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Effects of trimethoprim–sulfadiazine and detomidine on the function of equine K_V11.1 channels in a two-electrode voltage-clamp (TEVC) oocyte model

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The long QT syndrome (LQTS) is a channelopathy that can lead to severe arrhythmia and sudden cardiac death. Pharmacologically induced LQTS is caused by interaction between drugs and potassium channels, especially the K_V11.1 channel. Due to such interactions, numerous drugs have been withdrawn from the market or are administered with precautions in human medicine. However, some compounds, such as trimethoprim–sulfonamide combinations are still widely used in veterinarian medicine. Therefore, we investigate the effect of trimethoprim–sulfadiazine (TMS), trimethoprim, sulfadiazine, and detomidine on equine-specific K_V11.1 channels. K_V11.1 channels cloned from equine hearts were heterologously expressed in *Xenopus laevis* oocytes, and whole cell currents were measured by two-electrode voltage-clamp before and after drug application. TMS blocked equine K_V11.1 current with an IC_{50} of 3.74 mM (95% CI: 2.95–4.73 mM) and affected the kinetics of activation and inactivation. Similar was found for trimethoprim but not for sulfadiazine, suggesting the effect is due to trimethoprim. Detomidine did not affect equine K_V11.1 current. Thus, equine K_V11.1 channels are also susceptible to pharmacological block, indicating that some drugs may have the potential to affect repolarization in horse. However, *in vivo* studies are needed to assess the potential risk of these drugs to induce equine LQTS.

KEYWORDS
Repolarization, potassium channels, hERG, heart, acquired LQTS

1 | INTRODUCTION

Channelopathies have received much attention in human medicine. One of the best investigated channelopathies is the repolarization disorder known as long QT Syndrome (LQTS) (Crotti, Celano, Dagradi, & Schwartz, 2008; Lieve & Wilde, 2015; Roden, 2000). LQTS is associated with severe ventricular arrhythmias, such as torsade de pointes (TdP) that may lead to sudden cardiac death (SCD) (Crotti et al., 2008). As the name implies, LQTS is reflected on the body surface electrocardiogram (ECG) as an increased interval between the Q and the T waves. On a cellular level, this corresponds to a delayed repolarization of the cardiac action potential (AP).

Long QT Syndrome can be inherited or acquired. Inherited LQTS has been associated with mutations in ion channel genes (Tester & Ackerman, 2014), whereas the acquired form has been associated with pathophysiological changes in the heart, such as heart failure, or pharmaceuticals affecting ion channels. Pharmacologically induced LQTS is particularly associated with blockage of the K_V11.1 channel, also known as hERG channel in humans (Kallergis, Goudis, Simantirakis, Kochiadakis, & Vardas, 2012).

Much research has been undertaken in humans to assess the potential of different drugs to induce LQTS (Kallergis et al., 2012), and screening for K_V11.1 blocking activity is required for registration of new drugs (EMEA, 2005; FDA, U.S., 2005). In addition, several cardiac or noncardiac pharmaceutical compounds have been shown to increase QT intervals, and many of these compounds have been withdrawn from human medicine, including cisapride and terfenadine (Redfern et al., 2003; Roden, 2000). However, compounds affecting K_V11.1 channels in humans are still commonly used in veterinarian medicine (Finley, Lillich, Gilmour, & Freeman, 2003). Surprisingly, cases reports of cardiovascular side effects related to pharmacological treatments are sparse in equine medicine. To our knowledge, there are only few case reports on unexplained severe cardiac side effects including dysrhythmias, hypotension, collapse, and death during treatment with trimethoprim–sulfonamide combinations and detomidine (DET) in horses (Dick & White, 1987; Taylor, Rest, Duckham, & Wood, 1988; Van Duijkeren, Vulto, & Van Miert, 1994). However, veterinarian cases may be under-reported, as standardized ECG recordings have only recently become available, and compared to human medicine there has been less attention given to drug-induced sudden cardiac death. It is also important to keep in mind that even though the cases are tragic, they are still rare events. Trimethoprim–sulfamethoxazole combination drugs have been shown to prolong the QT interval in humans (Lopez et al., 1987; Wiener, Rubin, Martinez, Postman, & Herman, 1981), and it is advised to avoid its use in patients with congenital LQTS (Woosley, Heise, & Romero, 2017). Combinations of trimethoprim–sulfonamides are commonly used as broad-spectrum antimicrobials in equine medicine, and as mentioned the use of these antimicrobials in horses has also been associated with cases of cardiovascular side effects after intravenous injection (i.v.), especially in combination with alpha2 agonists (Dick & White, 1987; Taylor et al., 1988; Van Duijkeren, Vulto, Miert et al., 1994). Alpha2 agonists are widely used and considered safe tranquilizers in horses (Daunt & Steffey, 2002; Hubbell, 2007). The described cardiovascular side effects include a decreased heart rate, increased frequency of second-degree atrio-ventricular blocks, passing hypertension followed by a reduced blood pressure, reduced cardiac output, and increased peripheral resistance (Daunt & Steffey, 2002). In addition, as for trimethoprim–sulfonamides combinations, some clinical studies reported that dexmedetomidine, an alpha2 agonist used in humans, may prolong QT intervals and induce TdP (Char et al., 2013; Hammer et al., 2008; Woosley et al., 2017). However, other studies suggest that dexmedetomidine has no effect on QT interval duration (Ergul et al., 2015) and may even reduce the length of the QT intervals, making it beneficial in cases where drugs or other interventions have prolonged the QT interval (Kako, Krishna, Sebastian, Smith, & Tobias, 2015; Kim, Kim, Lee, Kong, & Han, 2014; Kim et al., 2016).

