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Inhibition of the rapid component of the delayed-rectifier K^+ current by therapeutic concentrations of the antispasmodic agent terodiline

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1 Prolongation of the QT interval and malignant ventricular arrhythmia have been observed in patients administered terodiline for urinary incontinence. Since this adverse reaction might be caused by inhibition of delayed-rectifier K^+ current (I_K), we investigated whether clinically relevant ($\leqslant 10\ \mu M$) concentrations of the drug modify I_K in guinea-pig ventricular myocytes.

2 Myocytes superfused with normal Tyrode's solution were pulsed from −40 mV to more positive test potentials (V) for 0.2–1 s to elicit tail I_K on repolarization and measure tail I_K-V relationships. I_{Kr} was distinguished from I_{Ks} by its sensitivity to the selective blocker E4031.

3 Inhibition of I_{Kr} by 5 μM E4031 was completely occluded by pretreatment with 3 μM terodiline. In addition, action potential lengthening by E4031 in guinea-pig papillary muscles ($29 \pm 3\%$) was abolished ($3 \pm 2\%$) ($P < 0.001$) by terodiline pretreatment.

4 Inhibition of I_{Kr} by terodiline appeared to be voltage-independent, and the parameters of the Hill equation describing the inhibition were $IC_{50} = 0.7\ \mu M$ and $n_H = 1.6$. High concentrations of the drug also affect I_{Ks}; in experiments with K^+-free Tyrode's, 10 μM terodiline inhibited tail I_{Ks} by $27 \pm 3\%$ ($n = 5$) ($P < 0.001$).

5 These data suggest that QT lengthening at therapeutic concentrations of the drug ($\approx 1.5\ \mu M$) is primarily due to inhibition of I_{Kr}. Inhibition of other K^+ currents such as I_{Ks} is likely to be important at higher concentrations.

Keywords: Guinea-pig ventricular myocytes; guinea-pig papillary muscles; K^+ currents; E4031; action potentials

Introduction

Terodiline is an antispasmodic drug with anticholinergic properties (Husted *et al*., 1980), that prior to its withdrawal in 1991, had become the drug of choice in Europe for management of unstable bladder (Langtry & McTavish, 1990; Davies *et al*., 1991). The withdrawal followed reports of adverse reactions that included sinus slowing, atrioventricular block, ventricular fibrillation, and torsades de pointes (Connolly *et al*., 1991; McLeod *et al*., 1991; Stewart *et al*., 1992). Terodiline-induced torsades de pointes is associated with a lengthening of the QT interval and predisposing factors that include heart disease, co-prescription of other drugs, and hypokalaemia (Stewart *et al*., 1992; Thomas *et al*., 1995; Hartigan-Go *et al*., 1996).

A common finding with drugs that cause QT lengthening and torsades de pointes is that they inhibit delayed-rectifier K^+ current (I_K) in ventricular muscle cells (Roden, 1993; Thomas, 1994; Woosley, 1996). There are two types of I_K, rapidly activating I_{Kr} and slowly activating I_{Ks} (Sanguinetti & Jurkiewicz, 1990), and, to date, torsades de pointes has been associated with drugs that selectively block I_{Kr} over I_{Ks} (e.g. quinidine (Balser *et al*., 1991); E4031 and sotalol (Sanguinetti & Jurkiewicz, 1990; Wettwer *et al*., 1992); dofetilide (Carmeliet, 1992; Williams & Beatch, 1997); terfenadine (Ming & Nordin, 1995; Salata *et al*., 1995; Berul & Morad, 1995). The objective of the present study was to evaluate whether terodiline (0.03–10 μM) has a preferential inhibitory action on I_{Kr} in guinea-pig ventricular myocytes.

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Methods

Adult guinea-pigs (ca. 250 g) of either sex were killed by cervical dislocation in accord with national and local regulations on animal experimentation. Hearts were rapidly removed, and ventricular myocytes and papillary muscles prepared as described below.

Ventricular myocytes

Single myocytes were enzymatically isolated from whole ventricles as described previously (Ogura *et al*., 1995). The excised hearts were mounted on a Langendorff column, and retrogradely perfused (37°C) through the aorta with Ca^{2+}-free Tyrode's solution containing collagenase (0.08–0.12 mg ml^{-1}: Yakult Pharmaceutical Co., Tokyo, Japan) for 10–15 min. The cells were dispersed and stored at ≈ 22°C in a high-K^+, low-Na^+ solution supplemented with 50 mM glutamic acid and 20 mM taurine. A few drops of the cell suspension were placed in a 0.3 ml perfusion chamber mounted on an inverted microscope stage, and the chamber was perfused (≈2 ml min^{-1}) with Tyrode's solution. Tyrode's solution contained (in mM) Nacl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 10, and N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) 5 (pH 7.4 with NaOH). In some experiments, KCl was omitted (K^+-free Tyrode's).

