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DPP10 is a new regulator of Nav1.5 channels in human heart

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ABSTRACT

Background: Cardiac accessory β-subunits are part of macromolecular Nav1.5 channel complexes modulating biophysical properties and contributing to arrhythmias. Recent studies demonstrated the structural interaction between β-subunits of Na^+ (Nav1.5) and K^+ (Kv4.3) channels. Here, we identified the dipeptidyl peptidase-like protein-10 (DPP10), which is known to modulate Kv4.3-current kinetics, as a new regulator of Nav1.5 channels.

Methods: We assessed DPP10 expression in the healthy and diseased human heart and we studied the functional effects of DPP10 on the Na^+ current in isolated rat cardiomyocytes expressing DPP10 after adenoviral gene-transfer (DPP10ad).

Results: DPP10 mRNA and proteins were detected in human ventricle, with higher levels in patients with heart failure. In rat cardiomyocytes, DPP10ad significantly reduced upstroke velocity of action potentials indicating reduction in Na^+-current density. DPP10 significantly shifted the voltage-dependent Na^+ channel activation and inactivation curve to more positive potentials, resulting in greater availability of Na^+ channels for activation, along with increasing window Na^+ current. In addition, time-to-peak Na^+ current was reduced, whereas time course of recovery from inactivation was significantly accelerated by DPP10ad. DPP10 co-immunoprecipitated with Nav1.5 channels in human ventricles, confirming their physical interaction.

Conclusion: We provide first evidence that DPP10 interacts with Nav1.5 channels, linking Na^+- and K^+-channel complexes in the heart. Our data suggest that increased ventricular DPP10 expression in heart failure might promote arrhythmias by decreasing peak Na^+ current, while increasing window Na^+ current and channel re-openings due to accelerated recovery from inactivation.

1. Introduction

Cardiac Na^+ channels (I_{Na}) are responsible for the rapid depolarization phase of the cardiac action potential (AP), controlling cardiac excitability and ensuring proper impulse propagation. The pore-forming α-subunit Nav1.5 of I_{Na} is regulated by several interacting proteins [1]. For instance β-subunits like the transmembrane Navβ1 can affect Nav1.5-channel trafficking or its anchoring to specific membrane compartments, along with the modulation of gating properties of Nav1.5. Mutations in the genes coding for Nav1.5-regulatory proteins such as Navβ1 have been identified in patients with Brugada syndrome (BrS), atrial fibrillation or cardiac conduction diseases. Unexpectedly β-subunits such as Navβ1 do not only interact with Nav1.5 channels, but also modulate properties of the transient outward K^+ current I_{to} via interaction with its pore-forming α-subunit Kv4.3 [2]. Inhibition of Navβ1 transcription in rat ventricular myocytes using small interfering RNA caused a reduction in expression levels of Nav1.5, Kv4.2 and Kv4.3 channel subunits [3], pointing to complex interaction patterns between Na^+- and K^+-channel subunits, which may have important implications for cardiac arrhythmogenesis.

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Mutations with enhanced expression of dipeptidyl peptidase-like protein-6 (DPP6), which lacks enzymatic activity, were found in patients with idiopathic ventricular fibrillation and altered DPP6 was associated with changes in I_{to} properties and AP shape [4–6]. The mRNA expression of DPP6 was significantly increased in patients with chronic heart failure (HF) [7]. DPP10 is another dipeptidyl peptidase-like protein, which was detected in human atria, but not studied in human ventricles [8,9]. DPP10 plays an important role for physiological properties of Kv4.3 channels, affecting their cell-surface expression, subcellular localization and electrophysiological properties [10]. In heterologous expression systems, DPP10 interacts with Kv4.3 channels, shifting the voltage-dependence of steady-state activation and inactivation to more negative potentials as well as accelerating kinetics of activation, inactivation and recovery from inactivation [8,11,12]. Here we discovered DPP10 as a new regulator of Nav1.5 channels, functionally linking Nav1.5- and Kv4.3-channel complexes. Ventricular DPP10 expression increases in human heart failure, potentially promoting arrhythmias by decreasing Na^+-current density, while increasing window Na^+ current and channel re-openings due to accelerated recovery from inactivation.

