Original Paper

4,4′-Diisothiocyanato-2,2′-Stilbenedisulfonic Acid (DIDS) Modulates the Activity of KCNQ1/KCNE1 Channels by an Interaction with the Central Pore Region

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Key Words
DIDS • Activator • Allosteric modulator • Molecular mechanism • KCNQ1

Abstract

Background/Aims: The cardiac current IKs is carried by the KCNQ1/KCNE1-channel complex. Genetic aberrations that affect the activity of KCNQ1/KCNE1 can lead to the Long QT Syndrome 1 and 5 and, thereby, to a predisposition to sudden cardiac death. This might be prevented by pharmacological modulation of KCNQ1/KCNE1. The prototypic KCNQ1/KCNE1 activator 4,4′-Diisothiocyanato-stilbene-2,2′-disulfonic acid (DIDS) represents a candidate drug. Here, we study the mechanism of DIDS action on KCNQ1/KCNE1. Methods: Channels were expressed in Xenopus oocytes and iPSC cardiomyocytes. The role of the central S6 region was investigated by alanin-screening of KCNQ1 residues 333-338. DIDS effects were measured by TEVC and MEA. Results: DIDS-action is influenced by the presence of KCNE1 but not by KCNQ1/KCNE1 stoichiometry. V334A produces a significant higher increase in current amplitude, whereas deactivation (slowdown) DIDS-sensitivity is affected by residues 334-338. Conclusion: We show that the central S6 region serves as a hub for allosteric channel activation by the drug and that DIDS shortens the pseudo QT interval in iPSC cardiomyocytes. The elucidation of the structural and mechanistic underpinnings of the DIDS action on KCNQ1/KCNE1 might allow for a targeted design of DIDS derivatives with improved potency and selectivity.

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Introduction

Long QT syndrome (LQTS) is associated with an increased risk of ventricular arrhythmias possibly leading to syncope or sudden cardiac death. Loss-of-function mutations in genes encoding KCNQ1/KCNE1, which represent the molecular basis of the cardiac IKS current may hamper cardiac repolarization and, thereby, result in congenital Long QT Syndrome [1]. Pharmacological intervention using small molecule KCNQ1/KCNE1 agonists might restore the faulty repolarization caused by KCNQ1/KCNE1 dysfunction [2]. Alternatively, in Long QT Syndromes 1 and 5 (LQT1/5), KCNQ1/KCNE1 channel activity might be enhanced by increasing the surface expression of channels. The direct pharmacological targeting of the channel promises to limit side effects of a potential drug therapy. KCNQ1 channels (also called Kv7.1 or KvLQT1) are built up by four homologous α-subunits that harbor six transmembrane helices (S1-S6). The central pore domain (PD) is formed by the tetrameric assembly of S5 and S6, whereas each subunit harbors a prototypical S1-S4 voltage-sensing domain (VSD). KCNQ1 is gated by depolarization of the membrane potential, which is sensed by positively charged residues in S4 causing the S4-segment to rotate in extracellular direction [3]. Voltage-dependent gating of KCNQ1 is facilitated by a bending motion in the central S6 helix [4] rather than by a S6 glycine hinge known from other Kv channels [4]. VSD-PD-coupling in KCNQ1 has been associated with an allosteric gating scheme. This assumes an independent movement of the VSDs; yet, the shift in balance towards the open state increases with the number of engaged VSDs [5, 6]. The linker between the S5- and S6-helix form the central pore domain including the selectivity filter constituting the TTIGYGD-motive in the pore loop; of note, the isoleucine residue is important for masking of channel inactivation mediated by the β-subunit KCNE1 [7]. Co-assembly of KCNQ1 with KCNE1 also slows down channel activation and shifts the voltage-dependence of activation towards more positive voltages, resembling the characteristics of the slowly repolarizing cardiac potassium current IKS [8, 9]. It has been proposed that KCNE1 binds to S1, S4 and S6 of adjacent KCNQ1-subunits [7, 10]. The KCNQ1/KCNE1 stoichiometry has however been a matter of debate. Some reports [11–15] suggest a fix ratio of 4:2, whereas other authors indicate a rather variable stoichiometry of up to 4:4 [16–20]. KCNQ1/KCNE1 agonists include the synthetic compounds R-L3, phenolboronic acid (PBA), hexachlorophene (HCP), mefenamic acid (MFA), ML277 [21], and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) [22, 23] as well as the naturally occurring Rottlerin (Mallotoxin) [24, 25], ω-6 and ω-9 polyunsaturated fatty acids [26], and gintonin [27]. R-L3, the first K+ channel agonist, whose binding site was revealed, increases the current amplitude, shifts the voltage-dependence of channel activation, and slows activation and deactivation kinetics of homomeric KCNQ1 channels; co-expression of KCNQ1 with KCNE1, however, strongly attenuates the potency/efficacy of the drug’s action on the channel [28]. Similarly, PBA and HCP act rather on homomeric channels [29, 30]. MFA and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) are well-known to target heteromeric KCNQ1/KCNE1 channels [22]. The binding site of DIDS has been predicted between the VSD and the N-terminal end of the KCNE1 transmembrane domain [31], which might explain the KCNE1-dependent action of the drug on the current amplitude. However, DIDS modulates the channel’s deactivation kinetics and voltage dependence also in the absence of KCNE1 [22], indicating that action of DIDS involves additional so far unknown drug-channel interactions. In fact, the mechanism underlying the action of DIDS on channel deactivation kinetics is unknown altogether. Moreover, it is unclear how the KCNQ1/KCNE1 stoichiometry affects the action of the drug. In the current study, we address these questions.
**Materials and Methods**