Whole-cell membrane currents were recorded using an EPC-7 amplifier (List Electronic, Darmstadt, Germany). Recording pipettes were fabricated from thick-walled borosilicate glass capillaries (H15/10/137, Jencons Scientific Ltd., Bedfordshire, U.K.) and filled with K^+ pipette solution that contained (in mM) KCl 40, potassium aspartate 106, Mg-ATP 1, K_2-ATP 4, ethylene glycol-bis (β-aminoethyl ether)-

N,N,N′,N′-tetraacetic acid (EGTA) 5, and HEPES 5 (pH 7.2 with KOH). The pipettes had resistances of 1.5–2.5 MΩ when filled with pipette solution, and liquid junction potentials between external and pipette-filling solution were nulled prior to patch formation. Series resistance ranged between 3 and 7 MΩ and was compensated by 60–80%. Membrane current signals were filtered at 3 kHz, and digitized with an A/D converter (Digidata 1200A, Axon Instruments, Foster City, CA, U.S.A.) and pCLAMP software (Axon Instruments) at a sampling rate of 8 kHz prior to analysis. The voltage clamp protocols used to measure K^+ currents are fully explained in the Results. All experiments were conducted at 36°C.

Papillary muscles

Excised hearts were placed in oxygenated (95% O_2–5% CO_2) Krebs' solution that contained (mM) NaCl 113.1, KCl 4.6, $CaCl_2$ 2.45, $MgCl_2$ 1.15, $NaHCO_3$ 21.9, NaH_2PO_4 3.48 and glucose 10.0 (pH 7.4). Papillary muscles dissected from right ventricles were mounted in a Perspex bath (0.25 ml volume) that was perfused with warmed (36±0.5°C) Krebs' solution at 4–6 ml min^{-1}. They were stimulated at 1 Hz with 3 ms long pulses of 1.2 times threshold strength *via* a bipolar Ag-AgCl electrode, and equilibrated for 60–90 min prior to data collection. Action potentials were recorded with a high-input impedance amplifier (model 750, WP Instruments, New Haven, CT, U.S.A.) using conventional microelectrodes filled with 3 M KCl (resistance 8–15 MΩ). Action potential duration was measured at 90% repolarization.

Drugs

Terodiline was generously supplied by Sepracor Inc. (Marlborough, MA, U.S.A.) and E4031 by Eisai (Tokyo, Japan). The drugs were freshly dissolved in dimethyl sulphoxide (DMSO) (Sigma Chemical Co., St. Louis, MO, U.S.A.) immediately prior to use. The highest final concentration of DMSO in the superfusate was 0.03%, a concentration that has little effect on membrane currents in guinea-pig ventricular myocytes (Ogura *et al*., 1995).

Statistics

Results are expressed as means±s.e.mean, and single comparisons were made using Student's *t*-test. Differences were considered significant when $P<0.05$.

Results

Measurement and stability of delayed-rectifier K^+ currents

The amplitude of tail I_K at −40 mV was monitored during standard pulsing (−40 to 0 mV for 200 ms at 0.1–0.2 Hz), and the dependence of tail I_K amplitude on test voltage (tail I_K-V) was periodically determined by applying sequences of test depolarizations at 0.1 Hz. Representative results in Figure 1 illustrate that tail I_K during regular pulsing and tail I_K on I-V determinations were generally stable. The shape of the tail I_K-V relationships ranged from near-linear (Figure 1c) to distinctly biphasic (an ascending phase between ca. −30 and +20 mV, and a second one at more positive potentials) (e.g. Figures 3b and 5a). The ascending phase at low voltages is primarily due to tail I_{Kr}, whereas the phase at high voltages reflects the recruitment of tail I_{Ks} (Sanguinetti & Jurkiewicz, 1990; Liu & Antzelevitch, 1995; Heath & Terrar, 1996a,b). The relative amplitudes of these two phases varied from myocyte to myocyte.

Identification of I_{Kr} as the primary I_K target of 3 μM terodiline

Voltage dependence of terodiline-sensitive I_K Figure 2a illustrates that 3 μM terodiline rapidly depressed the amplitude of tail I_K elicited during standard pulsing to 0 mV, and that recovery was gradual when the drug was removed. Subtraction of tail currents (control-terodiline) (Figure 2b), and average tail I_K-V relationships determined with 1000 ms depolarizations (Figure 2c), indicate that nearly all of the terodiline-sensitive tail current occurred in response to depolarization to potentials between −30 and +20 mV. This voltage dependence, and the finding that terodiline-sensitive current was smaller at +60 mV than at −40 mV (i.e. inward-rectification) (Figure 2d), point to I_{Kr} as the primary I_K target of the drug.