2. Methods

An extended description of employed methods is provided in the online data supplement.

2.1. Ethical approval

The investigation was approved by the ethics committee of Dresden University of Technology (No: EK114082002) and the Albert Szent-Gyorgyi Medical University Ethical Review Board (Szeged). The study conforms to the principles outlined in the Declaration of Helsinki. Each patient gave written informed consent.

2.2. Human cardiac tissue samples

Human heart tissue was derived from healthy non-failing (NF) donor hearts (four male, two female), which could not be used for transplantation due to technical reasons, or from explanted hearts of NYHA IV HF patients. Patient characteristics are summarized in Supplemental Table 1. Biopsies were excised from the central region of the anterior wall of left (LV) and right (RV) ventricles, were separated into subepi-(epicardial) and subendocardial (endocardial) layers.

2.3. Isolation of rat ventricular myocytes

The investigation complies with the ARRIVE guidelines and conforms to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and was approved by the Leipzig University Committee on the Use and Care of Animals. Epicardial cardiomyocytes were isolated enzymatically from adult male Wistar rats (8 weeks old) as previously described [13]. Cardiomyocytes were plated on poly-L-lysine coated wells and cultured for 48 h.

2.4. Adenoviral DPP10 expression

A bicistronic construct encoding V5-tagged DPP10 and green fluorescent protein (GFP) under control of the cytomegalovirus promoter (CMV) was generated by inserting the cDNA into the pShuttle vector (Q-BIOgene, USA). Adenoviruses were produced using the AdEasy system (Q-BIOgene, USA) according to the Manufacturer's Instructions. Freshly isolated rat cardiomyocytes were infected either with DPP10a-encoding adenovirus (DPP10ad) or with a control adenovirus encoding GFP only (control, GFPad) and cultured for 48 h.

2.5. CHO cells culture and transfection

The Chinese hamster ovary (CHO) cell line was cultured as previously published [8]. Transient transfection of plasmids encoding for hSCN5A-pCGI, hDPP10a-V5tag-pIRES-EGFP and pIRES-EGFP were done with a Roti-Fect® transfection reagent (Carl Roth, Germany). Cells were cultured for 48 h.

2.6. Biochemistry and molecular biology

After isolation of total RNA real-time RT-PCR was performed using the QuantiTect SYBR Green RT-PCR kit (Qiagen, Hilden, Germany) as previously described [7].

For protein quantification the specific primary DPP10 antibody (ab42084, Abcam) was used detecting the catalytic domain, but not other DPP proteins. We validated the antibody using dilution series of protein lysates detecting heterogeneously expressed DPP10 in HEK cells, but not DPP6 proteins (Supplemental Fig. 1).

For immunoprecipitation experiments, precleared lysates were incubated with 2 μg primary antibody for Nav1.5 (ASC-005, Alomone), DPP10 (Abcam) or rabbit IgG (Sigma) overnight at 4 °C followed by protein G Sepharose for 6 h. Protein–antibody complexes were collected, washed and denatured. Proteins were detected with antibodies against DPP10 (CHO cells: anti-V5tag ab27671, Abcam; human tissue: anti-DPP10, anti-Nav1.5) or anti-GAPDH (AB2302, Millipore) by immunoblotting.

2.7. Electrophysiology

Action potentials and Na^+ currents of rat cardiomyocytes were recorded using whole-cell patch-clamp technique at 23 °C with the MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Clamp-pulse generation, data collection and analysis were performed with pCLAMP 10 software. Mean series resistance was 14 ± 0.8 MΩ ($n = 22$) for rat cardiomyocytes, and was compensated by at least 75%. Recording solutions and conditions are described in the online Data Supplement.