However, aside from this partially conflicting clinical data, few electrophysiological data are available investigating the ion-channel-specific interaction of these drugs and subsequently describing their effect on AP duration. Furthermore, few studies have focused on cardiovascular side effect of drugs in veterinary medicine, even though K_V11.1 channels are involved in the repolarization of the cardiac AP in domestic animals (Finley et al., 2002; Pedersen et al., 2015). Therefore, the aim of this study was to investigate the effect of a trimethoprim–sulfonamides combination and of an alpha2 agonist on the electrophysiological function of equine K_V11.1 channels. Our hypothesis was that the commonly used equine drugs, trimethoprim–sulfadiazine (TMS) and DET will show blocking activity on the equine K_V11.1 channel and therefore may increase the repolarization period, and thereby predisposing horses to LQTS.

2 | MATERIALS AND METHODS

2.1 | Electrophysiology

Equine K_V11.1 channels were cloned form equine heart tissue as described in Pedersen et al. (2015). Briefly, K_V11.1, subcloned into a pXOOM expression vector, was linearized with Xbal (New England biolabs, Ipswich, Ma, USA) and was purified using the High Pure PCR Purification Kit (Roche, Mannheim, Germany). Messenger RNA (mRNA) was purchased by in-vitro transcription with the mMESSAGE mMACHINE kit from Ambion (Austin, Texas, USA) and was purified with the MEGAclear kit (Ambion) according to manufacturer's instructions.

Xenopus laevis oocytes were purchased (EcoCyte Bioscience, Castrop-Rauxel, Germany) and immediately placed into Kulori medium after arrival (4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 90 mM NaCl, 5 mM HEPES, pH:7.4). Oocytes were injected using a microinjector (Nanojet, Drummond Broomal, PA, USA) with 50 nl of a solution containing 20–25 ng/μl of equine K_V11.1 mRNA and incubated for 2–3 days at 19°C. Whole cell currents were recorded by two-electrode voltage-clamp (TEVC) at approximately 19°C in an air-conditioned room under continuous flow of Kulori medium; salt-bridges were used for compounds used in high concentrations to avoid that the alterations in ion concentration and/or osmolality affects the results. The intracellular micropipettes (resistance ranging between 0.5 and 1.5 MΩ) were pulled from glass capillaries (TW 120.3, World precision instruments, USA) on a programmable micropipette puller (P-97, Sutter Instruments, USA) and were filled with 3 M KCl. Voltage clamp experiments were performed using a Clamp Amplifier OC-725 B (Warner Instruments Corp., USA) connected to an Axon Digidata® 1440A digitizer (Axon Instruments, Molecular Devices, USA). Data were sampled at 2 kHz using the pClamp 10.4 acquisition software (Axon Instruments, Molecular Devices, USA), and varying voltage protocols were applied to address different aspects of the channel's kinetic properties (modified from Vandenberg et al. (2012)) and described in details in the electrophysiology protocols section). Results were confirmed in 2–3 different batches of oocytes, and un-injected oocytes were measured in parallel as controls.

2.2 | Drugs

Chemicals were purchased from Sigma-Aldrich (Denmark ApS, Broendby, DK). Pharmaceutical compounds were either

commercially available drugs licensed for use in equine medicine or purchased in pure form from Sigma-Aldrich. The concentrations of the compounds were chosen to cover the plasma concentrations reported after single i.v. injection in pharmacokinetic studies on horses (Gustafsson et al., 1999; Mama, Grimsrud, Snell, & Stanley, 2009). DET (Domosedan® VET, Orion Pharma Animal Health, Copenhagen, DK) was used at a concentration of 10 μM to include the maximal determined plasma concentration of 105.4 ng/ml after a single i.v. injection of 30 μg/kg (Mama et al., 2009). TMS (Norodine® VET, ScanVet Animal Health A/S, Fredensborg, DK) was used at a concentration of 10 mM, calculated based on the sulfadiazine (SULFA) content in the commercial compound. As the ratio of SULFA to TRIM is 5:1 in this commercial compound, this concentration of 10 mM SULFA corresponded to a concentration of 1.73 mM of trimethoprim (TRIM). The used concentration was chosen to include the maximal determined plasma concentration of 69.7 μg/ml of SULFA after the i.v. injection of 12.5 mg/kg (SULFA) of a combination product with SULFA:TRIM 5:1 (Gustafsson et al., 1999). A concentration of 5 mM of SULFA was used in the experiments to assess its specific effect, as solubility issues precluded use at higher concentrations. TRIM was dissolved in 1% DMSO and therefore control experiments with DMSO in a concentration of 1% were performed to exclude the solvent's effects on the channel properties.

2.3 | Electrophysiology protocols and data analysis

A step protocol (holding potential −90 mV, activation steps of 2,000 ms with test potentials varying between −100 and +40 mV in increases of 20 mV, followed by a step to −120 mV for 1,000 ms, see also Figure 2) was used to determine steady-state currents and tail currents. Tail currents were normalized to maximal, current and the relative tail currents were plotted against the test potentials (V_t). A Boltzmann fit ($I = 1/\{1 + e^{[\{V_{50} - V_t\}/k]}\}$) was applied to the relative tail currents, and the voltage required for half activation (V_{50}) and the slope factor (k) were determined. A mono-exponential fit ($I(t) = A\, e^{\{-t/\tau_a\}} + C$) was applied to the first 1,000 ms of the recordings to obtain the time constant of activation (τ_a). Only data determined between −20 mV and +20 mV steps were included as data outside this interval did not converge to a mono-exponential fit.

The proportional reduction in steady-state current in the presence of the chosen compounds was addressed with a pulse protocol (holding potential of −90 mV, activation steps to −20 mV for 2,000 ms, release to −100 mV for 2,000 ms, and back to −90 mV, repeated every 10 s, see Figure 1). Control recordings were obtained after 2 min in Kulori medium with continuous flow, followed by superfusion with the tested compounds for 5 min. Compounds showing a relevant effect on $K_V11.1$ were tested at decreasing concentrations to determine the concentration at which the current was reduced to half of the starting current (IC_{50}).