**Molecular biology**

DNA templates were linearized with an appropriate restriction enzyme (wildtype or mutant hKCNQ1/pSGEM with NheI, wildtype hKCNE1/pSP64 with EcoRI). *In vitro* synthesis of cRNA was performed using the mMessage mMachine T7 or SP6 kit (Invitrogen, Thermo Fisher Scientific, Waltham, USA). The cRNA quality was checked by agarose gel electrophoresis and concentrations were determined by photospectrometry.

**Two electrode voltage clamp (TEVC)**

Stage V-VI *Xenopus laevis* oocytes were purchased from EcoCyte Bioscience (Dortmund, Germany). The oocytes were injected with 8 ng KCNQ1-WT cRNA or co-injected with 4 ng KCNQ1-WT or mutant cRNA and 8 ng KCNE1-WT cRNA using a nanoliter injector 2000 (WPI, Berlin, Germany). To investigate the influence of KCNQ1/KCNE1 stoichiometry on DIDS action, the cRNA ratio was changed to 8 ng KCNQ1-WT and 5.3 ng KCNE1-WT cRNA. Injected oocytes were kept at 18°C in Barth’s solution (containing in mM: 88 NaCl, 1 KCl, 0.4 CaCl$_2$, 0.33 Ca(NO$_3$)$_2$, 0.8 MgSO$_4$, 5 TRIS-HCl, 2.4 NaHCO$_3$, supplemented with 80 mg l$^{-1}$ theophylline, 63 mg l$^{-1}$ penicillin, 40 mg l$^{-1}$ streptomycin and 100 mg l$^{-1}$ gentamycin). Two-electrode voltage clamp (TEVC) recordings were conducted at room temperature after 3-4 days of incubation using a *TURBO TEC 10CX* amplifier (npi electronic, Tamm, Germany), NI-USB-6221 AD/DA-interface (National Instruments, Austin, USA) and *GePulse* software (Dr. Michael Pusch, Genova, Italy). Currents were recorded at a constant flow rate in ND96 solution (containing in mM: 96 NaCl, 4 KCl, 1 CaCl$_2$, 1.8 MgCl$_2$, 5 HEPES, titrated to pH 7.4 with NaOH, adjusted to 0.1% (v/v) DMSO) using recording pipettes filled with 3 M KCl. Measuring solutions were freshly prepared by adding 4, 4'$-$Diisothiocyanato-2, 2'$-$stilbenedisulfonic acid (DIDS) to ND96 from a 10 mM or 50 mM DMSO stock solution. DIDS was obtained from Sigma-Aldrich, St. Louis, MO.

