

Delavirdine modifies action potential configuration via inhibition of I_{K_r} and I_{t_o} in canine ventricular myocytes

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ABSTRACT

Delavirdine is an anti-HIV agent with structural similarity (presence of methanesulfonamide group) to well-known rapid delayed rectifier potassium current (I_{K_r}) inhibitors like dofetilide and E-4031. In spite of this and the fact, that I_{K_r} blockade can lead to long QT syndrome with a risk of early afterdepolarization, cardiac arrhythmia and even sudden cardiac death development, there is no information on the cardiac electrophysiological effects of the delavirdine. Therefore, we examined the concentration-dependent acute effects of delavirdine on action potential morphology and on the underlying ion currents in enzymatically dispersed canine ventricular cardiomyocytes, as well as in a hERG-channel- and NaV1.5-expressing cell lines. Delavirdine application in the μM concentration range resulted in rapid, potent and reversible blockade of expressed hERG channels. NaV1.5 channels were slightly reduced by delavirdine. Moreover, APD increase, reduction of maximal rates of phases 1 and 3 and a small increase of Plateau20 amplitude were detected in canine left ventricular isolated cardiomyocytes in a concentration-dependent manner. Accordingly, the delavirdine-sensitive current contained an early and transient, as well as a late outward component corresponding to phases 1 and 3 of the AP. These results support the delavirdine-evoked inhibition of I_{K_r} , transient outward potassium current as well as sodium current. As these concentrations are similar to therapeutic plasma values (7–30 μM), delavirdine application might carry some risk of cardiac side effects especially in HIV-infected patients with altered cardiac function or patients with co-administration of drugs metabolized by certain cytochrome P450 isoforms.

1. Introduction

The rapid component of delayed rectifier potassium outward current (I_{K_r}) is a main player of late repolarization (phase 3) in ventricular myocytes [1–3]. Its pore-forming subunit is formed by Kv11.1 (hERG) protein [4,5]. The gain of function hERG mutations may lead to type 1 short QT syndrome [6], while the loss of function ones may result in type 2 long QT syndrome [7,8]. As I_{K_r} is key in determining the transition from phase 2–3 of the action potential (AP) its improper function can lead to cardiac arrhythmia. Class III antiarrhythmic agents of Vaughan

Williams classification act as inhibitors of I_{K_r} [9], leading to prolongation of the AP and therefore the refractory period, which is the mechanism behind the antiarrhythmic action of these compounds. The prolongation of the AP can however lead to the development of early afterdepolarizations (EADs) [10], a proarrhythmic event and might lead to a potentially lethal *torsade de pointes* (TdP) type of ventricular arrhythmia [7]. AP prolongation manifests itself as a QT interval increase on the ECG. As QT interval, just as AP duration, depends very much on heart rate, one should consider the QTc, the corrected QT interval [11–13]. Not just the prolongation of QTc is important in cardiac

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arrhythmia risk assessment but the short-term variability of the QTc is even more informative [13,14].

Delavirdine is a first generation non-nucleoside reverse transcriptase inhibitor approved by Food and Drug Administration (FDA) in 1997 and was applied in HIV infection and AIDS in combination with other antiretroviral agents with a recommended application of 400 mg delavirdine orally three times daily [15,16]. Cardiac side effects of delavirdine are rarely reported. QTc intervals increased in patients taking buprenorphine/naloxone in combination with 2×600 mg delavirdine (taken for 7 days) [17]. It must be noted that HIV infected patients can develop QTc prolongation due to HIV-associated dilated cardiomyopathy as a consequence of myocarditis associated with fibrous degeneration of the conduction system [18]. This already prolonged QT interval can be further aggravated when the patient takes delavirdine with or without other drugs potentially leading to QT prolongation like ABT-333 in simultaneous hepatitis C infection. Delavirdine's chemical structure contains a methanesulfonamide group (Fig. 1), which is present in some blockers of the hERG channel/ I_{Kr} current like dofetilide and E-4031. We have recently reported the electrophysiological actions of the anti-hepatitis C agent ABT-333 also containing a methanesulfonamide group in isolated canine left ventricular cells [19]. Another compound, the widely used serum cholesterol level reducer rosuvastatin, which also contains a methanesulfonamide group was reported to prolong the cardiac action potential, although in guinea-pigs [20]. Amsacrine is an agent used in chemotherapy and contains a methanesulfonamide group too. Amsacrine inhibits hERG channels in the open and inactivated states [21] and it is listed with a conditional risk of TdP in the CredibleMeds website (<https://www.crediblemeds.org/blog/five-drug-s-added-crediblemeds-qtdrugs-lists>).

Due to the presence of methanesulfonamide group in chemical structures of delavirdine and some blockers of the I_{Kr} current, we hypothesized that delavirdine inhibits hERG channels and I_{Kr} current. As there is absolutely no such information in the literature, we aimed to investigate the potential action of delavirdine on hERG channels and ventricular action potentials in its therapeutic concentration range.

2. Materials and methods

2.1. Conventional patch-clamp on HEK cells

HEK (human embryonic kidney) cells (a kind gift from Stefan Heinemann, Friedrich Schiller University, Jena, Germany) stably expressing the human sequence hERG (aka Kv11.1) channels were used up to maximum three passage number. Whole-cell currents of voltage-clamped HEK cells were recorded by manual patch-clamp technique according to standard protocols. Data were acquired using Axopatch 200B amplifiers connected to computers with Digidata 1550B digitizers (Molecular Devices, San Jose, CA, USA) at room temperature ranging between 20 and 24°C as described previously [19]. Solutions contained the following in mmol/L: choline chloride 140, KCl 5, MgCl₂ 2, CaCl₂ 2, glucose 20, HEPES 10, and CdCl₂ 0.1, pH= 7.35 (adjusted with NaOH)

in the perfusion and in mmol/L: KCl 140, HEPES 10, MgCl₂ 2, and EGTA 10, pH= 7.3 (adjusted with KOH) in patch pipette (internal solution).

For experiments on HEK cells we diluted delavirdine (Tocris, product no. 4149) to 1, 3, 10, 30, and 100 μ M concentrations from 10 mM stock dissolved in DMSO. 1 % DMSO was added to the control perfusion solution only before the application of 100 μ M delavirdine on HEK cells.

The concentration-response curve was fitted with the Hill equation ($RCF = 1/(1 + ([c]^{nH}/IC_{50}^{nH}))$), where RCF is the remaining tail current fraction, nH is the Hill coefficient, [c] is the concentration of delavirdine and the IC₅₀ is the concentration where half of the channels are inhibited.

2.2. Patch-clamp recordings on CHO cells

The effect of delavirdine was tested on human NaV1.5 channels (hSCN5A, a kind gift from Hugues Abriel, University of Bern, Bern, Switzerland) transiently expressed in Chinese Hamster Ovary (CHO) cells. Whole-cell patch clamp measurements were performed. Cells were depolarized to 0 mV every 5 seconds from a holding potential of -100 mV. Solutions contained the following in mmol/L: NaCl 145, KCl 5, glucose 5.5, HEPES 10, MgCl₂ 1, CaCl₂ 2.5, pH= 7.35 (adjusted with NaOH) in the perfusion and in mmol/L: NaCl 10, CsF 105, HEPES 10 M, EGTA 10, pH= 7.2 (adjusted with CsOH) in patch pipette (internal solution).

For experiments on CHO cells we diluted delavirdine to 10 μ M concentration from 10 mM stock dissolved in DMSO.

2.3. Isolation of single canine ventricular myocytes

Adult male and female mongrel dogs weighting 8–15 kgs were obtained from a local breeder and injected with intramuscular injections of 10 mg/kg ketamine hydrochloride (CP-Ketamin, Produlab Pharma BV, Raamsdonksveer, The Netherlands) + 1 mg/kg xylazine hydrochloride (CP-Xylazin, Produlab Pharma BV, Raamsdonksveer, The Netherlands) to reach complete narcosis according to a protocol approved by the local ethical committee (license No: 2/2020/DEMÁB) and conform to the principles outlined in the Declaration of Helsinki in 1964. We isolated cells with enzymatic digestion using the segment perfusion technique by as described previously [19].

2.4. Recording of action potentials with conventional microelectrode technique

Action potentials (APs) of rod-shaped viable cells showing clear striation were measured with conventional sharp microelectrodes at 37 °C as described previously [19]. Since the cytosol was not dialyzed, time dependent changes in action potential duration (APD) were negligible for the period of our experimental protocol [22]. Cells were continuously superfused with bicarbonate buffer containing Tyrode solution (BTY) containing (in mmol/L): NaCl 121; KCl 4; MgCl₂ 1; CaCl₂ 1.3; HEPES 10; glucose 10; NaHCO₃ 25; (pH=7.3; adjusted with NaOH).

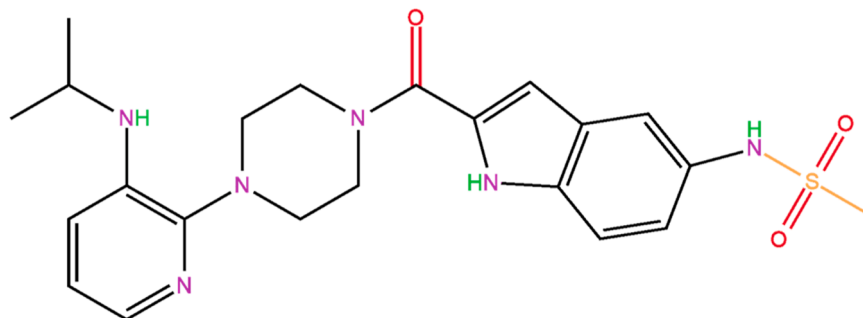


Fig. 1. Chemical structure of delavirdine, created by ChemDrawPro 12.0 software.