The rate of activation was addressed using an envelope-of-tails protocol (holding potential −90 mV, activation steps of increasing duration to 0 mV, release to −60 mV for 500 ms, and back to −90 mV, see Figure 3). The tail current, having been normalized to the maximal tail current and referred to as relative tail current, was plotted against duration of activation steps, and the points were fitted with a mono-exponential function.

To address the rate of onset of inactivation, a triple pulse protocol was applied (holding potential at −90 mV, activation step to +20 mV for 600 ms, a short (6 ms) step to −120 mV to release inactivation, and finally the test potentials of 125 ms varying from −20 to +40 mV in 10 mV increments, see also Figure 4). A mono-exponential fit to the current measured during the final test potential allowed extracting the time constant of onset of inactivation (τ_i).

The effect on channel rectification was determined for the different compounds. Therefore, the maximal tail current was measured using a two-pulse protocol (holding potential −90 mV, an activation step to +20 mV for 500 ms, followed by test potentials lasting 1,000 ms and varying between −120 and +40 mV in 20 mV increments, and finally a step back to −120 mV, see also Figure 5) and plotted against the test potential. The slope of a linear fit applied between −120 and −80 mV on the fully activated current–voltage relationship revealed the maximal conductance (G) and the reversal potential (E_{rev}), which were used to calculate the rectification factor ($R = I_{Kv11.1}/G \times n \times [V_t - E_{rev}]$; where n is the activation variable at 40 mV that equals 1.0).

The time constants of deactivation (τ_{dfast}, τ_{dslow}) were determined using a deactivation protocol (holding potential −90 mV, initial step to −80 mV for 1,000 ms, activation step to +20 mV for 1,700 ms, test potentials of 5,000 ms varying from −100 to −70 mV by 10 mV increases, followed by a release to −120 mV for 800 ms). A double-exponential function ($I(t) = \Sigma A_i\, e^{\{-t/\tau_i\}} + C$) was fitted to the 5,000 ms of decaying current.

For each test protocol, three measurements were done, for each of tested compound: Controls in Kulori medium, in the presence of the compound after a 5 min stabilization period and finally after washout for 5 min in Kulori medium (Kulori-WO).

Data are presented as mean ± *SEM*, and figures show representatives data from one recording day. Results in the absence or the presence of the compounds were compared by two-way ANOVA with a Bonferroni correction. Significance values were set at $P < .05$. The analyses were performed with ClampFit 10.4 (Axon Instruments, Molecular Devices, USA) and GraphPad Prism (version 5.01, GaphPad software, San Diego, CA USA, www.graphpad.com).

3 | RESULTS

Equine $K_V11.1$ channels were expressed in *Xenopus laevis* oocytes, and currents were recorded using two-electrode voltage-clamp. A voltage-protocol, as shown in Figure 2a, revealed robust currents activated by depolarization. The activating currents at a 2 s step to −20 mV was observed as well as large tail currents at the −100 mV step, reflecting release from inactivation of the channels, similarly to what has been described for human $K_V11.1$ (Pedersen et al., 2015). The pulse protocol was run continuously with a continuous flow of extracellular solution. After two minutes, control recordings were

obtained, and compounds were applied for 5 min. The effects of DET (10 μm), TMS (10 mM), TRIM (1.73 mM), SULFA (5 mM), and finally 1% DMSO were tested. The relative currents during the activation step were reduced to almost half of the initial currents in the presence of TMS or TRIM (0.49 ± 0.03 and 0.41 ± 0.03, respectively), whereas DET, SULFA, and DMSO showed no reduction in the currents (Figure 1b). The IC_{50} of TMS was 3.74 mM (95% Confidence interval: 2.95–4.73 mM; Figure 1d). The effects of the compounds on the tail currents were similar and confirmed the findings on steady-state currents (Figure 1c).

To investigate in detail how TMS, and its ingredients TRIM and SULFA, affected the function of the equine K_V11.1 channels, the standard step voltage-protocol was applied. The elicited currents were characterized by a rapid activation followed by rapid inactivation that was most prominent at positive potentials. The inactivation was released by a step to −120 mV, resulting in large, slowly deactivating currents. The maximal steady-state current was determined at the end of the voltage-step protocol (as indicated by black arrows in Figure 2, a-I), and when plotting the steady-state current as a function of voltage, the characteristic bell-shaped current–voltage (I/V) curve was determined (Figure 2b/c/d-I). Similarly, the maximal current recorded at the −120 mV tail-step was plotted as a function of voltage of the preceding step (Figure 2b/c/d-II). The tail currents were normalized to the maximal current and plotted as a function of voltage (Figure 2b/c/d-III). Data points were fitted to the Boltzmann equation, and the voltage corresponding to half-maximal activation (V_{50}) was determined. The speed of activation was determined by making mono-exponential fits to the activating currents in Figure 2a-I, and the time constants were determined. Using this standard protocol, we tested the effects of SULFA (5 mM), TRIM (1.73 mM), and TMS (10 mM) (Figure 2b–d). No significant effect of SULFA was found; however, application of TMS and TRIM resulted in a significant inhibition of equine K_V11.1 currents (Figure 2c,d-I and II). For all compounds, the V_{50} of activation significantly shifted left, reflecting that currents activated at less negative potentials. Furthermore, for TMS and TRIM, a reduction in τ_a was seen at −20 and 0 mV, indicating a faster activation of the currents (Figure 2c/d-IV). For the other tested compounds (DET 10 μM, or DMSO 1%), no effect on maximal steady-state and tail current could be measured (data not shown).

Both TMS and TRIM affected time constants of activation on the standard step protocol (Figure 2c/d-IV). During voltage steps of the standard protocol, the current amplitude is determined by the balance between current activation and the onset of inactivation. To separate these two processes, we employed an envelope-of-tail protocol (Figure 3): A step to 0 mV activates and inactivates currents, and the inactivation is then released by a step to −60 mV, this last process reflecting the amplitude of the activated current in the absence of inactivation. Representative recordings are shown in Figure 3a. As expected, both TMS and TRIM reduced current amplitudes. Maximal tail currents were normalized and plotted as function of the length of the preceding activation step (Figure 3b/c/d), revealing the time course of activation in the absence of inactivation. In agreement with the results in Figure 2, TMS induced a significantly faster activation of the channels, whereas TRIM or SULFA had no effect on onset of activation (Figure 3b/c/d).