2.8. Statistics

Data are presented as means ± S.E.M. Statistical analysis was performed with GraphPad Prism software (V5.1; San Diego, CA). Either unpaired Student's *t*-tests with equal variances and normal distribution, Welch's *t*-test with unequal variances and normal distribution or nonparametric Mann–Whitney *U* tests were used to determine statistical differences, which were considered significant if $p < 0.05$.

3. Results

3.1. DPP10 upregulation in human heart failure

First, we investigated expression of DPP10 mRNA in human ventricle. Since a transmural gradient was shown for several β-subunits, we assessed the distribution of DPP10 mRNA between subepi- and subendocardial tissues of LV and RV. DPP10 mRNA was expressed at equal levels in NF (Fig. 1A), whereas DPP10 mRNA was significantly higher in patients with HF compared to NF, with the strongest upregulation being detected in the epicardial layer of both ventricles and in endocardial tissue of the LV (Fig. 1A).

Protein expression of DPP10 was studied by immunoblotting (Fig. 1B). In addition to the expected band at 95 kDa, two additional bands at 100 and 105 kDa, likely corresponding to increased DPP10 glycosylation [8], were noted in lysates from HF samples. In accordance with mRNA levels, DPP10 protein was significantly upregulated in human HF tissues from the epi- and endocardial region of the LV compared to the respective regions in NF (Fig. 1C).

3.2. DPP10 affects AP and I_{Na} properties in rat epicardial cardiomyocytes

To evaluate the consequences of upregulated DPP10 in cardiomyocytes, we expressed DPP10 using adenovirus gene-transfer in rat epicardial cardiomyocytes, which have very low levels of endogenous DPP10 (Fig. 2A). Two days after infection with recombinant adenoviruses, we recorded APs from rat ventricular cardiomyocytes expressing DPP10ad or GFPad (control). Representative AP recordings are illustrated in Fig. 2B. DPP10ad expression significantly decreased the AP upstroke velocity pointing to a modulation of Nav1.5 channels (Fig. 2C). Resting membrane potential, AP amplitude and duration were not affected by DPP10ad (Fig. 2C, Supplemental Table 2).

To investigate the potential functional interaction of Na^+ channels with DPP10, I_{Na} was measured in rat epicardial cardiomyocytes infected with adenoviruses encoding GFP (control) or DPP10 (DPP10ad). Representative recordings at different membrane voltages are shown in Fig. 2D. I_{Na} density between −45 mV and −35 mV was significantly reduced by DPP10ad indicating an effect on Na^+-channel activation (Fig. 2E). Interestingly, the voltage-dependence of I_{Na} activation and inactivation was significantly shifted (by ~5 and 6 mV) to more positive voltages with DPP10ad compared to controls (Fig. 2F, Table 1). Accordingly, the calculated voltage (V_o) at the crossover of activation and inactivation curves was shifted to more positive potentials (Table 1). The overlap of both curves identifies a voltage range (window) where the channels have a small probability of being partially but not fully inactivated [14,15]. The product of the fitted activation and inactivation