We recorded APs during steady-state stimulation and analyzed off-line. In ten consecutive APs the following parameters were determined and averaged: APD₅₀ and APD₉₀ values (duration of the AP from the peak to 50 and 90 % of repolarization, respectively), maximal rate of voltage change in phase 0, 1 and 3 (V₊max, V_{ph1}max, and V₋max, respectively), resting membrane potential (RMP), overshoot potential (OSP), APA (action potential amplitude, determined as the difference between OSP and RMP) and membrane potential difference between the RMP and the membrane potential value of at the 20 and 50 % duration of APD₉₀ (Plateau₂₀ and Plateau₅₀ amplitudes).

Upon determination of short-term variability (SV) of APD we analyzed 50 consecutive APs and calculate the SV using the following formula:

$$SV = \frac{\sum_{i=1}^n (|APD_{i+1} - APD_i|)}{n\sqrt{2}}$$

where SV is short-term variability, APD_i and APD_{i+1} indicate the APD₉₀ values of the *i*th and *i* + 1th APs, respectively, and *n* denotes the number of consecutive beats analyzed [14,23].

Myocytes were perfused with 10 μM delavirdine for 10 min followed by a 15-min-long period of washout. Based on these experiments 4–5-min-long delavirdine application was sufficient to achieve steady-state effect. Therefore, concentration-dependent effects of delavirdine were determined by applying 1, 3, 10, and 30 μM delavirdine, each for 5 min in a cumulative manner.

For the experiments on cardiac myocytes delavirdine was dissolved in DMSO so as the maximal DMSO concentration (in case of 30 μM delavirdine) was 0.3 %. As shown previously, even 1.5 % DMSO did not modify any parameters of the canine left ventricular AP [19].

2.5. Recording of action potentials with patch-clamp

In some experiments (Fig. 7) the APs of each studied cells were recorded in the whole-cell configuration of the patch clamp in current-clamp mode at 37 °C. Cells were continuously superfused with bicarbonate buffer containing Tyrode solution (BTY, see above for composition). Micropipettes had tip resistance of 2–3 MΩ after filling with pipette solution containing (in mmol/L): K-aspartate 120, KCl 30, MgATP 3, HEPES 10, Na₂-phosphocreatine 3, EGTA 0.01, cAMP 0.002, KOH 10 at pH = 7.3. The series resistance was typically 4–6 MΩ. We discarded the experiments where series resistance was high or substantially increased during the measurement. We recorded APs during steady-state stimulation with 700 ms cycle length and analyzed off-line. In ten consecutive APs the previously mentioned AP parameters were determined and averaged. Some of these AP parameters (mainly the value of V_{ph1}max and the presence or absence of the spike-and-dome AP shape) were used to create two group of cells (*endo* and *non-endo*).

2.6. Action potential voltage clamp

Action potential voltage-clamp experiments were conducted according to the method described previously [3,19] after recording each cells own AP (as described in the previous chapter). A previously recorded typical AP (recorded with 700-ms-long cycle length pacing on a midmyocardial cell, termed as “canonic” AP) was applied to the voltage clamped cells in whole-cell configuration as command signal. Delavirdine-sensitive current was obtained by pharmacological subtraction calculated by deducting the current signals recorded in the presence of 10 μM delavirdine from those measured in control condition (BTY). Ion currents were normalized to cell capacitance values to calculate current densities. We determined cell capacitance using short hyperpolarizing pulses to –10 mV for 45 ms from 20-ms-long depolarization to 0 mV from the holding potential of –80 mV applied at 10 Hz. Average value of cell capacitance was 162.3 ± 9.9 pF in the 13 patch-clamped myocytes.

2.7. Statistics

We show the arithmetic means ± Standard Error of the Mean (SEM) of the studied parameters. Given the biological variability among cells, we treated each cell as independent in the statistical tests, although we could use more cells from the same animal. Statistical significance of differences was evaluated using paired or unpaired Student's *t*-test as appropriate after testing the distribution of data points with Shapiro-Wilk test. Differences were considered significant when *p* was less than 0.05 and indicated with asterisks on graphs.

3. Results

3.1. Effect of delavirdine on heterologously expressed hERG channels

Based on the presence of methanesulfonamide group in delavirdine we hypothesized that it blocks hERG channels. We tested this on HEK cells stably expressing hERG channels. Delavirdine rapidly and potently reduced the hERG current and its action was reversible (Fig. 2A–B). Testing various delavirdine concentrations, we created a concentration-response curve (Fig. 2C) from the tail currents measured during the repolarization to –40 mV after the activation of the current to +20 mV from the –80 mV holding potential (see pulse protocol on top of Fig. 2A). Fitting the data with the Hill equation yielded a Hill coefficient of 1.0 ± 0.1 and a half-inhibitory concentration (IC₅₀) of 11.0 ± 1.2 μM. Based on these results we continued to test the action of 10 μM delavirdine on canine left ventricular cells.

3.2. Effect of 10 μM delavirdine on left ventricular myocytes

First, we examined the time-dependent actions of 10 μM delavirdine on the action potential of left ventricular myocytes. Delavirdine perfusion lasted for at least 10 minutes to make sure that a steady-state effect developed, then we tested the reversibility of any potential effects with a 15-min-long washout period (Fig. 3). Several AP parameters were not altered by 10 μM delavirdine (Table 1).

10 μM delavirdine induced significant changes of six AP parameters (APD₉₀, the ratio of APD₅₀ and APD₉₀, the Plateau₂₀ amplitude, maximal rates of phases 0, 1, and 3) in the eight examined cells (Figs. 4–5).

Plateau₂₀ amplitude was also slightly (+3.5 %) but significantly altered by 10 μM delavirdine from 109.5 ± 2.6 to 113.2 ± 2.3 mV in a reversible manner (111.6 ± 2.2 mV after washout) (Fig. 4B). APD₉₀ increased from 203.7 ± 10.4 to 226 ± 12.7 ms (approximately +11 % change) in a reversible manner (211.3 ± 15.5 ms after washout) (Fig. 4C). As delavirdine did not alter APD₅₀ values (Table 1), the ratio of APD₅₀ and APD₉₀ reduced from 0.77 ± 0.02 to 0.72 ± 0.02 (–7 % change). Delavirdine action was again reversible (0.78 ± 0.02 of APD₅₀/APD₉₀ ratio after washout) (Fig. 4D). The maximal rates of AP phases were also significantly influenced by 10 μM delavirdine (Fig. 5). V₊max was reduced from 218.7 ± 25.8 to 188.3 ± 25.8 V/s (–25 % change) but, unlike the other delavirdine-influenced AP parameters, this effect was not reversible (160.7 ± 16.9 V/s after washout) (Fig. 5B). The maximal rates of phase 1, as well as phase 3 were also reduced in the presence of 10 μM delavirdine (from –6.2 ± 1.9 and –1.8 ± 0.1 to –3.6 ± 1.1 and –1.3 ± 0.2 V/s, respectively). These –34 and –28 % changes in case of V_{ph1}max and V₋max, respectively were reversible (–5.3 ± 1.6 and –1.9 ± 0.1 V/s after washout) (Fig. 5C–D). Based on these results we moved on and tested the potential actions of delavirdine on the cardiac AP in different concentrations, where cumulative concentration-dependent drug-effects were studied between 1 and 30 μM, increasing the concentration of delavirdine in half decade steps.

3.3. Concentration-dependent actions of delavirdine on cardiac AP

In the next experiments (Figs. 6–7), we applied delavirdine in a cumulative manner, between 1 and 30 μM. Even 1 μM delavirdine

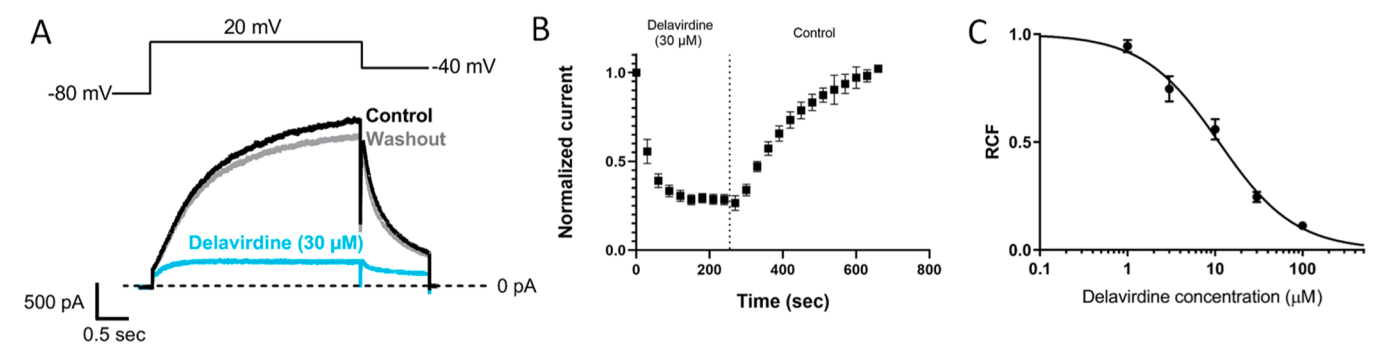


Fig. 2. Effects of delavirdine on hERG current expressed in HEK cells. (A) A representative patch-clamp recording in voltage-clamp mode with the applied voltage protocol (above). The black trace is the current measured in control condition, the cyan in 30 μM delavirdine, while the gray shows current after washout. (B) Time course of delavirdine effect in 4 cells. (C) Concentration-response curve of delavirdine, which was fitted on remaining current fractions (RCF) in the presence of 1, 3, 10, 30, and 100 μM concentrations ($n \geq 4$ for each concentration). The fit yielded an IC_{50} value of $11.0 \pm 1.2 \mu\text{M}$ and a Hill coefficient of 1.0 ± 0.1 (mean $\pm$ SEM).