To address the onset inactivation, the time constant (τ_i) was determined using a triple pulse protocol (Figure 4a-I). K_V11.1 currents were activated by a 20 mV step, the inactivation was released by a very brief step to −120 mV, followed by a series of steps to positive potentials to monitor the onset of inactivation (Figure 4a-II). Both

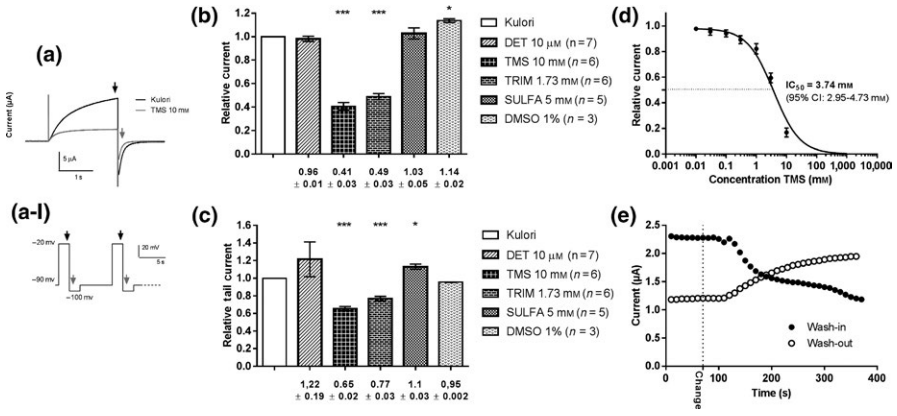

FIGURE 1 Equine K_V11.1 currents recorded in *Xenopus laevis* oocytes showing the effect of detomidine (DET), trimethoprim (TRIM), sulfadiazine (SULFA), trimethoprim–sulfadiazine (TMS), and dimethyl sulfoxide (DMSO). Currents were activated by successive test potentials to −20 mV every 10 s from a holding potential of −90 mV in the absence and the presence of drugs. (a) Representative current measurements in the absence and the presence of TMS. The insert (a-I) represents the applied voltage protocol, the black arrows indicate points of measurements for steady-state current and gray arrows indicate where the tail currents were measured. (b and c) Relative changes in steady-state currents (b) and tail currents (c) expressed as mean ± *SEM* in the presence of DET, TMS, TRIM, SULFA, and DMSO. (d) Increasing concentration of TMS was applied to establish the concentration acquired to reduce current to half of the starting current (IC_{50}). Mean and 95% confidence intervals (CI) are shown, $n = 6$. The dotted line indicates the point on the graph when the current was reduced to half of the starting current (IC_{50}). (e) Representative time course showing the reduction in the current during superfusion with TMS (●), and the increase in the currents during washout of the drug (○). Significant differences in *p*-values are symbolized as *<.05, **<.01, ***<.001