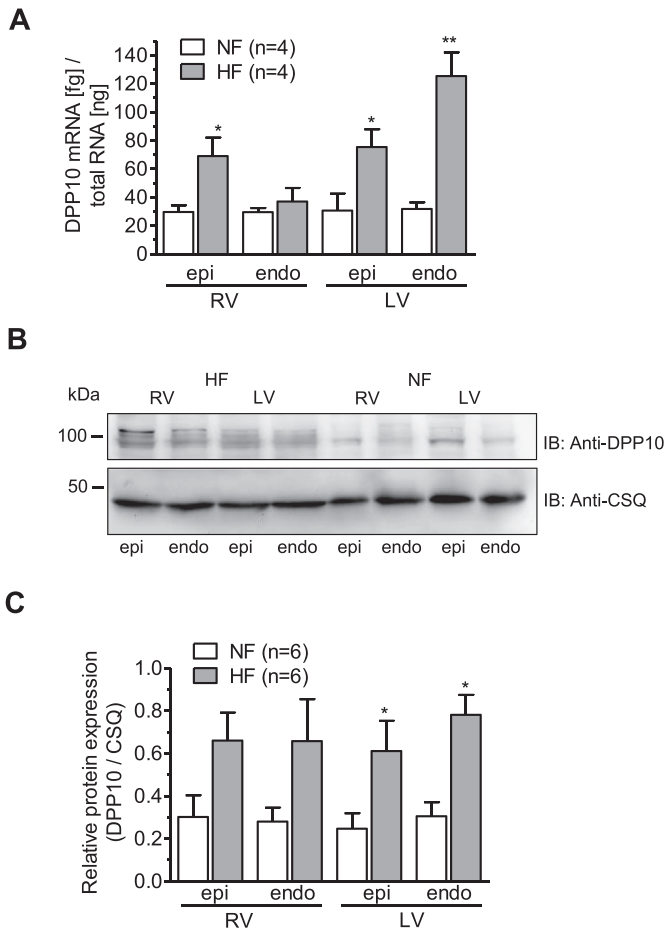

Fig. 1. DPP10 mRNA and protein are upregulated in human ventricles from patients with heart failure. (A) DPP10 mRNA expression in non-failing (NF) and failing human hearts (HF) in samples from subepi- and subendocardial layers of right (RV) and left ventricles (LV). (B) Representative Western blots showing DPP10 and CSQ protein expression in human ventricles. (C) DPP10 protein expression in NF and HF normalized to CSQ as loading control in samples from subepi- and subendocardial layers of RV and LV. $^{*}p < 0.05$, $^{**}p < 0.01$, NF vs. HF.

parameters represents the probability of being within this window and is plotted in Fig. 2G. The window I_{Na} current was enhanced with DPP10ad (Fig. 2G). DPP10ad also influenced native I_{Na} kinetics. The time-to-peak of Na^+ current was significantly prolonged with DPP10ad at −20 mV (Table 1). The recovery from inactivation of native Na^+ channels was significantly accelerated in DPP10ad infected cardiomyocytes compared to controls (Fig. 2H, Table 1).

3.3. Interaction of Nav1.5 channels with DPP10 in CHO cells

To assess a physical interaction of hDPP10 with hNav1.5 channels, we performed co-immunoprecipitation experiments (IP) in transfected CHO cells, expressing Nav1.5 with or without co-expression of V5-tagged DPP10. Nonimmune antibodies were used as negative controls. Using the Nav1.5 antibody for IP, DPP10 co-immunoprecipitated with Nav1.5 channels when both proteins were transiently expressed (Supplemental Fig. 2A). Conversely, when using the DPP10 antibody for IP, Nav1.5 co-immunoprecipitated with DPP10 (Supplemental Fig. 2B).

Furthermore, we studied the DPP10 influence on I_{Na} currents in CHO cells transiently co-expressing Nav1.5 and DPP10 to exclude possible counteracting ion channel interactions in cardiomyocytes, like Kv4 and KCNT channels [11,16]. In CHO cells DPP10 showed similar effects on Nav1.5 properties as observed in rat cardiomyocytes expressing DPP10ad reducing current density, positively shifting the activation and inactivation curve and hastening the time course of recovery from inactivation (Supplemental Fig. 2C–F).

3.4. DPP10 is part of Nav1.5-channel complexes in human heart

In order to validate the new interaction of Nav1.5 channels with DPP10 in human heart, we performed co-immunoprecipitation experiments in protein lysates from human ventricles using Nav1.5 (Fig. 3, left) or DPP10 antibodies. DPP10 co-immunoprecipitated with Nav1.5 when pulled down with anti-Nav1.5 (Fig. 3, left) in extracts of LV and RV, and Nav1.5 co-immunoprecipitated with DPP10 after pulldown with anti-DPP10 (Fig. 3, right), clearly indicating a physical interaction between DPP10 with Nav1.5 proteins in human heart.