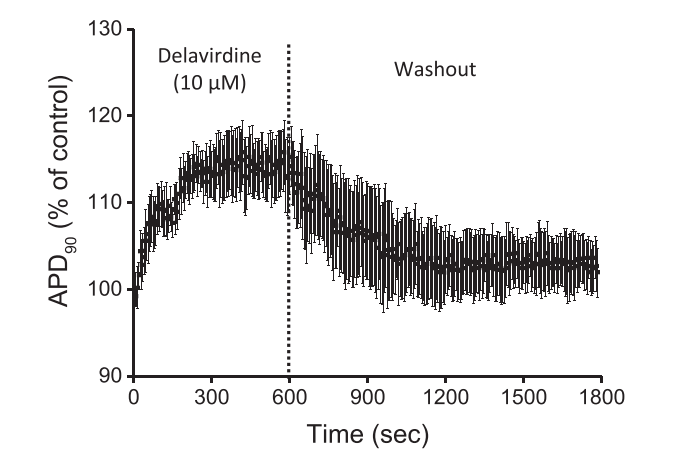


Fig. 3. Time dependent change of APD_{90} induced by delavirdine. APD_{90} values of 8 cells were normalized to those right before the application of delavirdine, averaged and plotted with the respective SEM values.

Table 1
Unchanged AP parameters obtained with 10 μM delavirdine.

AP parameter	BTY (control)	10 μM delavirdine	Washout
APA (mV)	124.9 ± 1.5	125.9 ± 1.4	124.7 ± 2.0
APD_{50} (ms)	158.1 ± 11.0	162.6 ± 9.6	166.5 ± 14.4
OSP (mV)	44.1 ± 1.5	44.1 ± 1.7	41.5 ± 2.8
Plateau ₅₀ amplitude (mV)	91.1 ± 2.9	89.6 ± 2.3	92.0 ± 2.3
RMP (mV)	-80.8 ± 1.0	-81.8 ± 1.4	-83.2 ± 2.3
SV (ms)	2.7 ± 0.3	2.6 ± 0.3	3.1 ± 0.6

Data are mean $\pm$ SEM from eight cells obtained from six animals. Explanation of abbreviations is in the list of abbreviations.

modified the values of APD_{90} and V_{Ph1max} in a significant manner (Figs. 6C and 7C). APD_{90} was increased to 222.1 ± 13.7 from 204.8 ± 10.6 ms (+8 % change) while V_{Ph1max} was reduced to -7.9 ± 1.4 from -9.0 ± 1.5 V/s (−13 % change) in the presence of 1 μM delavirdine. The actions of larger delavirdine concentrations were even more pronounced for APD_{90} (+15, +23, and +37 % changes for 3, 10, and 30 μM respectively) (Fig. 6C) and also for V_{Ph1max} (−21, −39, and −55 % change for 3, 10, and 30 μM respectively) (Fig. 7C). No other AP parameters were influenced by 1 μM delavirdine, but Plateau₂₀ amplitude and V_{max} values were significantly changed in the presence of 3 μM delavirdine (Figs. 6E and 7D). Plateau₂₀ amplitude increased from 100.2 ± 1.9 to 103.9 ± 2.4 mV (+4 % change) while V_{max} reduced from -1.72

± 0.11 to -1.57 ± 0.13 V/s (−10 % change) in the presence of 3 μM delavirdine. Similarly to APD_{90} values, V_{max} was also affected in a concentration-dependent manner (−26, and −45 % change in 10, and 30 μM delavirdine respectively). Unlike the values of APD_{90} and V_{max} , Plateau₂₀ amplitude was only significantly increased in 10 but not in 30 μM delavirdine (Fig. 6E). Just as seen with the experiments where only 10 μM delavirdine was tested (Figs. 4–5), $\text{APD}_{50}/\text{APD}_{90}$ ratio and V_{max} were significantly reduced in 10 μM delavirdine (from 0.78 ± 0.01 and 255.9 ± 29.1 V/s to 0.74 ± 0.02 and 216.1 ± 28.6 V/s, −5 % and −16 % changes, respectively). 30 μM delavirdine led to a further reduction of $\text{APD}_{50}/\text{APD}_{90}$ ratio to 0.68 ± 0.03 (−13 % change) (Fig. 6D) whereas the 201.2 ± 35.3 V/s value of V_{max} did not reach the level of significance (Fig. 7B). Regarding the reversibility, delavirdine action was reversible (values in washout did not differ from those in control) in all AP parameters except that of V_{max} . Unlike with the previously described 10 μM delavirdine experiments (Table 1), APD_{50} was also significantly increased by 1–3–10–30 μM delavirdine from 159.6 ± 9.7 to 173.3 ± 11.4 , 182.1 ± 11.2 , 185.2 ± 9.6 , and 183.3 ± 9.0 ms, respectively (+9, +14, +17, and +17 % change, respectively) (Fig. 6B). Similarly, OSP was significantly different in 1 and 3 μM but not higher delavirdine concentrations (approximately −3 % change with each concentration, from 33.4 ± 2.5 to 32.5 ± 2.7 and 32.5 ± 2.6). RMP values were significantly different in 3 and 10 μM delavirdine concentrations only (from -84.4 ± 1.1 to -86.1 ± 1.1 and -85.9 ± 1.2 mV). Delavirdine did not change other investigated AP parameters in the tested concentration range, only a tendency of SV increase could be detected (Table 2). Our data indicate that delavirdine potentially influences APD_{90} and V_{Ph1max} , which could be the consequence of the hypothesized hERG/ I_{Kr} inhibition, and additionally a transient outward potassium current (I_{to}) blockade. To test whether this is the case, we conducted further experiments where we recorded delavirdine-sensitive current in AP voltage clamp condition, a method capable of recording the influence of a tested compound on all currents shaping the AP simultaneously.

3.4. Delavirdine-sensitive current profile with AP voltage clamp (APVC)

In the following experiments (Fig. 8) we have chosen to apply 10 μM delavirdine, as most AP parameters were significantly modified by that concentration (see above). During these experiments, we always stimulated each cell with a previously recorded AP (canonic AP) to be able to compare the delavirdine-sensitive current among the tested cells with various AP shapes. The average of the delavirdine-sensitive current in all studied cells (Fig. 8B) possessed an early and a late outward component with densities of 0.9 ± 0.39 and 0.46 ± 0.08 pA/pF. We tried to correlate the peak (both early and late) current density values of the delavirdine-