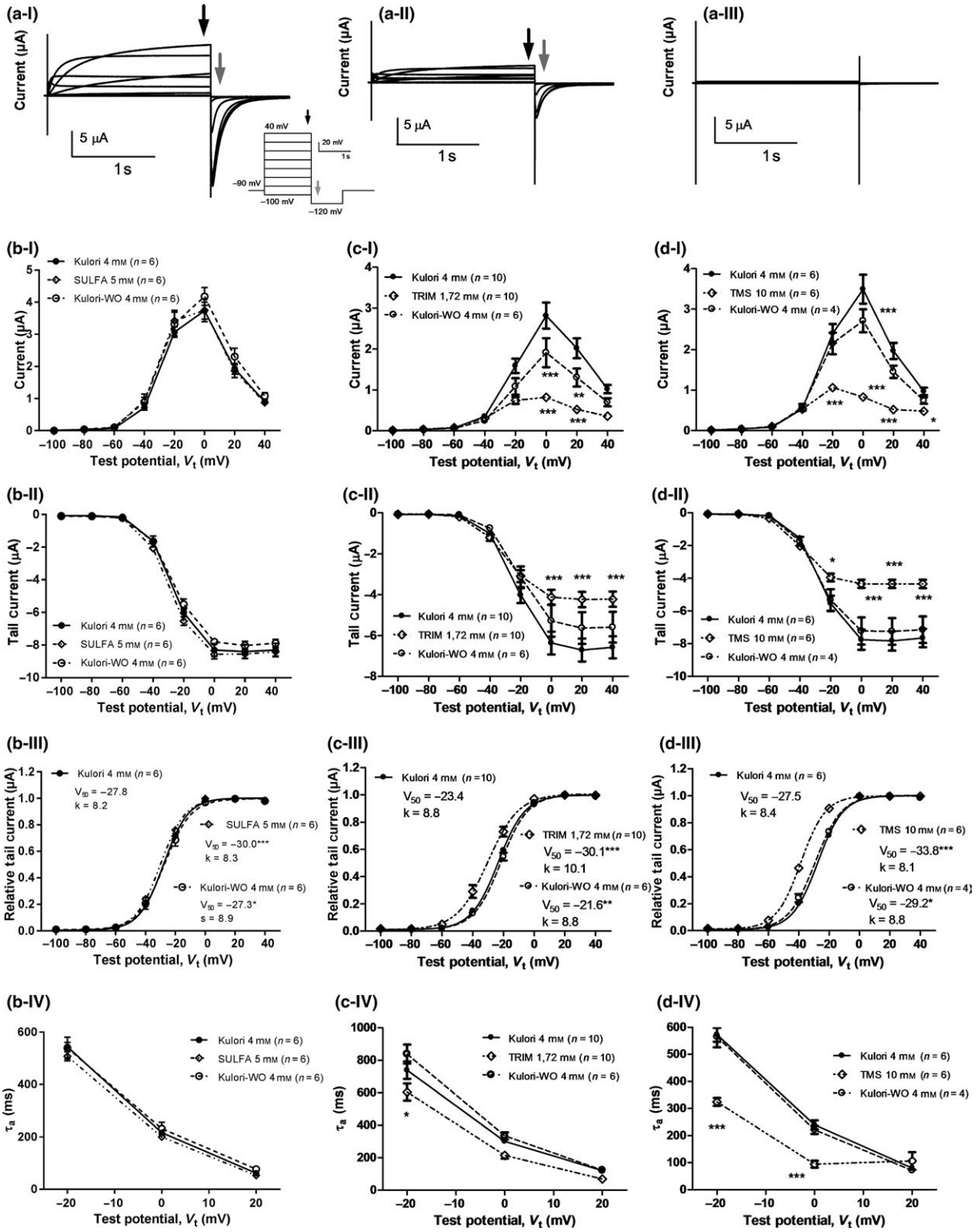

FIGURE 2 Equine $K_v11.1$ currents recorded in *Xenopus laevis* oocytes showing the effect of sulfadiazine (SULFA), trimethoprim (TRIM), and trimethoprim–sulfadiazine (TMS) on maximal steady-state currents, absolute and relative tail currents and, time constants of activation (τ_a). The first row shows representative recordings of equine $K_v11.1$ currents determined in the absence (a-I) and in the presence (a-II) of TMS, and in an un-injected control oocyte (a-III). The insert shows the voltage protocol applied. Black arrows indicate points of measurements of steady-state currents and gray arrows indicate where the tail currents were measured. Current–voltage graphs (I–III) and time constant of activation (IV) for SULFA (b), TRIM (c), and TMS (d) show that TRIM and TMS reduce equine $K_v11.1$ current, whereas SULFA has no effect. Significant differences in *p*-values are symbolized as *<.05, **<.01, ***<.001

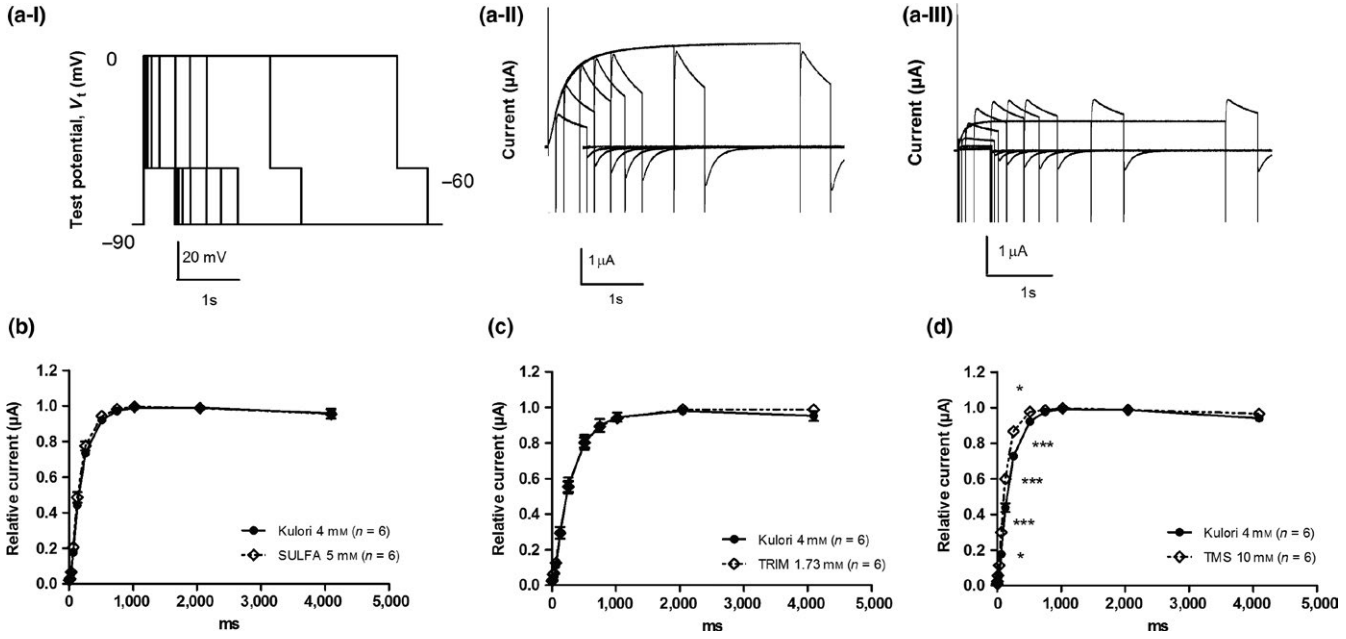

FIGURE 3 The effect of sulfadiazine (SULFA), trimethoprim (TRIM), and trimethoprim–sulfadiazine (TMS) on activation of equine K_V11.1 expressed in *Xenopus laevis* oocytes. An envelope-of-tail protocol was employed. The upper row shows the applied test protocol (a-I) and representative current recordings in the absence (a-II) and the presence (a-III) of trimethoprim–sulfadiazine (TMS). The lower panels show the normalized tail currents plotted as a function of the duration of the preceding activation step in the absence or the presence of drug. These panels show that the activation is faster for equine K_V11.1 channels in the presence of TMS (d), but not in the presence of sulfadiazine (SULFA; (b)) or trimethoprim (TRIM; (c)). Significant differences in *p*-values are symbolized as *<.05, **<.01, ***<.001

TRIM and TMS reduced τ_i, reflecting a faster onset of inactivation; SULFA showed no effect (Figure 4b/c/d).