Starting from a holding potential of -80 mV, different voltage-pulse protocols were applied as described in the following: (1) To analyze the concentration dependent effect of DIDS on KCNQ1/KCNE1 current amplitude, currents were elicited by a 8 s pulse to +40 mV followed by -120 mV pulse for 5 s. For oocytes injected with 8 ng KCNQ1-WT, pulse durations were modified to 3.5 s and 4 s respectively. (2) To study the effect of DIDS on voltage dependence of channel activation, currents were elicited with 8 s pulses to potentials between -120 and +80 mV in 20 mV increments, and tail currents were recorded at -120 mV for 5 s. (3) The effect of DIDS on KCNQ1/KCNE1 channel deactivation was investigated using 10.9 s repolarizing pulses to potentials of -60 to -120 mV in 20 mV increments after a 8 s depolarizing pulse to +40 mV.

**Data analysis**

Data were analyzed using Ana software (Dr. Michael Pusch, Genova, Italy), Microsoft Excel (Microsoft, Redmond, WA, USA) and OriginPro 2019 (OriginLab Corporation, Northampton, USA). Channel activation was determined at the end of 8 s pulse to +40 mV: $y = (I_{DIDS}/I_{Control} - 1) * 100$, where $I_{DIDS}$ is the current amplitude in presence of DIDS and $I_{Control}$ is the current amplitude before drug application. The half maximal effective concentration (EC$_{50}$) was calculated by logistic function curve fitting: $y = A_2 + (A_1 - A_2) / (1 + (x/x_0)^H)$. $A_1$ is the maximum rate of activation, $A_2$ is the minimal rate of activation, $x_0$ is the drug concentration, $x$ is the EC$_{50}$ and $H$ the Hill coefficient.

The current-voltage curves were constructed by plotting normalized tail currents vs. the respective test potential. For comparing the effect of DIDS on voltage-dependent channel activation at varying KCNQ1-WT/KCNE1-WT stoichiometry, tail currents were normalized to tail currents at +60 mV before drug application. The voltage of half-maximal activation was individually determined using a Boltzmann equation of the form: $y = A_1 + (A_2 - A_1) / (1 + exp ((x-x_0) / sf))$, whereas $y$ is the normalized tail current, $x_0$ is the voltage of half-maximal activation, $x$ is the test voltage, $A_1$ and $A_2$ are minimal and maximal tail current amplitudes, $sf$ is the slope factor. Tail currents for comparison of different KCNQ1 mutants plus KCNE1-WT were additionally normalized to the averaged normalized tail current of each mutant at +80 mV after application of ND96.

Deactivation time constants were obtained by fitting tail currents to a single exponential function: $y = A_s + A_t * exp (-t/τ)$, where $A_s$ is the steady-state amplitude, $A_t$ is the current amplitude and $τ$ is the time constant of deactivation.

Data are expressed as mean ± SEM, n indicates the number of oocytes tested. Differences were considered statistically significant when unpaired t-test or ANOVA-test resulted in a p-value < 0.05.
Of note, mutants V334A and F335A showed significant run-up. This run-up was determined in the absence of DIDS. When the run-up was subtracted from the DIDS-induced current increase, V334A still showed significant effects.