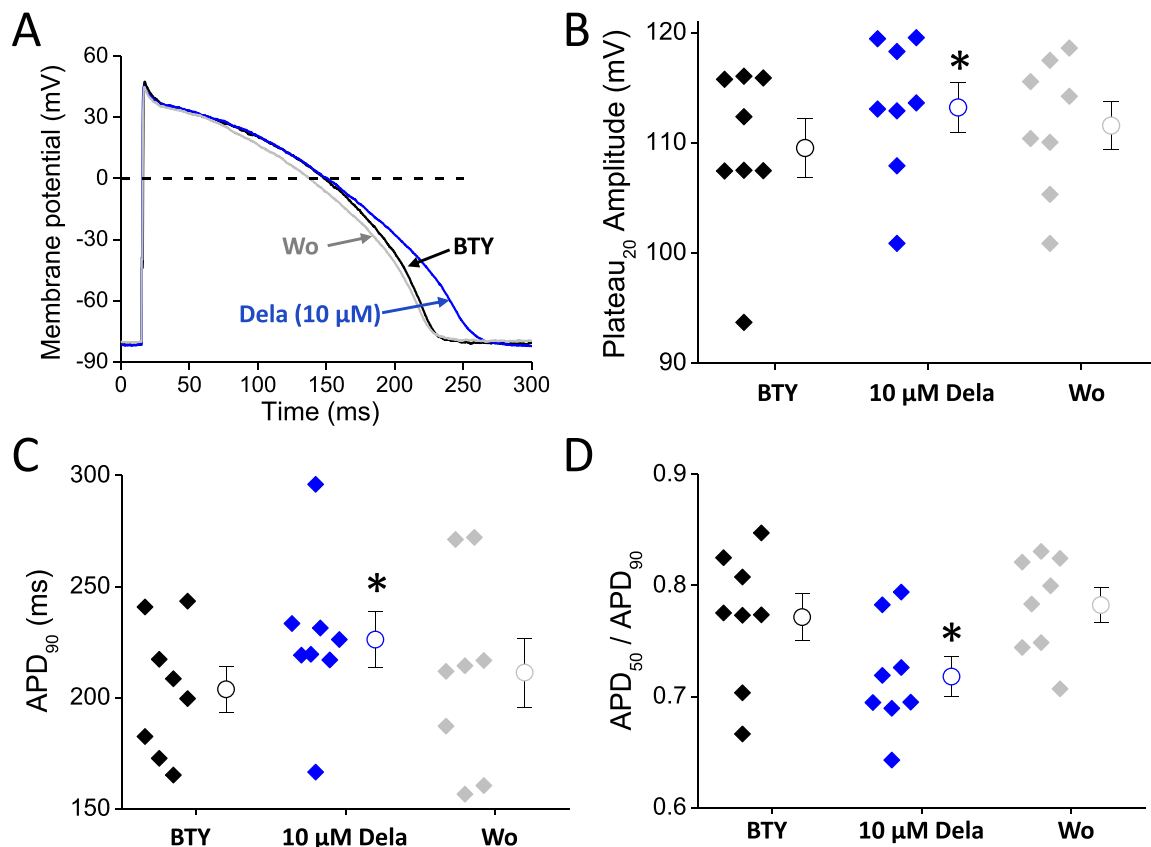


Fig. 4. Effects of 10 μ M delavirdine (Dela) on left ventricular AP. (A) Representative action potentials. The black trace is the AP measured in BTY (control solution), the blue in the presence of 10 μ M delavirdine, while the gray is after washout (Wo). (B–D) Diagrams showing AP parameters in control condition (BTY, black), in the presence of delavirdine (10 μ M Dela, blue) and after washout of delavirdine (Wo, gray) where filled diamonds, empty circles, and whiskers show the individual values, means, and $\pm$ SEM, respectively. Values of Plateau₂₀ amplitudes (difference between resting membrane potential and the membrane potential at 20 % of APD₉₀, B), action potential duration measured at 90 % of repolarization (APD₉₀, C), and APD₅₀/APD₉₀ ratios of action potential duration measured at 50 and 90 % of repolarization (APD₅₀/APD₉₀, D). Results were obtained from 8 examined cells isolated from 6 animals. Asterisks show statistically significant difference from control ($p < 0.05$).

sensitive current with various parameters of the studied cell own APs (value and time of V_{Ph1max} and V_{max}). A significant correlation could be detected between the density values of the late peak current and the V_{max} values of the cell's own AP (Fig. 8C). Due to the well-known transmural gradient of I_{to} [24] we separated the studied 13 cells based on their AP parameters (mainly the value of V_{Ph1max} and the presence or absence of the spike-and-dome AP shape) to two groups.

Cells with V_{Ph1max} values between 0–(–2) V/s and without spike-and-dome AP configuration were considered as subendocardial (*endo*) cells, whereas all other cells were aggregated into the *non-endo* group. None of the examined AP parameters were significantly different between the *endo* and *non-endo* cells except the V_{Ph1max} values ($p = 0.0014$) (Table 3).

These parameters were measured from the cells' own APs in whole-cell configuration, current-clamp mode before starting the APVC experiments. Data are mean $\pm$ SEM from the indicated number of cells obtained from four, six, and nine animals for *endo*, *non-endo* and all cells, respectively. Bold and underlined values indicate a significant difference between *endo* vs *non-endo* cells. Explanation of abbreviations is in the list of abbreviations.

There was a marked difference between the *endo* and *non-endo* cells regarding the early component of delavirdine-sensitive currents, because only the *non-endo* cells exhibited the early outward current component (Fig. 8D). However, there was no difference either in the position or in the density of the late peak current between the two groups (Fig. 8E). Moreover, there were no significant differences in the values of baseline (f1), mid-plateau (f2), and end (f3) current densities

between the two groups (Fig. 8F). The position of the early current peak in *non-endo* cells was 5.5 ± 0.7 ms after the V_{max} value, and 5.2 ± 0.7 ms before the incisura of the canonic AP, respectively. The early peak density was 1.8 ± 0.4 pA/pF in the *non-endo* group. In all 13 studied cells, the position of the late peak was 0.3 ± 1.2 ms after the position of the V_{max} of the canonic AP.

3.5. Effect of delavirdine on expressed NaV1.5 channels

Based on the results of action potentials (reduction of V_{max}) delavirdine could potentially inhibit sodium current. As NaV1.5 is responsible for cardiac sodium current [25,26] we tested the action of 10 μ M delavirdine on NaV1.5 current expressed in CHO cells (Fig. 9). When applied extracellularly via the perfusion system, 10 μ M delavirdine caused a slight reduction of the whole-cell NaV1.5 current ($\text{RCF} = 0.91 \pm 0.03$, $n = 5$). The inhibition developed quickly and was reversible (Fig. 9B).

4. Discussion

We carried out detailed characterization of delavirdine-induced action on hERG channels and left ventricular cardiomyocytes obtained from dogs, a model species having very similar to human heart in regard of cardiac electrophysiology [27,28].

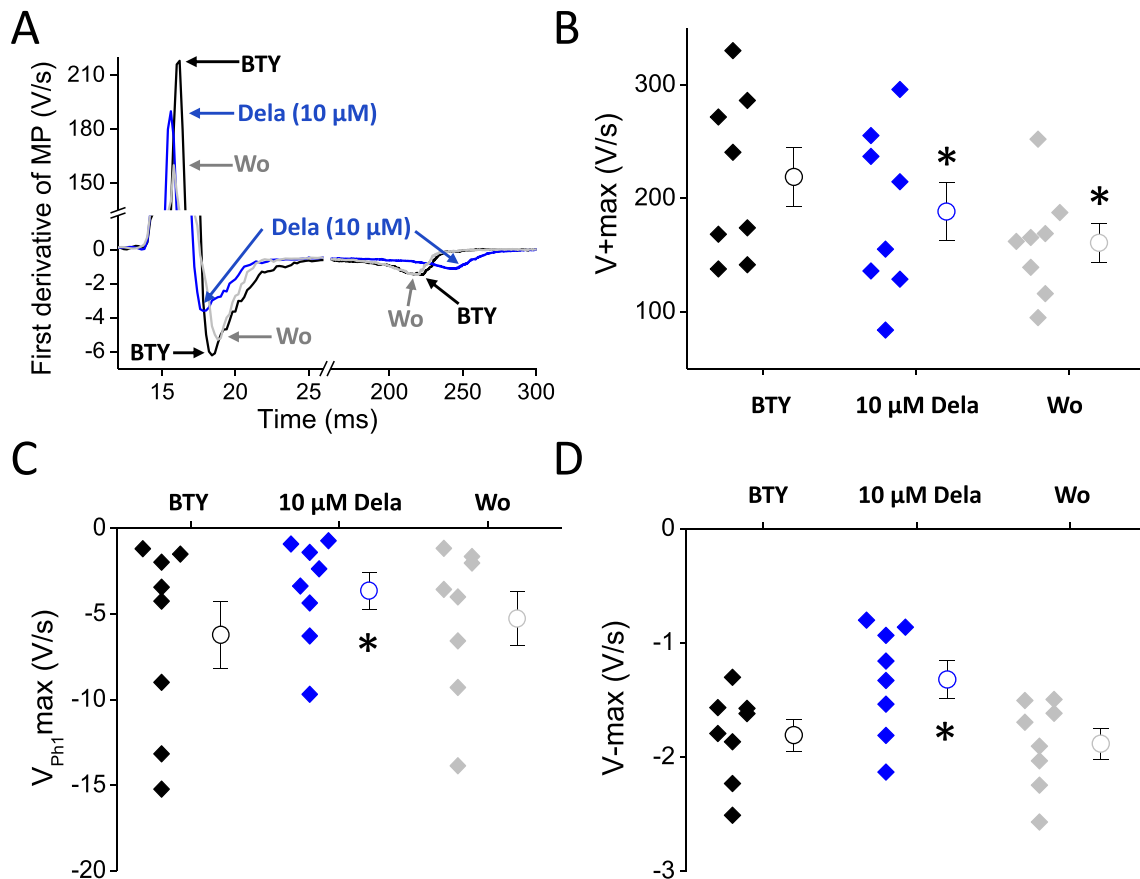


Fig. 5. Effects of 10 μM delavirdine (Dela) on maximal rates of left ventricular AP. (A) Representative first derivative of action potentials shown on Fig. 4A emphasizing the maximal rates of phases 0, 1, and 3. The black trace is measured in BTY (control solution), the blue in the presence of 10 μM delavirdine, while the gray is after washout (Wo). (B–D) Diagrams showing AP parameters in control condition (BTY, black), in the presence of delavirdine (10 μM Dela, blue) and after washout of delavirdine (Wo, gray) where filled diamonds, empty circles, and whiskers show the individual values, means, and $\pm$ SEM, respectively. Values of maximal rate of depolarization (V_{+max} , B), maximal rate of early repolarization (V_{ph1max} , C), and maximal rate of late repolarization (V_{-max} , D). Results were obtained from 8 examined cells isolated from 6 animals. Asterisks show statistically significant difference from control ($p < 0.05$).

4.1. Effect of delavirdine on expressed channels

Due to the presence of the methanesulfonamide group in the structure of delavirdine, we thought that it might reduce the ion current of hERG channels responsible for the generation of I_{Kr} . To confirm this, we carried out experiments on HEK cells stably expressing hERG channels. To our best knowledge even the highest DMSO concentration (1 %) has little effect on hERG channel [29]. The hERG-mediated current was indeed rapidly reduced by delavirdine in a reversible and concentration dependent manner with an IC_{50} of 11 μM and Hill coefficient of 1.0 ± 0.1 (Fig. 2A). Moreover, NaV1.5 channels expressed in CHO cells were also reduced by delavirdine, although the amount of this effect was much smaller. This reduction was also rapidly developed and reversed upon washout.