To further assess the effect of the compounds on inactivation, a rectification protocol was used. Currents are activated by a step to +20 mV, followed by a series of voltage steps at potentials ranging from −120 mV to +40 mV, in 20 mV increments. Maximal currents during the voltage steps were plotted as a function of voltage. From these curves, the reversal potential was determined (the voltage where the graph crosses the zero current line), and the maximal conductance, *G*, was calculated by fitting a straight line to the recordings determined at more negative potentials (Figure 5b/c/d-I). There were no significant effects on the reversal potential of K_V11.1 by SULFA, TRIM, or TMS, suggesting that the compounds do not affect the selectivity of the channels. As expected, TRIM and TMS reduced G but the difference reached statistical significance only for TMS (Figure 5c/d-I). The rectification factors were calculated as described in the Method section and plotted as a function of voltage (Figure 4b/c/d-II). Treatment with TMS and TRIM resulted in a left shift of the rectification factor curve, reflecting that in the presence of the compounds, the inactivation would be more prominent compared to in the absence of compounds at physiologically relevant potentials. SULFA had no effect on inactivation. Deactivation, assessed by the time constants (τ_{dfast}, τ_{dslow}) of a final hyperpolarizing step, was not affected by any of the tested compounds (data not shown).

For all experiments, washout could reverse the effects of the compounds.

4 | DISCUSSION

Trimethoprim–sulfadiazine (TMS) is a commercially available compound that consists of a combination of TRIM and SULFA. Both TMS and TRIM inhibited equine K_V11.1 channels expressed in *Xenopus laevis* oocytes. TMS and TRIM reduced both steady-state and tail currents to almost half of control currents in a concentration of 10 mM and 1.73 mM, respectively. Moreover, the kinetics of activation and inactivation were affected—the presence of TMS or TRIM accelerated the onset of activation and inactivation, and the rectification factors demonstrated that the onset of inactivation is shifted towards more negative values. The faster onset of inactivation as well as the left shift of rectification agrees with the inhibitory effect on K_V11.1 currents (Vandenberg et al., 2012). Furthermore, the reported effects on gating kinetics suggest that the compounds are not just simple pore blockers, but rather interact allosterically with the channel proteins. However, K_V11.1 mutants are required to assess the exact binding site of TRIM and TMS to the channel.

Sulfadiazine (SULFA) alone showed no effect on the function of equine K_V11.1 channels, suggesting that all effects of TMS can be ascribed to TRIM. We also tested DET and found no effect on function of equine K_V11.1.

The results with TRIM and TMS are particularly interesting in the light of two case reports from human medicine, reporting prolonged QT intervals and severe arrhythmias in humans treated with a trimethoprim–sulfamethoxazole combination (Lopez et al., 1987; Wiener et al., 1981). However, in these two case reports, a

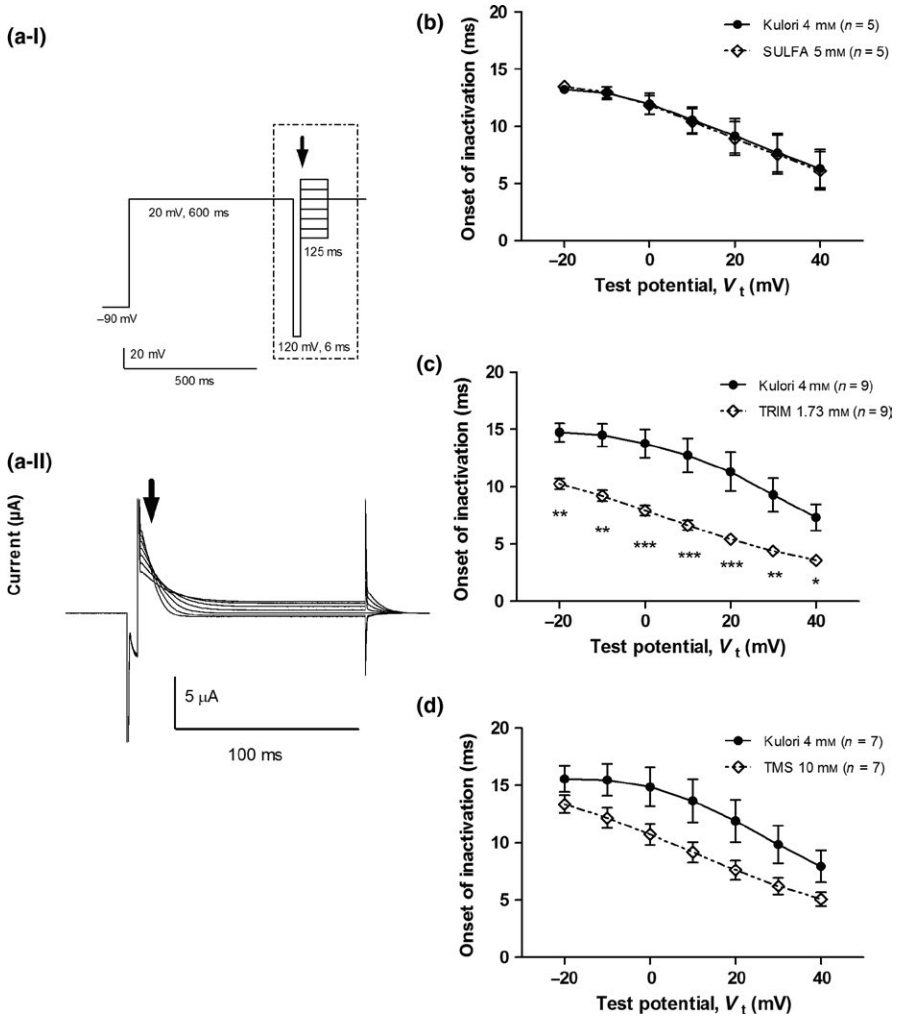

FIGURE 4 The effect of sulfadiazine (SULFA), trimethoprim (TRIM) and trimethoprim-sulfadiazine (TMS) on the onset of inactivation. Currents were activated by the voltage protocol shown in (a-I), and (a-II) shows a representative current recording from the area enclosed by the square on the voltage protocol in panel a-I. Time constants of onset of inactivation were determined by mono-exponential fits to the inactivation currents (indicated by an arrow) and the time constants were plotted as a function of test potentials in the absence or the presence of SULFA (b), TRIM (c), and TMS (d). Significant differences in *p*-values are symbolized as *<.05, **<.01, ***<.001