**hiPSC culture**

The hiPSC cardiomyocytes were generated as described before [32]. In brief, the hiPSC-line Sendai Foreskin 1 (SFS.1) was used as starting material for the generation of hiPSC-derived cardiomyocytes. SFS.1 were cultured in FTDA-Medium consisting of (DMEM/F12 (Invitrogen #21331020), 5 µg/ml ITS (Becton Dickinson #354350), 0.1% human serum albumin (Biological Industries #05-720-1B), 1X CD Lipid Concentrate (Invitrogen #19050301), 1X Penicillin/ Streptomyacin/ Glutamine (Life Technologies #10378016), 10 ng/ml FGF2 (PeproTech #100-18B), 5 ng/ml Activin A (eBioscience #34-8993-85), 0.4 µg/ml TGFβ1 (eBioscience #34-8348-82), and 50 nM Dorsomorphin [Santa Cruz #sc-200689] [33]. Medium was changed daily and cell passaging was performed every 4th day. The replating is done under the use of Accutase® solution (Sigma #A6964) supplemented with 10 µM Y-27632 (abcamBiochemicals #ab120129). After 10 min of Accutase® treatment cells are gently washed with FTDA-Medium supplemented with 10 µM Y-27632 and centrifuged at 1200 rpm for 2 min. Cells are resuspended in FTDA medium supplemented with 10 µM Y-27632 and seeded in a density of 600,000 cells into Matrigel coated 6-Wells (matrigel dilution 1:75).

**Cardiomyocyte differentiation and maturation**

Detached SFS.1 are seeded into 1:75 matrigel coated 24-wells in a density of 500,000 cells/well in Day-0 differentiation medium consisting of (KO-DMEM (Life Technologies #10829018), 1X Penicillin/ Streptomyacin/ Glutamine, 5 µg/ml ITS, 10 µM Y-27632, 20 ng/ml FGF2, 1 nM CHIR-99021 (Axon Medchem #Axon1386), and 1 nM BMP-4 (R&D # 314-BP-010). The next day, exactly after 24h, medium is changed to TS-ASC medium (KO-DMEM, 5.5 mg/L Transferrin (Sigma #T8158-100MG), 6.75 µg/L Selenium (Sigma #S5261-10G), 1X Penicillin/ Streptomyacin/ Glutamine, 250 µM ascorbate (Sigma #49752-10G)). On day 2 and day 3 of differentiation, cells get additionally supplemented with 0.5 µM C59 (Tocris #5148). Finally, the cells are kept in TS-ASC until spontaneous beating of the whole cell layer occurs. This usually took place between day 8 and day 9 of differentiation.

Young Cardiomyocytes were detached from 24-wells with 1x TrypLE Select and reseeded into new 24-wells coated with 1:150 matrigel / 0.1% gelatin in a dilution of 1:4. Cell medium was changed from TS-ASC to KO-THAI (KO-DMEM, 1X Penicillin/ Streptomycin/ Glutamine, 0.2% human serum albumin, 250 µM ascorbate, 5 µg/ml ITS, and 0.004% (v/v) Thioglycerol). Cells were kept in culture for 30 more days for maturation including media change every third day. Under this condition iPSC cardiomyocytes contract spontaneously and express KCNQ1, an indication that cardiomyocytes are "mature" enough to analyze $I_{\text{Kr}}$ [34–36].