4.2. Effects of 10 μM delavirdine on the cardiac AP

Although hERG is the pore-forming subunit of the I_{Kr} current [4], the native current might contain other regulatory subunits such as minK or MIRP [30,31]. The action of a compound might not be the same on native channels as on the expressed pore-forming subunit (containing no other regulatory proteins) as we have shown previously for the action of TTX on calcium channels [32]. Therefore, we tested the action of 10 μM delavirdine on the ventricular AP, where I_{Kr} is a major current determining late repolarization. We have first chosen 10 μM concentration as it is close to the IC_{50} of delavirdine on hERG channels. As we expected, 10 μM delavirdine prolonged the AP and reduced V_{-max} , which

(especially the increase of APD) corresponds well with the inhibition of I_{Kr} , just as seen with I_{Kr} blockers like dofetilide [14,33] and E-4031 [34, 35].

Other marked effects of 10 μM delavirdine were the reduction of V_{ph1max} , V_{+max} , and the APD_{50}/APD_{90} ratio values. These actions could be due to inhibition of I_{to} , sodium current (I_{Na}), inward rectifier potassium current (I_{K1}), respectively. The increase of Plateau₂₀ amplitude (Fig. 4B), might be due to the reduction of I_{to} . As we experienced V_{+max} reduction in 10 μM delavirdine we tested its effect on NaV1.5 channels expressed in CHO cells. We only observed a small (approximately 9 %) reduction of NaV1.5 current, which can support the V_{+max} reduction. However, as 10 μM delavirdine-induced NaV1.5 reduction was reversible unlike the reduction of V_{+max} the reduction of the latter might be a delavirdine-unrelated effect. Moreover, as only 10, but neither smaller nor higher concentrations reduced V_{+max} in the experiments where multiple delavirdine concentrations were applied (Fig. 7B), a definitive delavirdine-induced I_{Na} block is still questionable. Also, if delavirdine altered the sodium current it would most likely alter OSP and APA, neither of which was detected (Table 1). The lack of OSP and APA reduction however can be due to the inhibition of I_{to} . The APD_{50}/APD_{90} ratio reduces by a greater extent in case of inhibition of I_{K1} than of I_{Kr} [14], which raises the possibility of delavirdine-induced I_{K1} reduction. This is, however, unlikely as RMP was not altered by delavirdine, (slightly but not significantly more negative values of RMP) is actually the opposite of what one might expect from I_{K1} inhibition. Additionally, the reduction in V_{-max} can be attributed not only to I_{K1} but also to significant I_{Kr} inhibition. During terminal repolarization,

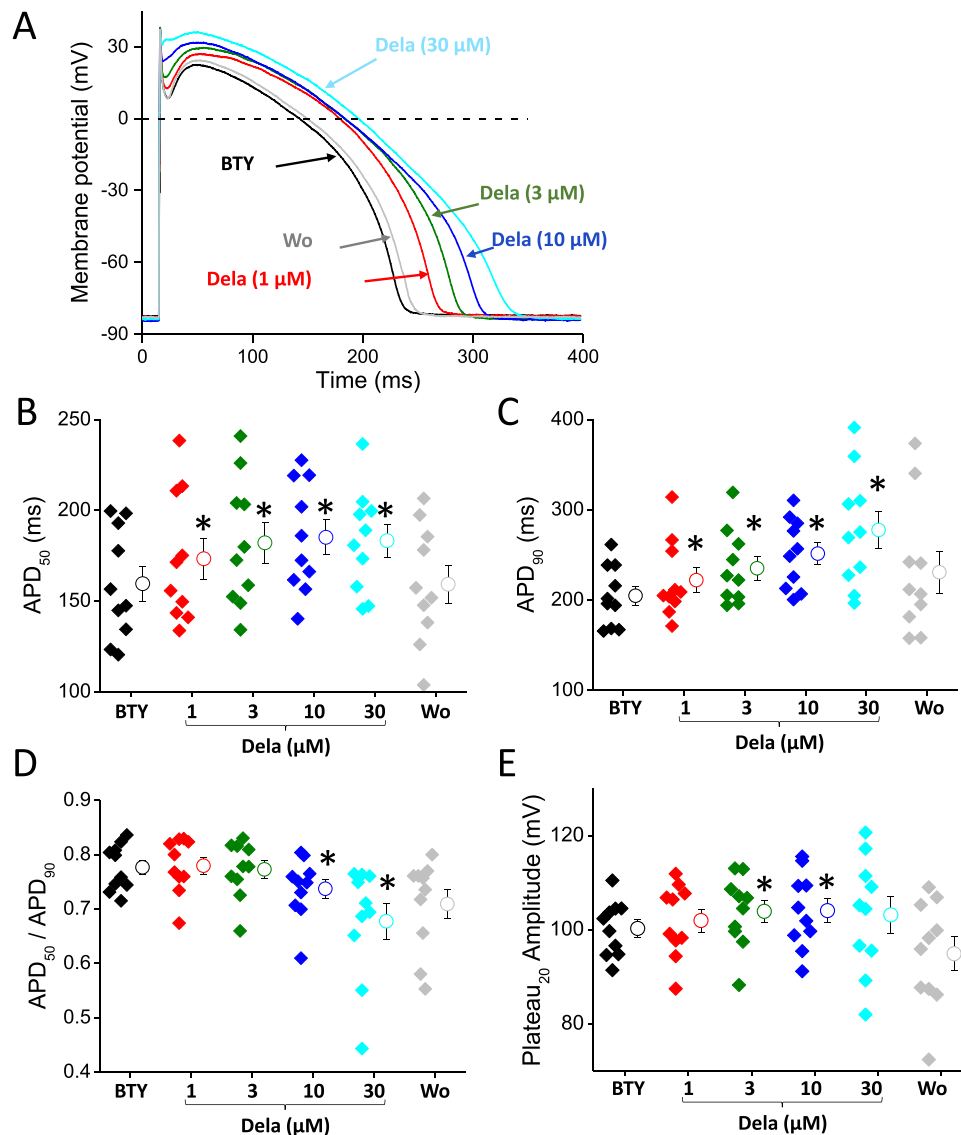


Fig. 6. Concentration-dependent delavirdine (Dela) actions on left ventricular AP. (A) Representative action potentials. The black trace is the AP measured in BTY (control solution), the color coded ones are in the presence of increasing delavirdine concentrations, while the gray is after washout (Wo). (B–D) Diagrams showing AP parameters in control condition (BTY, black), in the presence of delavirdine (various concentrations using color coding) and after washout of delavirdine (Wo, gray) where filled diamonds, empty circles, and whiskers show the individual values, means, and $\pm$ SEM, respectively. Values of action potential duration measured at 50 % of repolarization (APD₅₀, B), action potential duration measured at 90 % of repolarization (APD₉₀, C), ratios of action potential duration measured at 50 and 90 % of repolarization (APD₅₀/APD₉₀ D), and plateau₂₀ amplitudes (difference between resting membrane potential and the membrane potential at 20 % of APD₉₀, E). Results were obtained from 10 examined cells isolated from 5 animals. Asterisks show statistically significant difference from control ($p < 0.05$).

where V-max appears, approximately 75 % of the net membrane current is due to I_{K1} , with the remaining contribution coming from I_{Kr} . [1].

Delavirdine-induced increase of Plateau₂₀ amplitude can also be due to the reduction of I_{to} as, despite the rapid inactivation of I_{to} (with APVC recordings, around 20–25 ms as a 4-aminopyridine(4-AP)-sensitive current [2], or up to 100 ms when examined as a chromanol-sensitive current [36]), it can contribute to the early but not late plateau potentials. Indeed, although there is a discrepancy in the literature regarding the effect of I_{to} inhibition on APD, its action on early plateau height is uniformly reported as an increase [37,38].

4.3. Concentration-dependent delavirdine effects

Some AP actions seen with 10 μM delavirdine could also be detected by lower drug concentrations such as the increase of APD₉₀ and the reduction of V_{Ph1}max in ≥ 1 μM delavirdine (Figs. 6C and 7B). V-max was significantly changed by ≥ 3 μM delavirdine (Fig. 7D), while

APD₅₀/APD₉₀ ratio significantly changed by ≥ 10 μM delavirdine (Fig. 5D). The increase of Plateau₂₀ amplitude values was only significant in 3–10 μM delavirdine (Fig. 6E). These changes, except for that of V-max, were reversible during the 15-min-long washout period despite the cumulative application of the drug suggesting an easy recovery of delavirdine actions. On top of the actions of 10 μM delavirdine the cumulative application of 1–3–10–30 μM delavirdine resulted in the concentration-dependent and reversible increase of APD₅₀ which supports again its I_{Kr} inhibitory potential as seen with dofetilide [14,33]. OSP values were only slightly smaller (–3 %) and only in 1 and 3 μM (but not higher) delavirdine concentrations. OSP reduction in theory can be due to reduction of I_{Na} and/or increase (more rapid activation) of I_{to} . The latter one is unlikely (reduction of V_{Ph1}max actually suggests the opposite), but I_{Na} reduction is supported by the V-max reduction and the small reduction of NaV1.5 current. Of note, OSP reduction was significant only in 1 and 3, whereas V-max reduction only in 10 μM delavirdine. The statistically significant but only minor (less than 2 %