combination product containing another sulfonamide molecule was used. While we cannot fully exclude that the different structure of that sulfonamide molecule could also affect K_V11.1, the aim of our study was to assess the effect of a commercially available, equine-specific compound on equine-specific potassium channels. Furthermore, in our experimental setup, SULFA was used at a lower concentration (5 mM) than that found in the TMS compound (10 mM), due to poor solubility. Thus, we cannot exclude that SULFA at a higher concentration (as used in the experiments with TMS) may have some effect on equine K_V11.1 channels that could not be reproduced with SULFA alone. Additionally, we found an IC_{50} on K_V11.1 channels of 3.74 mM for the commercially available TMS. This concentration of SULFA (10 mM) in the combination product corresponds to a concentration of 0.65 mM of TRIM. As the expected TRIM plasma concentration after a single i.v. injection is between 0.012 and 0.034 mM (Alexander & Collett, 1975; Gustafsson et al., 1999; Van Duijkeren, Vulto, Sloet van Oldruitenborghoosterbaan et al., 1994), the clinical relevance of this finding is speculative. Even though dosage recommendations vary greatly and up to 30 mg/mg i.v. BID or TID are used (Corley & Hollis, 2009; Haggett & Wilson, 2008), it is unlikely that concentrations as high as the IC_{50} can be achieved. Furthermore, TRIM has a relatively short half-life (2.8–4.6 hr) (Gustafsson et al., 1999; Van Duijkeren, Vulto, Sloet van Oldruitenborghoosterbaan et al., 1994) and is metabolized before it is eliminated, mainly through the kidney (Van Duijkeren, Vulto, Miert et al., 1994). Therefore, accumulation is unlikely, unless severe impairment of renal and/or hepatic function is present. Similarly, due to its high binding to proteins (Gustafsson et al., 1999; Van Duijkeren, Vulto, Miert et al., 1994), higher TRIM plasma concentration could be reached in clinical cases with decreased plasma proteins, but probably not as high to reach the range of the IC_{50}. However, a safety margin of 30-fold between free plasma concentration and IC_{50} has been advocated (Redfern et al., 2003). Furthermore, the best cutoff in this safety margin to predict prolonged QT intervals has been calculated to be a factor of 45 (Gintant, 2011). As the plasma binding of TRIM is between 35 and 50% (Gustafsson et al., 1999; Van Duijkeren, Vulto, Miert et al., 1994), the safety margin for TRIM based on the estimated free plasma concentration seen in horses would be between 30- and 40-fold and therefore just above the recommended safety margin.

Trimethoprim–sulfadiazine and TRIM showed an approximately 50% blocking activity at millimolar concentrations of equine K_V11.1 channels expressed in *Xenopus laevis* oocytes. The compounds could be washed off, and currents recovered almost completely within 5 min. Terfenadine, a pulse-dependent, frequency-independent