**Electrophysiological characterization on MEA**

The characterization of 40 days matured ventricular-like cardiomyocytes was done on micro-electrode arrays (USB-MEA256 9Well Multichannel systems) as described before [37]. In brief, MEA chips were plasma treated for complete surface cleansing. Subsequently all 9 wells were coated with 0.2% gelatin solution for 1h at room temperature. Shortly before cell seeding the MEA chip was pre-warmed in a humidified cell-culture incubator at 37°C. The 40 days matured hiPSC ventricular-like cardiomyocytes were detached from culture plates with 10x TrypLE Select, centrifuged at 1200 rpm and re-suspended in cardiomyocyte KO-THAI culture medium supplemented with 10 µM Y-27632. Gelatin coating solution was removed from the MEA wells and 200,000 ventricular-like hiPSC cardiomyocytes were seeded into each well. Cells were allowed to attach overnight. The next day the medium was changed to KO-THAI w/o 10 µM Y-27632 and cells were kept on the MEA chip for two days. Electrophysiological recordings were performed on the third day after re-plating. The recordings were performed under cellular stimulation with a pulse of 1000 mV for 500 ms in an interval of 999 ms. Cells were measured for 10 min under 10 µM Isoprenaline supplementation followed by 10 min measurement under additional DIDS supplementation. Data were filtered a 100 Hz. QT-Intervals of both conditions were measured using the software Cardio2D+ (Multichannel Systems). Using the analysis of the “QT-related interval” the field potential duration corresponds with the action potential duration and can be correlated to a QT-like interval in an electrocardiogram, which is measured from the
minimum of the Na⁺ peak current to the minimum of the Iᵢᵥ current peak. The QT-like interval is measured from the heartbeat timestamp to an extremum within the post HB interval.

Results

The action of DIDS is independent of the KCNQ1/KCNE1 stoichiometry

As it has been described in the literature that DIDS increases the current amplitude of KCNQ1/KCNE1 channels and modifies gating kinetics of KCNQ1 channels regardless of the presence of KCNE1 [22], we first investigated the action of DIDS on KCNQ1 and KCNQ1/KCNE1 (expression ratio 1:2) channels expressed in Xenopus laevis oocytes. Confirming the previous results, DIDS (10 µM) increased the amplitude of KCNQ1/KCNE1 currents by 95.6 ± 7.0 % (n = 38), whereas the drug did not affect the amplitude of currents carried by KCNQ1 (Fig. 1). Next, we studied the action of DIDS on KCNQ1/KCNE1 channels upon expression of KCNQ1 and KCNE1 at different ratios. At 10 µM, the drug evoked a similar increase in current amplitude and left-shift of the voltage-dependence of activation upon expression of KCNQ1/KCNE1 at a ratio of 1:0.67 and 1:2; the current increase at +40 mV was 96.6 ± 14.6 % (n = 21) versus 95.6 ± 7.0 % (n = 38) and the shift in Vₑ/₂ was 21.1 ± 2.2 mV (33.5 ± 2.1 mV to 12.4 ± 2.2 mV, n = 14) versus 19.1 ± 2.2 mV (40.2 ± 1.9 mV to 21.2 ± 2.6 mV, n = 6), respectively. Moreover, at +40 mV, DIDS increased the KCNQ1/KCNE1 current amplitudes in a dose-dependent fashion with an EC₅₀ that was rather independent of the expression ratio (Fig. 2) (3.6 µM at 1:2 and 5.1 µM at 1:0.67 expression ratio, n = 3-38). Finally, also the DIDS-mediated slowdown of the deactivation kinetics was independent of the expression ratio (Fig. 3). We conclude that the presence of KCNE1 but not the KCNQ1/KCNE1 stoichiometry affects the DIDS action on the channel.

DIDS action on KCNQ1 pore-domain mutants

It has been proposed that DIDS activates KCNQ1/KCNE1 channels by binding between the voltage-sensing domain and the outer boundary of the KCNE1 transmembrane domain [31]. To elucidate the mechanism underlying the DIDS-mediated slowdown of channel deactivation, we examined whether and how the drug's action is affected by mutation of residues that are suspected to be involved in deactivation (S333A, V334A, F335A, A336C, I337A, and S338A), i.e. residues near to the central S6-region containing the pore domains. The action of DIDS on the current amplitude of a specific mutant and on the wildtype channel was tested side-by-side in identical