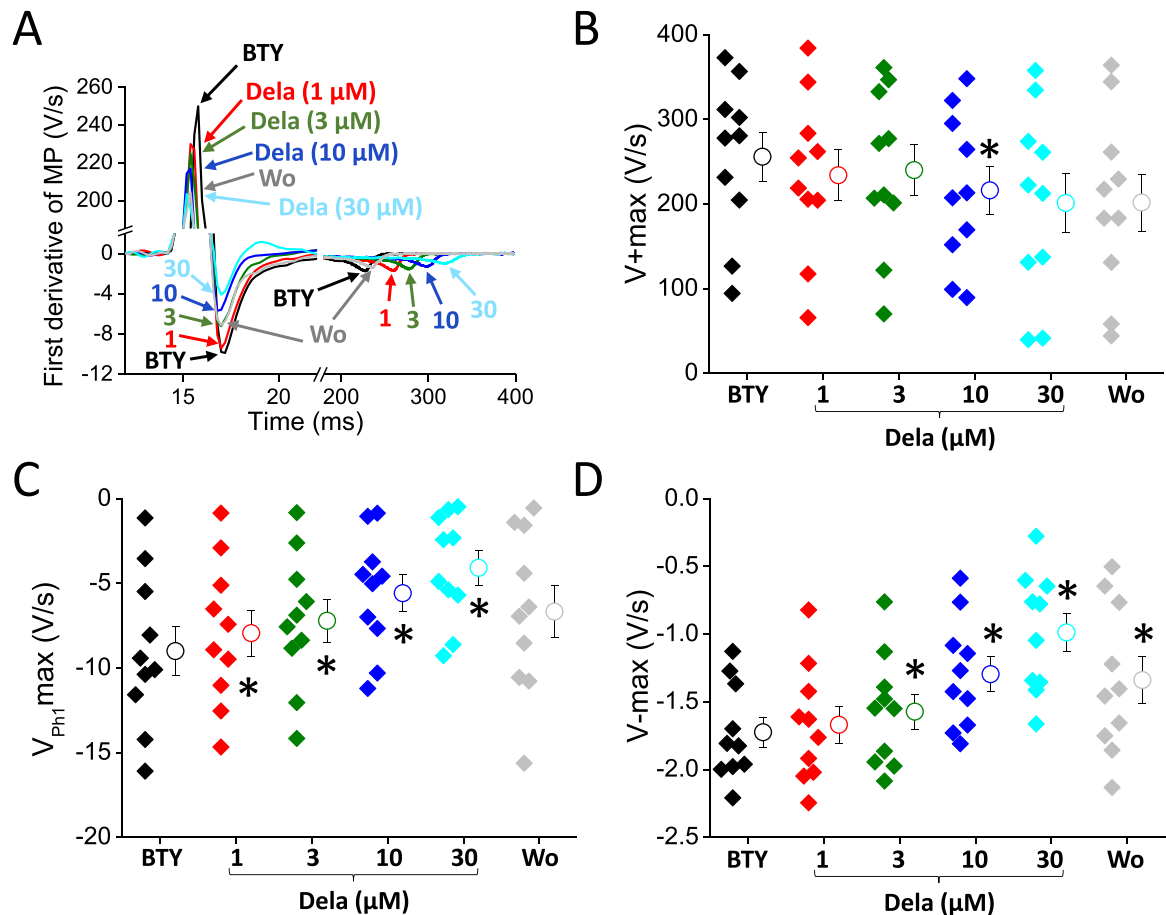


Fig. 7. Concentration-dependent delavirdine (Dela) actions on maximal rates of left ventricular AP. (A) Representative first derivative of action potentials shown on Fig. 6A emphasizing the maximal rates of phases 0, 1, and 3. The black trace is the AP measured in BTY (control solution), the color coded ones are in the presence of increasing delavirdine concentrations, while the gray is after washout (Wo). (B–D) Diagrams showing AP parameters in control condition (BTY, black), in the presence of delavirdine (various concentrations using color coding) and after washout of delavirdine (Wo, gray) where filled diamonds, empty circles, and whiskers show the individual values, means, and $\pm$ SEM, respectively. Values of maximal rate of depolarization (V_{+max} , B), maximal rate of early repolarization (V_{ph1max} , C), and maximal rate of late repolarization (V_{-max} , D). Results were obtained from 10 examined cells isolated from 5 animals. Asterisks show statistically significant difference from control ($p < 0.05$).

Table 2
Unchanged AP parameters obtained with various delavirdine concentrations.

AP parameter	BTY (control)	1 μ M Dela	3 μ M Dela	10 μ M Dela	30 μ M Dela	Washout
APA (mV)	117.8 ± 2.6	117.5 ± 2.9	118.5 ± 2.8	117.9 ± 2.8	115.2 ± 5.4	113.2 ± 5.5
Plateau ₅₀ amplitude (mV)	83.6 ± 2.2	84.5 ± 2.9	85.2 ± 3.0	82.5 ± 3.2	76.5 ± 5.3	74.3 ± 4.2
SV (ms)	2.5 $\pm$ 0.3	3.1 ± 0.4	3.0 ± 0.3	3.1 ± 0.4	3.7 ± 0.7	4.2 $\pm$ 0.8

Data are mean $\pm$ SEM from ten cells obtained from five animals. Explanation of abbreviations is in the list of abbreviations.

change) hyperpolarizing effect seen with only 3 and 10 μ M delavirdine might be the consequence of increased Na/K pump current, activation of ATP-dependent K^+ channels or increase of I_{K1} . The last one is unlikely based on our results seen with other AP parameters and the profile of delavirdine-sensitive sensitive current in APVC (see next chapter). The first two cannot be excluded but the question arises why the hyperpolarization was not detected with 30 μ M delavirdine, where actually the resting membrane potential was less negative (-84.0 ± 1.4 mV) than in control -84.4 ± 1.1 mV.

Based on these and previous results (see: *Effects of 10 μ M delavirdine*

on the cardiac AP) it seems that delavirdine blocks I_{Kr} , I_{to} , I_{K1} and might activate an inward current flowing during the plateau phase of the AP. Two possible candidates for this inward current are the late I_{Na} and $I_{Ca,L}$. The former one is not supported by the slight delavirdine-induced reduction of NaV1.5 current, V_{+max} and OSP values.

4.4. Delavirdine-sensitive current profile recorded by APVC

Based on our current-clamp results, delavirdine could influence several ion currents including I_{to} , I_{K1} , late I_{Na} , and $I_{Ca,L}$, on top of I_{Kr} . We have used the APVC method which has the advantage of simultaneously recording all ion currents which are influenced (either reduced or increased) by the tested compound [2], in this case delavirdine. One must remember some facts for the interpretation of delavirdine-sensitive current traces with APVC results. First, delavirdine-sensitive current is determined by subtracting the current recorded in the presence of delavirdine (after the development of its actions) from that recorded right before the application of delavirdine, therefore contains all currents modified by delavirdine. Second, drug-sensitive current is an outward current not only if the drug blocks an outward current, but also if it increases an inward current during the AP such as seen with ABT-333 [19]. Third, knowing the characteristic profile/fingerprint of the ion currents during the course of the AP [1–3,39] allows us to identify the currents modified by delavirdine.