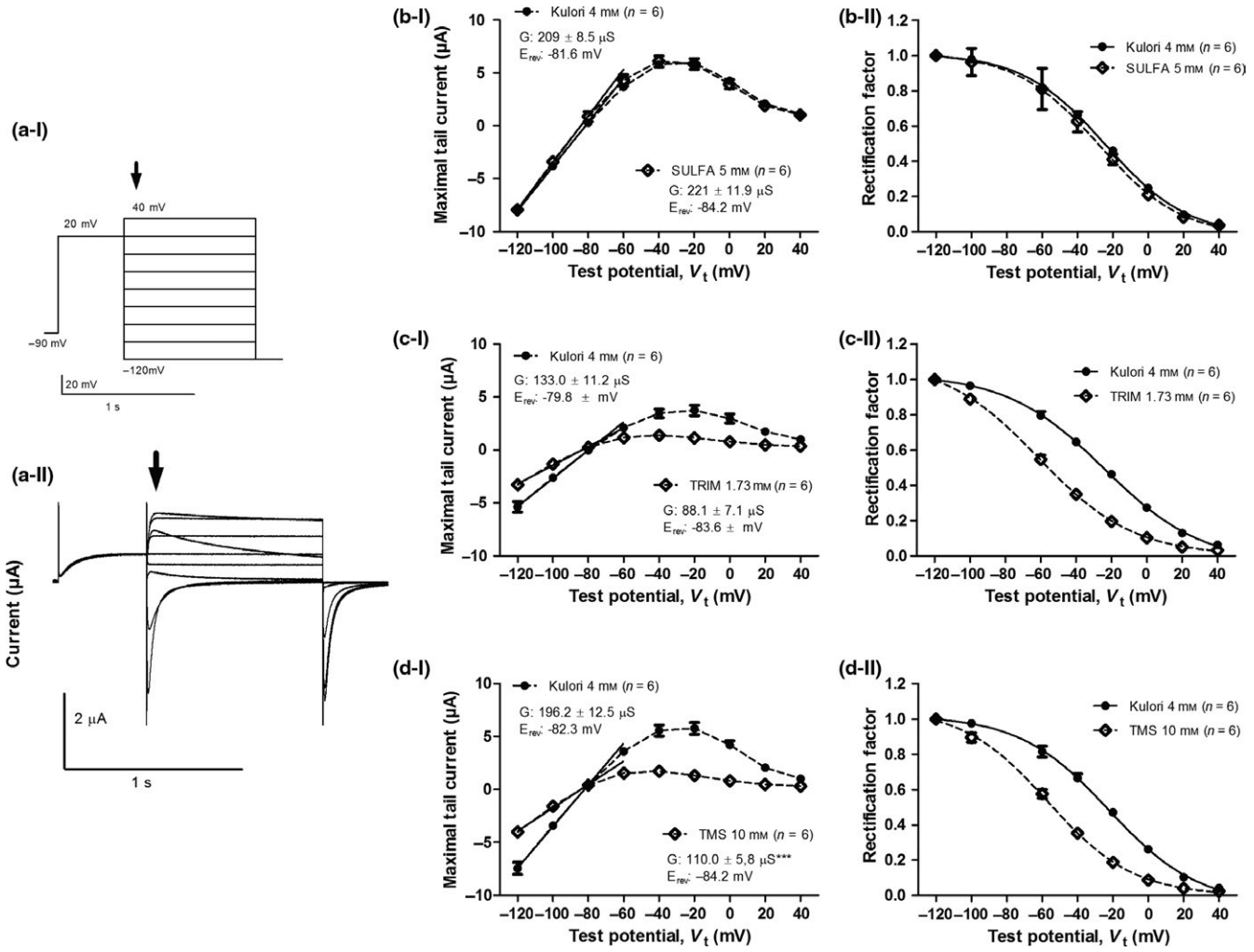

FIGURE 5 The effect of sulfadiazine (SULFA), trimethoprim (TRIM) and trimethoprim-sulfadiazine (TMS) on the voltage dependency of inactivation and channel rectification. The applied voltage protocol and a representative current recording are shown in (a-I) and (a-II), respectively. Black arrows indicate point of measurement of the maximal tail currents. Rectification factors *R* were calculated based on the formula $R = I_{Kv11.1}/G \times n \times [V_t - E_{rev}]$; where, $I_{Kv11.1}$ is the measured tail current, *G* is the maximal conductance, and E_{rev} the reversal potential determined from the maximal current–voltage relationship, V_t represents the applied test potential, *n* is the activation variable at 40 mV that equals 1.0. Significant differences in *p*-values are symbolized as *<.05, **<.01, ***<.001

K_V11.1 blocker with ultra-slow recovery (Stork et al., 2007), and cisapride, a pulse-dependent, frequency-dependent and voltage-dependent K_V11.1 blocker with a fast recovery (Mohammad, Zhou, Gong, & January, 1997; Stork et al., 2007), reduced the measured currents to approximately 80% at micromolar concentrations in a similar TEVC oocyte model (Kamiya, Niwa, Morishima, Honjo, & Sanguinetti, 2008). Furthermore, the IC_{50} for these drugs (Kamiya et al., 2008) were much lower than the IC_{50} for TMS established in our experiments. Ketamine, a partially reversible, open-state voltage-dependent K_V11.1 blocker showed also blocking activity at lower concentrations (Zhang et al., 2013) than those found for TMS or TRIM in our study. Therefore, TRIM and TMS might be considered moderate K_V11.1 blockers with fast recovery, suggesting binding to the closed state.

Trimethoprim was dissolved in 1% DMSO due to poor water solubility. This concentration of DMSO is higher than the normally advised upper limit of 0.3%. To assess the possible effects of this high concentration of DMSO, we ran all the protocols in the presence of 1% DMSO. In our experiments, 1% DMSO showed a slight effect on maximal measured currents with the pulse protocol (Figure 1) but had no effects on the other protocols. Therefore in accordance with a former study (Du et al., 2006), the presence of DMSO did not affect our results.

Detomidine, a wieldy used alpha2 agonist in equine medicine, showed no effect on the function of equine K_V11.1 channels expressed in *Xenopus laevis* oocytes. This result aligns with some clinical studies using dexmedetomidine in human medicine (Ergul et al., 2015), where dexmedetomidine showed no effect on the QT intervals. However, the results from human studies with dexmedetomidine are inconsistent (Kako et al., 2015; Kim et al., 2014, 2016) and the reported prolongations of the QT intervals might be related to other mechanisms. Therefore, interaction with other ion channels should be considered to fully explain the reported effect of alpha2 agonists on repolarization.

In the present study, we investigated the effect of different drugs commonly used in equine medicine on equine K_V11.1

channels heterologously expressed in an oocyte model. Based on the results, we conclude that, as in human medicine, drugs can interact with equine $K_v11.1$ channels and reduce their function in vitro. Most notably, TMS and TRIM reduced the function of equine $K_v11.1$ channels. The IC_{50} was higher than the normally achieved plasma concentration after a single i.v. injection at clinically relevant dosages, but the relation of free plasma concentration to IC_{50} was close to the recommended safety margin for $K_v11.1$ blockers (Gintant, 2011; Redfern et al., 2003). Therefore, TRIM could be classified in the category of drugs associated with isolated reported cases of TdP (Lopez et al., 1987; Wiener et al., 1981) and with a marginal safety margin (Redfern et al., 2003). This conclusion could also explain the relatively low number of reported clinical cases related to TMS and TRIM in equine medicine. $K_v11.1$ channels are only one of the potassium channels involved in AP repolarization in the equine heart (Finley et al., 2003), and blocking of $K_v11.1$ channels might not be the only mechanism leading to LQTS in horses. Therefore, clinical studies measuring the QT intervals during drug administration are needed to assess the clinical relevance of our laboratory findings.

This is the first report showing that drugs known to increase the risk of LQTS in humans can affect the function of heterologously expressed equine potassium channels and thus have the potential to induce severe life-threatening arrhythmias in horses. As several other drugs known to increase the risk of LQTS in humans are commonly used in veterinary medicine, awareness of the possible side effects should be increased and further investigations are needed to establish whether these drugs are safe or may increase the risk of induced LQTS in veterinarian patients.

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CONFLICT OF INTERESTS

The authors have no conflict of interests.

AUTHORS' CONTRIBUTION

DST, MAT, PJP, JKK, RB, KC, DAK contributed to the hypothesis generation and to the experimental design; DST, MAT, VGC, JKK, KC, DKA contributed to the conduction of the experiments, interpretation and analyzing of the data; DST, MAT, VGC, PJP, JKK, RB, KC, DAK contributed to the drafting of the manuscript. All authors have read and approved the final manuscript.

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