Fig. 1. Effect of DIDS on KCNQ1 channels depends on the presence of KCNE1. A. Structural depiction of 4,4'-Diisothiocyanato-2,2'-stilbenedisulfonic acid (DIDS) as structural formula and as space fill representation (CPK coloring). The symmetric structure of DIDS contains two aromatic benzyl rings, two outer amides and two sulfonic groups. The molecule thus combines aromatic regions with charged regions. B. Representative current traces of KCNQ1 or KCNQ1/KCNE1 expressed at a ratio of 1 to 2 recorded in Xenopus laevis oocytes before (black) and after (gray) application of 10 µM DIDS.
Fig. 2. Impact of varying KCNQ1/KCNE1 stoichiometry on concentration dependence of DIDS action and change in current/voltage relationship. A-B. Representative current traces of KCNQ1-WT/KCNE1-WT at two KCNQ1/KCNE1 ratios before and after application of 10 µM DIDS. C-D. Tail currents were analyzed at -120 mV and normalized to tail currents at +80 mV before application of 10 µM DIDS (Q1/E1 1:0.67 n = 7; Q1/E1 1:2 n = 6 ± SEM). E-F. Dose-response relationship for activation of KCNQ1/KCNE1 channel currents at two KCNQ1/KCNE1 ratios. Activation was determined as percent change in current amplitude at +40 mV. Current amplitudes were measured before and after a series of 15 voltage equilibration pulses. Data were fitted to a logistical function to calculate EC50-value. (Q1/E1 1:0.67 n = 3-21; Q1/E1 1:2 n = 3-38; ± SEM). G. V1/2-values were calculated by Boltzmann-fits of each individual oocyte’s normalized peak tail currents before and after application of 10 µM DIDS (n = 6-14, ± SEM). Even though the currents did not saturate V1/2 values can be approximated. DIDS causes a shift in voltage-dependence of KCNQ1/KCNE1 channel activation (Q1/E1 1: 0.67 – V1/2 = 33.5 ± 2.1 mV shifted to V1/2 = 12.4 ± 2.2 mV (n = 14), Q1/E1 1: 2 V1/2 = 40.2 ± 1.9 mV shifted to V1/2 = 21.2 ± 2.6 mV (n = 6)). Shift in half maximal activation voltage V1/2 is not significantly influenced by varying the KCNQ1/KCNE1 ratios. Data were tested for significance by performing two sample t-test.

Fig. 3. Activation of KCNQ1/KCNE1 by DIDS is associated with a reduced rate of channel deactivation. A-B. Examples of KCNQ1/KCNE1 deactivating current traces before and after application of 10 µM DIDS at two different KCNQ1/KCNE1 ratios. Currents were recorded using the deactivation protocol described in methods section. C-D. DIDS increases the time constant of deactivation determined by fitting traces to a single exponential function (Q1/E1 1:0.67 n = 13-23; Q1/E1 1:2 n = 7-11, ± SEM).
batches of oocytes and normalized to wildtype activation. DIDS enhanced the amplitude of KCNQ1(V334A)/KCNE1 channels to a much greater extent than that of KCNQ1-WT/KCNE1 channels (Fig. 4). DIDS evoked increased voltage-dependence of channel activation appeared in oocytes co-expressing KCNQ1 and the S333A, V334A and I337A KCNQ1 mutants (Fig. 4).

Interestingly, F335A impacted deactivation (slowdown) DIDS-sensitivity but did not significantly affect the amplitude effect, whereas V334A is key to amplitude effects but reduced deactivation DIDS-sensitivity (Fig. 4, 5). Furthermore, we found that only in S333A mutant channels, DIDS evoked a similar slowdown of deactivation as in KCNQ1-WT/KCNE1 channels. DIDS did not cause a consistent shift in deactivation slowdown in A336C, I337A and S338A mutants. Therefore, it is obvious that individual residues in the central S6 region are key for mediating the different DIDS effects highlighting the necessity of this region for KCNQ1/KCNE1 pharmacology. Summarizing, only V334A produces a significant higher increase in current amplitude, whereas deactivation (slowdown) DIDS-sensitivity is affected by residues 334-338.