4. Discussion

Here we discovered a new regulator of Nav1.5 channels in heart. We found that a) expression of DPP10 is significantly upregulated in human ventricles from HF patients, b) in rat epicardial cardiomyocytes DPP10 reduced AP upstroke velocity and decreased peak I_{Na}, while increasing window I_{Na} and channel reopenings due to accelerating recovery from inactivation, and c) DPP10 co-immunoprecipitated with Nav1.5 in human heart pointing a direct physical interaction between the two proteins. Thus in addition to Kv4 channels, Nav1.5 channels are also regulated by DPP10. Overall, our findings suggest that the increase in ventricular DPP10 expression in human HF might promote arrhythmias by decreasing I_{Na} density, thereby slowing impulse propagation and increasing abnormal impulse formation (triggered activity) by the higher probability of I_{Na} re-openings. In rat ventricular cardiomyocytes, sufficient Na^+ channel reopening may produce slow inactivation potentially contributing to the plateau of cardiac AP [17].

4.1. DPP10 effects on Nav1.5 channels

In rat epicardial cardiomyocytes we noted that DPP10ad reduced peak I_{Na} and shifted the voltage-dependence of I_{Na} activation and inactivation to more positive potentials, resulting in enhanced availability of Na^+ channels for activation compared to controls. Further detailed analysis of the biophysical DPP10 effects on Nav1.5 channel activation and re-activation should be done in future studies. Interestingly, the transmembrane β-subunit Navβ1 was shown to shift the voltage-dependence of Nav1.5 channels in opposite direction towards negative potentials after co-expression [18,19]. The time-to-peak of I_{Na} was slowed, whereas the time course of recovery from inactivation was accelerated in DPP10ad expressing rat cardiomyocytes. A short domain of the DPP10 N-terminus has been proposed to mediate the DPP10 effects on Kv4 kinetics [11,20]. As observed for DPP10, the time course of recovery from inactivation was also altered by Navβ1 [18,21]. Thus, the dynamic regulation of cardiac Nav1.5 by both DPP10 and Navβ subunits should be detailed in future studies.

4.2. The physiological relevance of DPP10 in mammalian ventricle

Previous work showed that DPP10 is expressed in human atria [9]. Here, we showed for the first time that DPP10 is abundant in human ventricles, but at a lower level than that of DPP6 [7].

Our data provide new insights into the functional association of I_{Na} and I_{to} that are essential for normal depolarization and early repolarization in the heart. During HF, the expression of I_{to} and I_{Na} channel subunits is decreased causing a reduction of both currents [22]. In contrast, DPP6 and DPP10 (this study) were upregulated in ventricles of patients with HF compared to NF donors [7]. Patients with HF exhibit prolonged APs and a high risk for lethal ventricular arrhythmias partially due to the imbalance of I_{Na} and I_{to} currents [22]. We interpret our results to suggest that the increased DPP10 expression and the related changes in I_{Na} properties might contribute to the imbalance of