Delavirdine-sensitive current contained a late outward component in

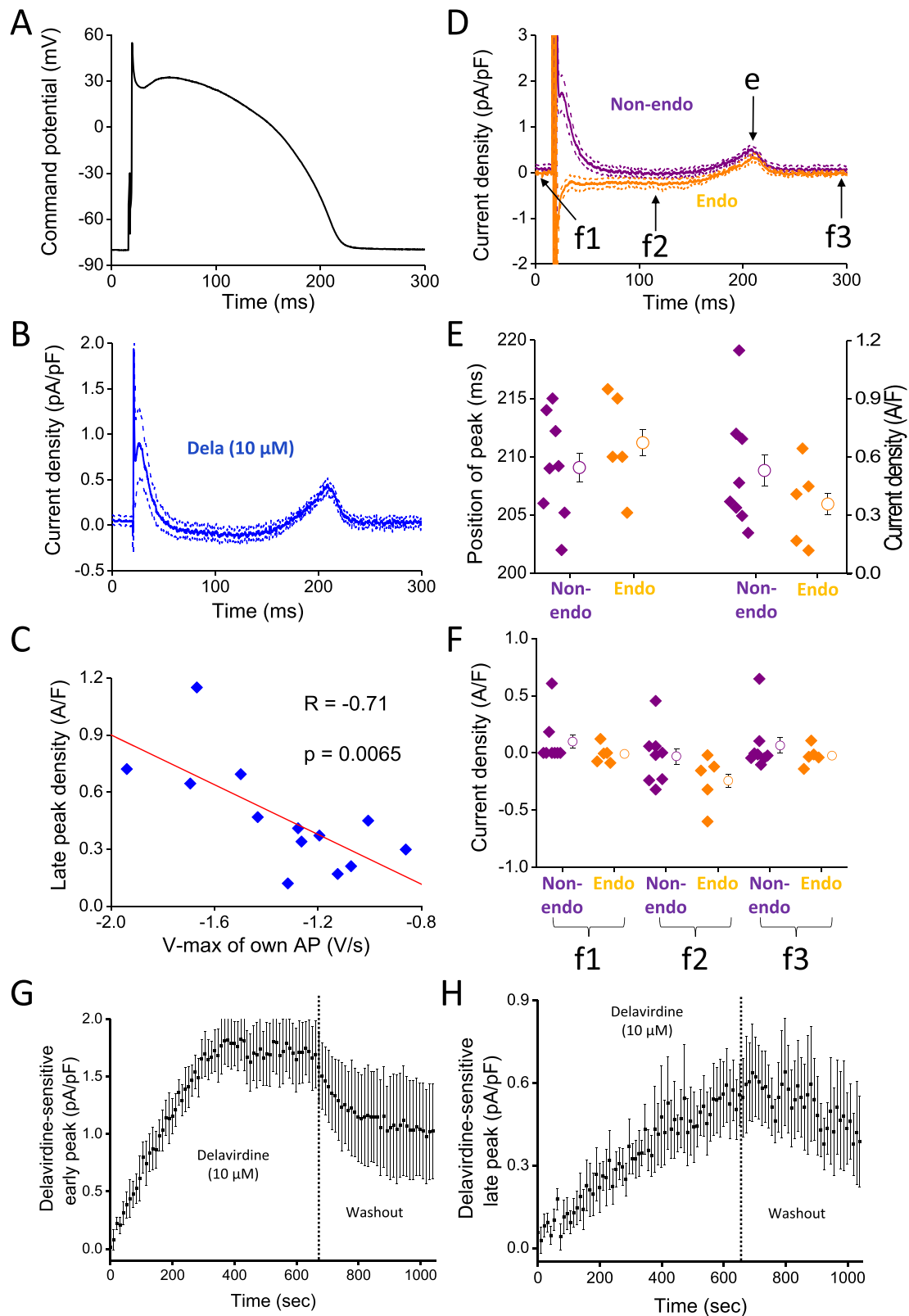


Fig. 8. Delavirdine-sensitive current in APVC condition. (A) The canonic action potential, which was used as stimulus command. (B) Average (solid trace) $\pm$ SEM (dotted lines) of 10 μ M delavirdine-sensitive current obtained in 13 examined cells isolated from 9 animals. (C) Correlation of late outward peak current density values with V-max of the given cell's own APs. (D) Average (solid trace) $\pm$ SEM (dotted lines) of 10 μ M delavirdine-sensitive current recorded in sudendocardial (Endo, 5 cells isolated from 5 animals) and other cells (Non-endo, 8 cells isolated from 6 animals). (E) The position (left) and current density (right) values of late delavirdine-sensitive peak (indicated with e on panel D). (F) Values of delavirdine-sensitive current densities at baseline, mid-plateau, and end indicated with f1, f2, and f3 on panel D, respectively. The average $\pm$ SEM values of early- (G) and late (H) outward peaks of the 10 μ M delavirdine-sensitive current.

Table 3
AP parameters of the cells that are used for APVC experiments.

AP parameter	Endo cells (n = 5)	Non-endo cells (n = 8)	All cells (n = 13)
APA (mV)	126.2 ± 1.9	122.4 ± 3.2	123.9 ± 2.1
APD ₅₀ (ms)	252.9 ± 15.8	260.4 ± 12.5	257.5 ± 9.4
APD ₉₀ (ms)	338.3 ± 17.2	344.5 ± 14.6	342.1 ± 10.7
APD ₅₀ /APD ₉₀	0.75 ± 0.02	0.76 ± 0.02	0.75 ± 0.01
OSP (mV)	50.9 ± 2.9	48.1 ± 3.7	49.2 ± 2.5
V _{ph1} max (V/s)	-1.46 ± 0.26	-8.81 ± 1.65	-5.99 ± 1.43
Plateau ₂₀ amplitude (mV)	106.1 ± 1.9	101.6 ± 2.0	103.3 ± 1.5
Plateau ₅₀ amplitude (mV)	86.9 ± 2.6	83.7 ± 2.1	84.9 ± 1.6
RMP (mV)	-75.3 ± 2.3	-74.3 ± 1.0	-74.7 ± 1.0
V+max (V/s)	398.4 ± 45.5	485.7 ± 72.8	452.1 ± 48.1
V-max (V/s)	-1.28 ± 0.12	-1.37 ± 0.12	-1.33 ± 0.08

every cell examined and in some cells an early outward component too. Those cells with the early outward current component also had a spike-and-dome configuration AP. Cells with V_{ph1}max values between 0-(−2) V/s and without spike-and-dome AP configuration were considered as subendocardial (“endo”) cells, whereas all other cells were aggregated into the “non-endo” group (Table 3). Besides the marked difference in the value of V_{ph1}max between *non-endo* and *endo* cells, some other AP parameters tended to be different such as APA, which can be slightly higher in canine endocardium than epicardium [40,41] in agreement with the slightly higher APA values in endo cells. APD₉₀ values of canine endocardium was larger than epicardium [40,41], in contrast to the tendency observed in this study. A possible explanation for this discrepancy can be twofold. On the one hand, the cited canine studies used right ventricular papillary muscles (as subendocardium) and epicardial strips (as epicardium) instead of isolated cells in our study, on the other hand we could only separate *endo* cells from *non-endo* ones based on AP shape. As *non-endo* cells definitely contained mid-myocardial (probably also M cells) having the longest APD [42,43] and can be found more abundantly in the ventricular wall compared to subepicardial cells, it is not surprising that APD₉₀ was larger in *non-endo* cells. The possible reason for the lack of significantly larger APD₉₀ in *non-endo* than in *endo* cells can be the presence of at least 3 subepicardial cells in the *non-endo* group.

The position of the peak of the early component in *non-endo* cells was almost exactly halfway between the positions of the V+max value and the incisura of the canonic AP. This is in good agreement with the

position of 4-AP-sensitive current peak, which is considered as I_{to} [1]. Of note, the early peak density value of 1.8 ± 0.4 pA/pF for the delavirdine sensitive current is smaller than the 3.0 ± 0.23 pA/pF peak density of 4-AP-sensitive current. The reason of this can be either due to the smaller I_{to} inhibition elicited by 10 μM delavirdine than by 1 mM 4-AP or simply the smaller I_{to} currents in cells of the current study (probably due to fewer large I_{to} expressing subepicardial cells in the studied population) than with 1 mM 4-AP [1]. Comparing the decay of the early component of the delavirdine-sensitive current with that of 4-AP-sensitive one, they both similarly reached nearly 0 value by 50 ms.

Late peak density of delavirdine-sensitive current (0.46 ± 0.08 pA/pF) was only slightly smaller than that of 1 μM E-4031-sensitive one representing I_{Kr} (approximately 0.5–0.6 pA/pF) [1,3]. Taking into account the result of hERG measurements where 10 μM delavirdine evoked almost 50 % reduction and also the fact that 1 μM E-4031 reduces I_{Kr} with 80 % [35] it seems possible that late component of delavirdine-sensitive current contains I_{K1} too. Further support for this is the location of the late delavirdine-sensitive peak (0.3 ± 1.2 ms after the position of the V-max of the canonic AP). This point is later than that seen with E-4031-sensitive current peak (7–11 ms before the position of the V-max) but earlier than in 50 μM BaCl₂-sensitive current (1–3.5 ms later than the position of V-max) [1,3]. Moreover, the lack of correlation between positions of V-max of own APs and delavirdine-sensitive late peak also suggest the involvement of a current other than I_{Kr} (likely I_{K1}) in the late peak as a very strong correlation was described in case of E-4031-sensitive current [1]. Delavirdine-sensitive late peak density correlated well with the values of V-max of own APs (Fig. 8C) which was the case for BaCl₂-sensitive current, representing I_{K1} [1] strengthening the possibility of I_{K1} involvement in late component of delavirdine-sensitive current. That component started to rise at + 8.6 ± 1.5 mV of the canonic AP and reached its peak at −59.9 ± 1.9 mV. The membrane potential where E-4031-sensitive current started to rise was + 17 mV and reached its peak at around −50 mV. For I_{K1} (BaCl₂-sensitive current) these potential values were −22 mV (at start of rise) and around −65 mV (at the peak) [1,3]. These membrane potential values also suggest the presence of I_{K1} in the delavirdine-sensitive current on top of I_{Kr}. There are, however, some observations making the contamination of late delavirdine-sensitive peak with I_{K1} unlikely: lack of positive shift of RMP, lack of diastolic outward current (a characteristic feature of BaCl₂-sensitive current [1,3]) and finally the percentage of late delavirdine-sensitive peak (26.6 ± 4.4 %) out of the net membrane current at the position of V-max of the canonic AP. That percentage value is the same for I_{Kr} (25 %) but much different from that for I_{K1} (at least 75 %) [1,3].

Another issue needs discussion regarding the delavirdine-sensitive

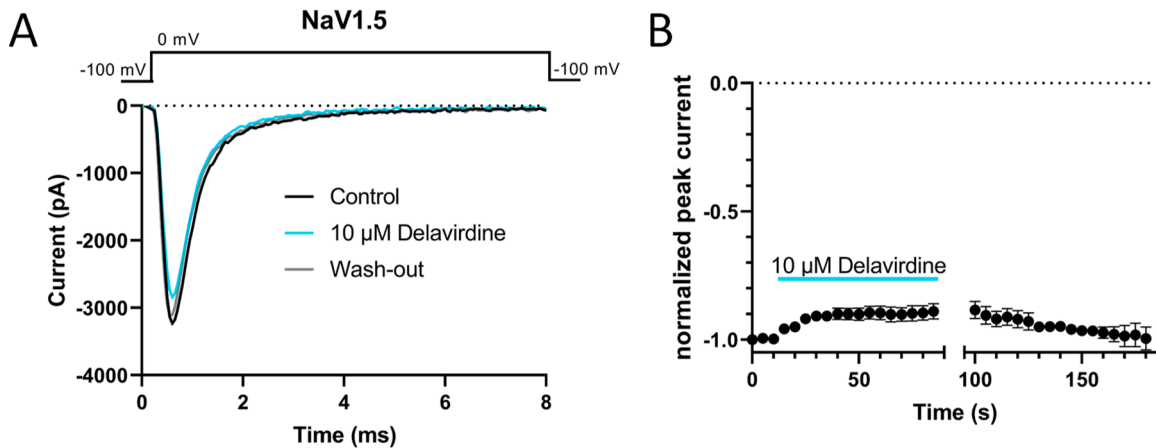


Fig. 9. Effects of delavirdine on Nav1.5 channels expressed in CHO cells. (A) A representative patch-clamp recording in voltage-clamp mode with the applied voltage protocol (above). The black trace is the current measured in control condition, the cyan in 10 μM delavirdine, while the gray shows current after washout. (B) Time course of delavirdine effect indicated the average ± SEM of normalized peak currents recorded in 5 cells.