**Effect of DIDS on ventricular repolarization**

Next, we evaluated the action of DIDS in a human cardiac-ventricular environment. To this end, we analyzed cardiac repolarization in hiPSC cardiomyocytes [25]. We trans-differentiated a human iPSC line to yield beating cardiomyocytes expressing hKCNQ1/KCNE1. These cells were stimulated by Isoprenaline [37] and the electrical activity of hiPSC-cardiomyocyte clusters were monitored by the multi-electrode array technique. The extracellularly recorded local field potential is comparable to the human ventricular ECG and

![Fig. 4. Influence of KCNQ1 pore domain mutations on DIDS-effect on voltage-dependence of channel activation and change in current amplitude. A. Effect of 10 µM DIDS on the current-voltage relationship of wildtype and mutant KCNQ1/KCNE1 channels (KCNQ1-KCNE1 - ratio 1:2). Tail currents were recorded at -120 mV and additionally normalized to the averaged normalized tail current at +80 mV after application of ND96 containing 0.1 % DMSO instead of DIDS (voltage pulse-protocol (2) in methods section). Similar normalized tail currents after application of ND96, 0.1 % DMSO served as control (Q1 wt n = 4-6; S333A n = 4; V334A n = 3; F335A n = 9-13; A336C n = 7; I337A n = 11-14, S338A n = 3-4, ± SEM). Representative currents are shown as small inlay representations. B. Activation of wildtype and mutant KCNQ1/KCNE1 by 10 µM DIDS. Activation was defined as percent change in current amplitude at the end of the +40 mV depolarizing test pulse, using voltage pulse-protocol (1) described in methods section (n = 3-14, ± SEM). Activation (DIDS-stimulation) of mutant KCNQ1 coexpressed with KCNE1 at a ratio of 1 to 2 was normalized to activation of oocytes coexpressed with wildtype KCNQ1/KCNE1 from respective batches to minimize variation by preparation. Data were tested for significance using unpaired t-test and *** indicates p = 0.001.
a pseudo QT interval can be studied. Therefore, this pseudo QT interval is a readout of cardiac cell repolarization. DIDS (10 μM) shortened the pseudo QT interval (Fig. 6), presumably by activation of KCNQ1/KCNE1.

Discussion

We confirm that DIDS activates KCNQ1/KCNE1 channels [22, 23, 31]. Here, we investigated KCNQ1/KCNE1 at two cRNA ratios that showed a similar EC50 and deactivation kinetics and no significant difference in the shift in voltage-dependence of activation. KCNQ1/KCNE1 stoichiometry has been described to be up to a ratio of 4:4 in a chimeric approach and
some arguments suggest a variable stoichiometry [16]. This indicates that KCNQ1/KCNE1 stoichiometry at least within the range tested here does not markedly alter DIDS action. Thus, the presence of one KCNE1 β-subunit within the KCNQ1-KCNE1 complex seems sufficient for full DIDS sensitivity. As coexpression with KCNE1 is not necessary for DIDS action on channel kinetics, it appears possible that these effects are predominantly carried by KCNQ1 α-subunits rather than the amount of coexpressed KCNE1. On the contrary, the fact that the presence of KCNE1 is necessary to produce the combination of current facilitation and prominent shift in gating towards the open state indicates that KCNE1 can couple these functions in the channel complex. A structural KCNQ1 region described to be relevant for current amplitude, deactivation and KCNE1 interaction is the central pore domain [10, 38, 39]. The mediator in this region of the selectivity filter on one side and the voltage sensor domain associated with the KCNE1-transmembrane segment on the other side is the central KCNQ1 S6 transmembrane region. Bending in this region allows for (de-)activation gating and stabilization of the selectivity filter [40]. In a simplified gating scheme DIDS action can be assumed to decrease the rate of deactivation and is thus affecting the deactivation process depending on these structures (Fig. 7). For this reason we scanned this region by mutations to alanine or cysteine and tested for DIDS sensitivity. As none of the KCNQ1 pore domain mutants investigated leads to a decrease in DIDS action on current amplitude, a direct