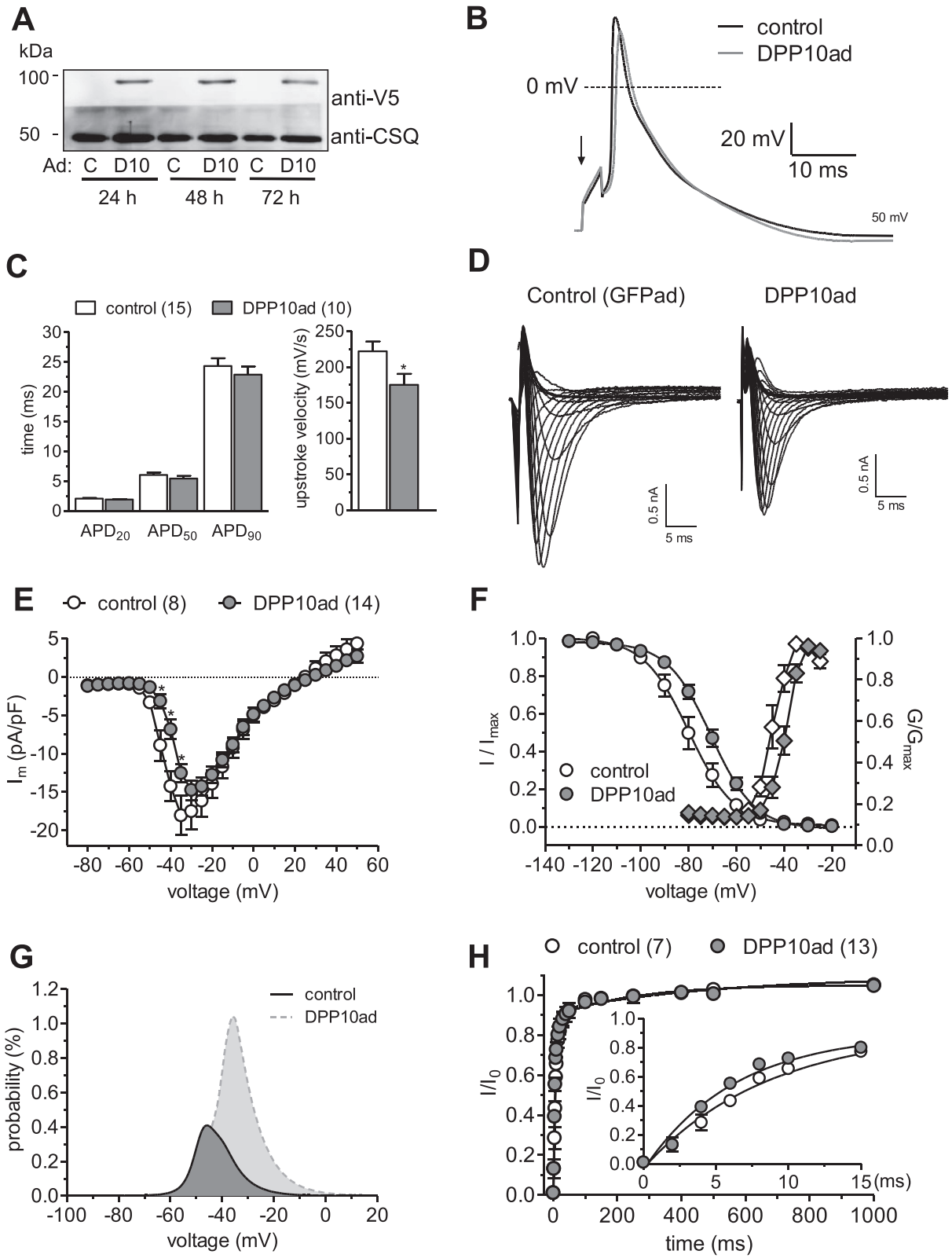

Fig. 2. DPP10ad affects action potential (AP) and I_{Na} in rat epicardial cardiomyocytes. (A) Immunoblot of rat cardiomyocytes infected with DPP10- (D10) or GFP-encoding adenoviruses (C), and maintained for the indicated times after infection. (B) Representative recordings of APs in cardiomyocytes infected with GFPad (control) or DPP10ad. (C) Effect of DPP10ad expression on AP duration at 20%, 50% and 90% of repolarization and on the maximum upstroke velocity (dV/dt_{max}). (D) Whole-cell I_{Na} of control cardiomyocytes (left) and cardiomyocytes expressing DPP10ad (right) at different voltages. (E) Current-voltage relationships. (F) Normalized voltage-dependence of activation (G/G_{max}) and of steady-state inactivation (I/I_{max}). Boltzmann functions were fitted to mean data to estimate half-maximum voltage ($V_{0.5}$). (G) Window current was calculated from the overlap of activation and inactivation curves. The probability of Nav1.5 channels being within this window in cardiomyocytes infected with GFPad (black continuous line) or DPP10ad (grey dotted line) is plotted. (H) Recovery from inactivation at −20 mV from holding potential of −120 mV as determined by a double-pulse protocol with a variable interpulse interval. Inset: time course of recovery from inactivation at an expanded time scale. Bi-exponential functions were fitted to data points. (n) Number of cardiomyocytes. $^*p < 0.05$ for control vs. DPP10ad.

