Antagonistic molecular effects of AF and HDAC-inhibition, respectively, provide the cellular electrophysiological basis for a novel rhythm control paradigm. Of note, in this study we employed a non-selective HDAC inhibitor, resulting in cumulative effects of multiple HDACs on cardiac cellular electrophysiology. Future translation of this epigenetics-based concept will require the identification of specific HDACs involved in proarrhythmic ion channel remodeling during AF.

Significance of HDAC inhibition in ventricular electrophysiology and safety pharmacology

The clinical application of HDAC inhibitors for anticancer treatment is associated with QT prolongation and ventricular arrhythmias [21-24, 48]. TSA-induced APD prolongation in cardiomyocytes is consistent with QT interval prolongation observed in humans during clinical application of small molecule HDAC inhibitors. While extrapolations from experiments in atrial HL-1 cells to ventricular repolarization are limited by differences in ion channel expression between atrial and ventricular cardiomyocytes, the majority of channels that are relevant to safety pharmacology are present in the cells investigated here (i.e., KCNQ1 and KCNH2). In particular, reduced KCNQ1 levels (conducting the slowly activating component of the delayed rectifier K^+ current in ventricular myocytes, I_{Ks}) could account for ventricular proarrhythmia during clinical application of TSA. Of note, *Kcnh2* mRNA encoding the rapidly activating I_{Kr} that is frequently affected by QT-prolonging compounds [49-52] was not reduced by TSA. Finally, *KCNJ3* and *KCNJ5* mRNAs display very low ventricular expression in humans [7]. Therefore, TSA- and HDAC-dependent modulation of encoded $I_{K,ACh}$ is not expected to contribute significantly to ventricular electrophysiology. Based on these findings we suggest that effects of HDAC inhibitors on KCNQ1 subunits should be carefully considered during preclinical safety assessment.

Potential limitations and future directions

The present approach does not allow for the identification of specific HDAC isoforms responsible for the regulation of potassium channel expression. TSA was applied to achieve broad inhibition of multiple class I/II HDACs to assess cumulative HDAC effects in HL-1 atrial cardiomyocytes. Given that different HDAC isoforms may govern specific gene expression programs in physiology and disease, the future investigation of isoform-selective inhibitors may identify compounds with improved target specificity, efficacy and safety. Furthermore, alterations of protein acetylation may directly affect cardiac ion channel biophysics and regulation that were not addressed in this investigation.

In the present study we used a 12 h tachypacing period with a moderate pacing rate to mimic typical episodes of paroxysmal atrial fibrillation. The lack of transcriptional effects on potassium channel subunits may be attributable to the short duration of tachypacing, as K^+ channel remodeling is time- and rate-dependent under experimental conditions [53]. Therefore, we cannot exclude that additional electrical alterations may occur during longer AF simulation periods that may more closely resemble chronic AF. Furthermore, neurohumoral proarrhythmic mechanisms may not be fully represented by rapid pacing of cultured cells.

Finally, the study focused on the emerging role of histone modification in cardiac arrhythmogenesis and antiarrhythmic therapy. In addition to HDAC-dependent transcriptional regulation, DNA hypermethylation was observed in AF patients, and pharmacologic hypomethylation suppressed atrial arrhythmias in a rat model [54, 55]. We suggest that mechanisms related to methylation and other epigenetic factors involved in electrical remodeling are beyond the scope of the present work and remain to be investigated in separate approaches.

Conclusion

Effects of HDAC inhibition and increased histone acetylation on potassium channel expression and cardiomyocyte APD highlight previously unrecognized epigenetic regulation of cardiac electrophysiology. Pharmacological targeting of specific HDACs may exert antiarrhythmic effects by preventing or correcting ion channel remodeling. Potential proarrhythmic effects of broad-spectrum HDAC inhibitors on ventricular repolarization require consideration during preclinical drug safety screening.

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Disclosure Statement

The authors declare to have no competing interests.

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