current: the nature of the small inward current flowing through the plateau phase with a -0.11 ± 0.07 pA/pF mid-plateau density value in the average of all 13 studied cells (Figs. 8D, 8F). Based on our previous results this small inward current is about 50 % of the late I_{Na} and sodium-calcium exchanger currents and about 25 % of the amplitude of $I_{Ca,L}$ recorded at this point of the canonic AP [3]. If one assumes that delavirdine slightly reduces any of these inward currents or some combination of them that would, however, lead to a reduction of plateau potential, which was not the case. In spite of this, early plateau potential (just like Plateau₂₀ amplitude) was elevated by delavirdine whereas mid-plateau potential (and Plateau₅₀ amplitude) was unchanged. Moreover, the mid-plateau value of delavirdine-sensitive current density tended to be more negative (larger) in *endo* cells, although it was not statistically significant ($p = 0.072$), suggesting larger inward currents. In canine ventricular cells the late I_{Na} was approximately 1.5 times larger in midmyocardial cells than in epi and *endo* ones [40] whereas the descending order of the sodium-calcium exchanger current within the ventricular wall was midmyocardial > subepicardial > subendocardial [41]. $I_{Ca,L}$ was similar in canine subepicardial and midmyocardial cells when studied with conventional voltage pulses [42], but larger overall current (higher amount of charge) was detected in subepicardial than in subendocardial cells with APVC technique [39]. On the contrary, another study reported higher subendocardial $I_{Ca,L}$ compared to subepicardial cells with conventional voltage pulses [43]. Based on these findings, if delavirdine blocked any of the three currents (late I_{Na} , sodium-calcium exchanger current, $I_{Ca,L}$) that would be expected to be smaller in *endo* than in *non-endo* cells, which was not the case. It must be mentioned that delavirdine actually slightly reduced the current of expressed NaV1.5 channels, the major component of cardiac sodium current. Therefore, it is unlikely that delavirdine blocks sodium-calcium exchanger current or $I_{Ca,L}$ but the small inward mid-plateau current can be due to delavirdine-induced sodium current reduction.

4.5. Lack of additional consequences of delavirdine application

Based on our results delavirdine inhibits I_{Kr} (which was expected from its chemical structure and was proven by hERG channel experiments) and I_{to} too (reduction of V_{ph1max} , early outward component of APVC-recorded delavirdine-sensitive current). On top NaV1.5 channels were also slightly inhibited by delavirdine. The consequence of these resulted in the characteristic change of AP shape (APD increase, reduction of phase-1, Plateau₂₀ amplitude increase). AP and as a consequence QT prolongation per se can lead to increased risk of cardiac arrhythmia due to development of EADs [10,44]. During our experiments EADs were absent even in the presence of the highest applied delavirdine concentration of 30 μ M. In contrast, ABT-333, a methanesulfonamide group-containing compound used in hepatitis C infection, led to the appearance of EADs [19]. As ABT-333 also blocked I_{Kr} and I_{to} (blocked hERG current, increased APD, reduced V_{ph1max} , Plateau₂₀ amplitude increase) but generated EADs there should be some difference in the cardiac actions of the two drugs. The difference is the lack of the sustained outward current during the plateau of the AP in the delavirdine-sensitive current (Figs. 8B, 8D in this study) compared to the ABT-333-sensitive one (see Fig. 6B in [19]). That maintained outward plateau current can be due to the ABT-333-induced activation of an inward current (most likely $I_{Ca,L}$) leading to the elevation of early plateau potential, reactivation of calcium channels and generation of EADs. In case of delavirdine, the small inward mid plateau current detected with APVC measurements is the opposite what we observed with ABT-333 and might be due to the delavirdine-induced NaV1.5 reduction. This sodium current reduction can balance the inhibition of outward currents (I_{Kr} and I_{to}) which reduces the arrhythmic risk of delavirdine.

Another parameter representing higher arrhythmia risk is the increase of SV [13,14]. This, however, did not increase in the presence of delavirdine, we detected only a tendency for SV increase. According to

our previous results I_{to} , sodium current (as well as I_{K1}) inhibition reduces SV, which can balance its increase by I_{Kr} inhibition [14]. In summary, it seems that delavirdine does not carry additional risk of cardiac arrhythmias (no SV increase, no EADs).

4.6. Clinical relevance

In our experiments 1–30 μ M delavirdine exerted significant action on canine ventricular AP, on hERG channels and to a small extent on NaV1.5 channels. Delavirdine inhibits HIV-1 activity in vitro with half and 90 % effectivity in 0.01 and 0.05 μ M, respectively [45]. In 12 HIV patients the maximal plasma concentration (C_{max}) was developed after approximately one hour of the intake of a single 400 mg tablet, reaching a value of 7.22 ± 4.0 μ M and declined with an 2.39 ± 0.82 hour half-life [46]. When delavirdine was given as recommended (400 mg every 8 hours for 14 days) its C_{max} was 35.5 ± 17 μ M in 11 HIV patients, while lower (22.4 ± 11 μ M) when given together with didanosine [47] and also lower when taken with food compared to taken on an empty stomach (23.0 ± 8.61 vs 29.6 ± 13.6 μ M) [48]. It must be noted, that although the C_{max} was lower when delavirdine was taken with food, other steady-state pharmacokinetic parameters (minimal concentration of approximately 10 μ M, oral clearance) did not differ in the presence or absence of food intake [48]. When delavirdine concentration was determined on a pooled number of patients (67) the mean of minimal, steady-state, and maximal plasma concentration values were 12, 21, and 33 μ M, respectively and these values were almost the same when 600 mg delavirdine was used twice a day [49].

Cytochrome P450 (CYP) 3 A enzyme N-dealkylates delavirdine [50], which enzyme is actually inhibited by delavirdine itself with and a half inhibitory concentration (IC_{50}) of 0.9 μ M [51]. Other CYP isoforms (2D6) are also probably involved in the metabolism of delavirdine [52]. Due to these actions, several drugs such as lovastatin, simvastatin, rifabutin, rifampicin, sildenafil, ergot derivatives, quinidine, midazolam, carbamazepine, phenobarbital or phenytoin are not recommended to be taken with delavirdine [53].

QTc intervals were only increased in patients taking buprenorphine/naloxone in combination with 2×600 mg delavirdine (taken for 7 days) [17]. Apart from that study, delavirdine-induced QT prolongation was not reported, but HIV infected patients can develop QTc prolongation due to HIV-associated dilated cardiomyopathy [18], which prolongation can be worsened in case of delavirdine application.

4.7. Summary and conclusions

In summary, acute application of delavirdine in the clinically relevant concentrations between 1 and 30 μ M resulted in rapid and reversible blockade of expressed hERG and NaV1.5 channels, as well as mostly reversible APD increase, reduction of maximal rates of phases 1 and 3 and a small increase of Plateau₂₀ amplitude in canine left ventricular isolated cardiomyocytes. Accordingly, delavirdine-sensitive current contained an early and transient, a late outward component corresponding to phases 1 and 3 of the AP, respectively as well as an inward current present under the plateau phase. Based on the plasma concentrations of delavirdine (7–30 μ M) we can conclude that delavirdine modifies cardiac electrophysiological parameters in the therapeutically relevant concentrations. This can only lead to cardiac arrhythmias in HIV-infected patients with already altered cardiac function or patients with co-administration of drugs metabolized by certain CYP isoforms. Luckily, as delavirdine application was without increase of SV or EAD appearance its arrhythmic risk does not increase any further.

List of abbreviations

4-AP	4-aminopyridine
AP	action potential

APA	action potential amplitude
APD	action potential duration
APD ₅₀	action potential duration at 50 % of repolarization
APD ₉₀	action potential duration at 90 % of repolarization
APVC	action potential voltage clamp
BTY	bicarbonate buffer containing Tyrode solution
Cmax	maximal plasma concentration
CYP	cytochrome P450 enzyme
EAD	early afterdepolarization
HIV	human immunodeficiency virus
HEK	human embryonic kidney
IC ₅₀	half maximal inhibitory concentration
I _{Ca,L}	L-type calcium current
I _{K1}	inward rectifier potassium current
I _{Kr}	rapid component of the delayed rectifier potassium current
I _{Na}	sodium current
I _{to}	transient outward potassium current
LQTS2	long QT syndrome type 2
OSP	overshoot potential
Plateau ₂₀	amplitude difference between RMP and membrane potential at 20 % duration of APD ₉₀
Plateau ₅₀	amplitude difference between RMP and membrane potential at 50 % duration of APD ₉₀
RCF	remaining current fraction
RMP	resting membrane potential
SV	short-term variability of repolarization
TdP	torsade de pointes
V+max	maximal rate of phase 0 depolarization
V _{ph1} max	maximal rate of phase 1 repolarization
V-max	maximal rate of phase 3 repolarization

CRedit authorship contribution statement

Dienes Csaba: Writing – review & editing, Methodology, Investigation, Formal analysis. **Kovács Zsigmond Máté:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Bányász Tamás:** Writing – review & editing. **Magyar János:** Writing – review & editing. **Szentandrassy Norbert:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Varga Zoltan:** Writing – review & editing. **Óvári József:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Nánási Péter Pál:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Feher Adam:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Horváth Balázs:** Writing – review & editing. **Domingos Geraldo Jorge:** Formal analysis, Investigation, Methodology.

Declaration of Competing Interest

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