Table 1
Effects of DPP10ad on endogenous Na^+ currents in rat epicardial cardiomyocytes. $I_{m,peak}$ (pA/pF), maximum Na^+-current density; $V_{0.5}$ (mV), half-maximum voltage of activation or steady-state inactivation curves with respective slope factors; Window current, the overlap of the activation and steady-state inactivation curve with the overlap voltage (V_o) and the window current amplitude (%); TtP (ms), time-to-peak of I_{Na} maximum; τ_{inact} (ms), time constant of inactivation fitted with mono-exponential functions; τ_1, τ_2 (ms), time constants of recovery from inactivation fitted with bi-exponential functions; n, number of cardiomyocytes. Values represent means ± SEM; $^*p < 0.05$, $^{**}p < 0.01$, control (GFPad) vs. DPP10ad.

Parameter I_{Na}	Control (GFPad)	n	DPP10ad	n	
Capacity (pF)	157 ± 14	8	143 ± 11	14	
$I_{m,peak}$ (pA/pF) at −30 mV	−18 ± 2	8	−15 ± 1	14	
Steady-state activation					
$V_{0.5}$ (mV)	−44 ± 1.4	8	−39 ± 1.0	14	**
Slope (mV)	2.3 ± 0.3	8	2.4 ± 0.3	14	
Steady-state inactivation					
$V_{0.5}$ (mV)	−78 ± 2.6	9	−72 ± 1.7	15	*
Slope (mV)	7.1 ± 0.4	9	7.9 ± 0.3	15	*
Window current					
V_o (mV)	−53 ± 2.5	8	−46 ± 1.1	14	**
Amplitude (%)	0.40 ± 0.19	8	1.04 ± 0.18	14	*
Activation at −30 mV					
TtP (ms)	3.6 ± 0.3	8	4.7 ± 0.3	13	*
Inactivation at −30 mV					
τ_{inact} (ms)	2.1 ± 0.2	7	2.8 ± 0.2	14	
Recovery from inactivation					
τ_1 (ms)	7.5 ± 0.5	7	5.8 ± 0.3	13	*
τ_2 (ms)	482 ± 100	7	213 ± 41	13	*

I_{Na} and I_{to} in human HF. A reduction in AP upstroke velocity and a decrease in transmural conduction have been reported for human HF and HF models [23–27]. Similar to DPP10 effects in this study, a reduced I_{Na} density, a positive shift of the I_{Na} steady-state activation curve and slowed inactivation have been observed in a mouse HF model, resulting in a reduced AP amplitude and a slower upstroke velocity [28]. In human HF, peak I_{Na} was significantly decreased by 43%. The I_{Na} inactivation curve was slightly shifted to more positive potentials while the activation curve was unchanged in non-failing compared to HF cells [29]. In rat epicardial myocytes DPP10 did not affect APD, although prolonged APD could be expected due to the enhanced window I_{Na} [30], likely because of additional DPP10 effects on the dominant epicardial I_{to} in rat, shifting its voltage-dependence to more negative potentials, thereby accelerating I_{to} kinetics [8]. The DPP10-induced faster recovery of I_{Na} from inactivation and the positive shift of steady-state inactivation curve may promote the development of automaticity as well as early and delayed afterdepolarisations, potentially leading to lethal arrhythmias, particularly in patients with HF [31]. This notion is supported by data obtained with a Na^+ channel mutation identified in patients with idiopathic ventricular fibrillation, which also caused a shift of the I_{Na} inactivation curve to more positive potentials and accelerated the recovery of I_{Na} from inactivation [32]. The putative proarrhythmic effects of DPP10 upregulation in HF need direct demonstration in subsequent work.