![Fig. 7. Model of DIDS effects. A. Scheme of a KCNQ1 and a KCNQ1/KCNE1 channel composed of four subunits with and without KCNE1 in yellow. VS = voltage sensing domain, PD = pore domain. Orange arrows indicate interactions of the voltage sensors with the pore domains. The about position of KCNE1 transmembrane segment is depicted in yellow. B Scheme of pore domain composed of two opposing KCNQ1 channel subunits with and without KCNE1. The region on the S6-segments suspected to be involved in deactivation kinetics is colored red. The PAG motif of the gate and the selectivity filter are labeled PAG and SF, respectively. C. Effect of DIDS on a simplified basic KCNQ1/KCNE1 gating scheme. DIDS shifts the balance towards open states. D. Model of KCNQ1/KCNE1. Key residues suspected to affect the mechanism of action of DIDS on channel deactivation kinetics are highlighted in red (dark blue respectively). Residues that significantly alter DIDS sensitivity if mutated are colored dark blue, residues located on the neighboring subunit which are supposed to interact with key residues are colored in green.](#)
interaction of DIDS with the S6-hinge seems unlikely. The predicted binding site between the VSD paddle motif and the N-terminal end of the KCNE1 transmembrane part supports this assumption [31]. An allosteric mechanism of DIDS action on KCNQ1/KCNE1-gating has also been suggested before [22]. In contrast to other mutants, the V334A mutant produced a significant increase of activation current. This could be due to altered gating kinetics when KCNQ1 (V334A) is coexpressed with KCNE1, which produces a more noticeable DIDS-effect on voltage-dependence of activation, too. Indeed, V334 contacts the selectivity filter (residue T311), S5 (residue L266), S6 (residues C331, F332, S333, F335, A336, I337, S338 and F340) spanning multiple subunits in silico, suggesting a high importance for the lower selectivity filter conformation and conduction rate. Consistently, DIDS stimulation of macroscopic conductance depends on this residue.

As the S6-hinge region around residue A336 has been suspected to influence KCNQ1 gating kinetics [4], it is consistent that this region is highly relevant for DIDS effects on voltage dependency and deactivation gating. It can be speculated that the mutants studied here are involved in mediation of allosteric DIDS action on KCNQ1/KCNE1 gating. However, the low number of mutations studied might not allow a valid statement. As mentioned before, direct binding to these residues is unlikely, however cannot be excluded because none of the mutants disrupts DIDS effects completely. As the regions involved in functional KCNQ1-KCNE1 interaction are involved in DIDS action as well, it seems as if DIDS would boost the KCNE1-KCNQ1 interactions.

Conclusion

In summary we have identified the central S6-transmembrane region as a key hanger connecting selectivity filter, gate, voltage sensors and KCNE1 transmembrane segment to allow for DIDS modulation of KCNQ1/KCNE1 channels. The DIDS scaffold is potentially suitable to generate potent KCNQ1/KCNE1 selective drug candidates. Activation of KCNQ1/KCNE1 may be a strategy to recover function of KCNQ1/KCNE1 in LQTS. Supporting the hypothesis that DIDS-analogs may be beneficial for treatment in LQTS our experiments on hiPSC cardiomyocytes clearly show that DIDS shortens ventricular repolarization. Therefore, DIDS or optimized derivatives hold a potential as QT interval correcting drugs.

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Supplementary Material
All data are included into the article. Raw data are available on reasonable request.

Disclosure Statement
The authors declare no conflict of interest exist.
References


26 Bohannon BM, Perez ME, Liin SI, Larsson HP: ω-6 and ω-9 polyunsaturated fatty acids with double bonds near the carbonyl head have the highest affinity and largest effects on the cardiac IKs potassium channel. Acta Physiol (Oxf) 2019;225:e13186.