4.3. Potential limitations

We studied DPP10 function in rat epicardial cardiomyocytes. However, the role of DPP10 may vary between mammalian species. Thus, our results may not reflect the physiological interactions of DPP10 in human in all details. In general, expression levels after adenoviral gene transfer might be higher than the (patho)physiological levels. However, DPP10 association with Kv4 proteins showed a preferred stoichiometry of 2:4 independent from expression levels [32]. In addition, we titrated DPP10ad concentrations as low as possible. Using these conditions, we did not observe any increase in Kv4-subunit carried K^+ current amplitude (Supplemental Fig. 3), which was previously reported [33]. We interpret this finding that the achieved expression of DPP10 in cardiomyocytes was rather too low to affect Kv4-mediated K^+ currents although it was sufficient to alter Na^+ currents. Finally, we observed similar DPP10 effects on Na^+ currents in CHO cells co-expressing Nav1.5 and DPP10 (1:1 ratio) as in rat cardiomyocytes. Altogether, this makes supra-pathophysiological expression of DPP10 rather unlikely. However, a selective DPP10 knockdown in human or rat ventricular cardiomyocytes would be the best strategy to uncover its precise contribution to cardiac depolarization and repolarization currents, but these approaches are unfortunately not feasible due to difficulties in maintaining human ventricular cardiomyocytes in culture or due to very low endogenous DPP10 levels in rat hearts. Since I_{Na} function depends on temperature the DPP10 effects DPP10 on Nav1.5 channels might differ at physiological temperature [34]. Despite these limitations, our findings could establish that DPP10 interacts with cardiac Nav1.5 and alters I_{Na} properties, which might have important clinical implications for the promotion of cardiac arrhythmias.

5. Conclusion

Here we have detected DPP10 as a novel regulator of Nav1.5-bearing Na^+ channels, linking Na^+ and K^+ channel multiprotein complexes in heart. We establish increased DPP10 expression as a putative promoter of arrhythmias in HF by decreasing peak I_{Na}, while increasing window I_{Na} and channel re-openings due to accelerated recovery from inactivation. Our novel findings expand our understanding of Na^+ channel regulation, which might have important implications for the development of new therapeutic strategies for HF and arrhythmias.

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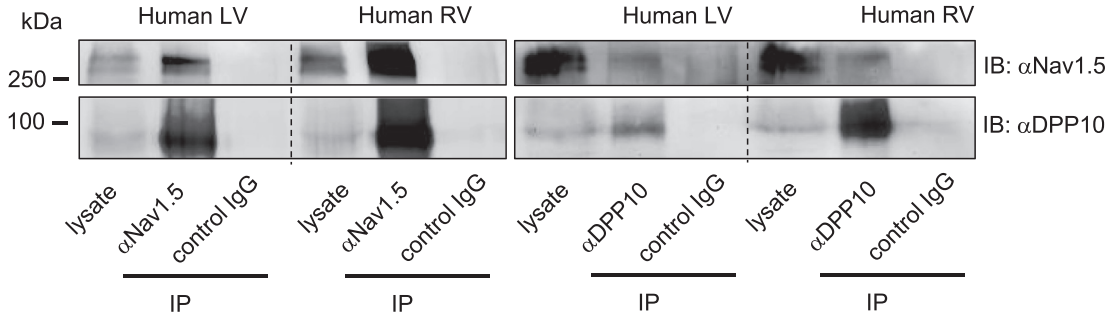

Fig. 3. DPP10 co-immunoprecipitates with Nav1.5 channels in human heart ventricles. Pulldown (IP) of human endogenous Nav1.5 channel complexes with anti-Nav1.5 (A) or anti-DPP10 (B) in protein lysates from right (RV) and left ventricle (LV). Non-immune antibodies (rabbit IgG) were used as negative control. After separation, Nav1.5 and DPP10 proteins were detected by Western blot (IB: anti-Nav1.5, anti-DPP10).

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Conflicts of interests

The authors report no relationships that could be construed as a conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.12.072.

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