Comparison with specific I_{Kr} *inhibitor E4031* A 5 μM concentration of E4031 (IC_{50} ≈0.4 μM: Sanguinetti & Jurkiewicz, 1990) was used to delineate the effects of I_{Kr} inhibition on tail I_K. The results from representative myocytes in Figure 3 indicate that E4031 quickly reduced tail I_K amplitude during regular 200 ms pulsing to 0 mV, and

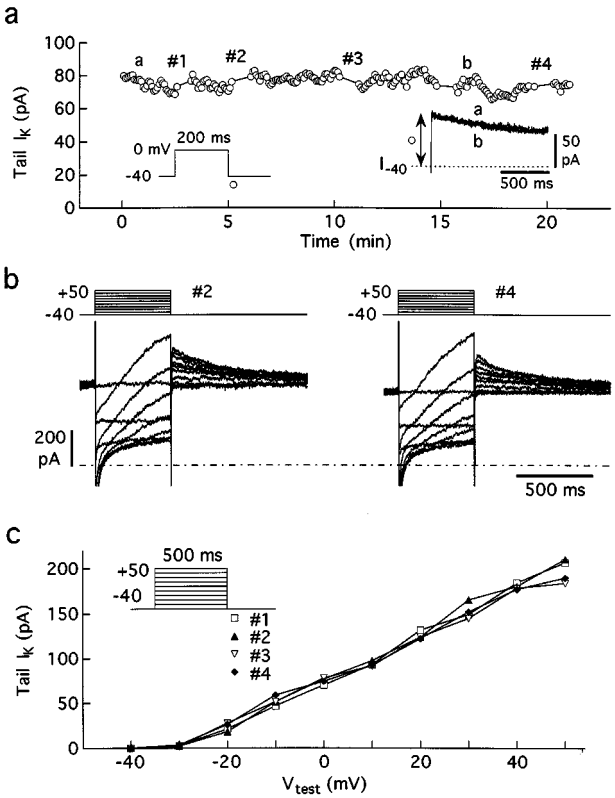

Figure 1 Stability of delayed-rectifier I_K in a representative myocyte bathed in normal Tyrode's solution. The myocyte was held at −40 mV and pulsed for 200 ms to 0 mV at 0.2 Hz except for four tail I_K-V determinations conducted at 0.1 Hz with 500 ms pulses to potentials between −40 and +50 mV. (a) Time course of the amplitude of tail I_K elicited on repolarizations from 200 ms pulses to 0 mV. Tail amplitudes were measured with respect to the current level at −40 mV prior to the pulse (see inset) in this and all other experiments. (b) Current records obtained during the second and fourth I-V determinations at the times indicated in plot a. The horizontal broken line indicates zero current level. (c) Tail I_K-V relationships determined at the times indicated in plot a.

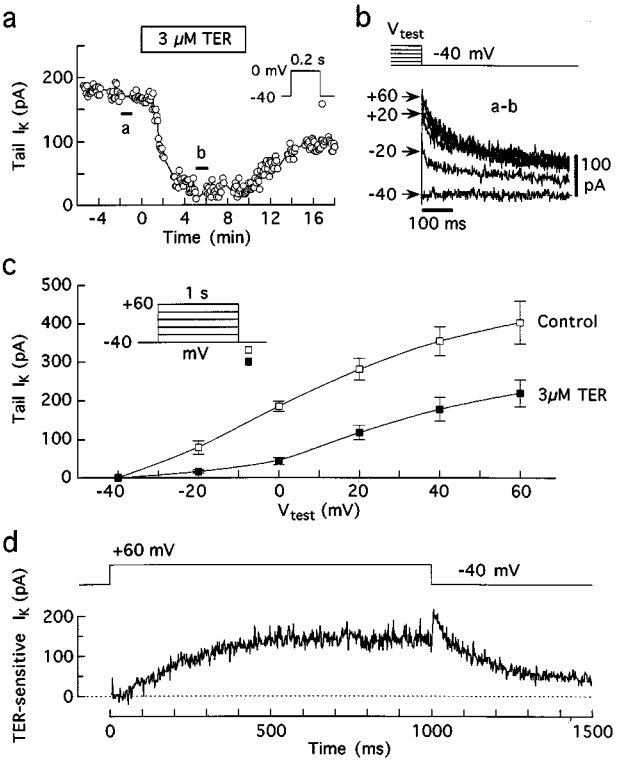

Figure 2 Tail I_K in myocytes treated with 3 μM terodiline. The myocytes were held at -80 mV and depolarized from prepulse -40 to 0 mV for 200 ms at 0.2 Hz except for sequences of 1 s pulses to potentials between -40 and $+60$ mV. (a) Time course of changes in tail current amplitude at -40 mV during regular pulsing to 0 mV. (b) Terodiline-sensitive tail I_K obtained by digital subtraction of records obtained on I-V runs before and during drug treatment (see a). (c) Average tail I_K-V relationships from six myocytes. (d) Terodiline-sensitive I_K at $+60$ mV and -40 mV from one of the myocytes in c.

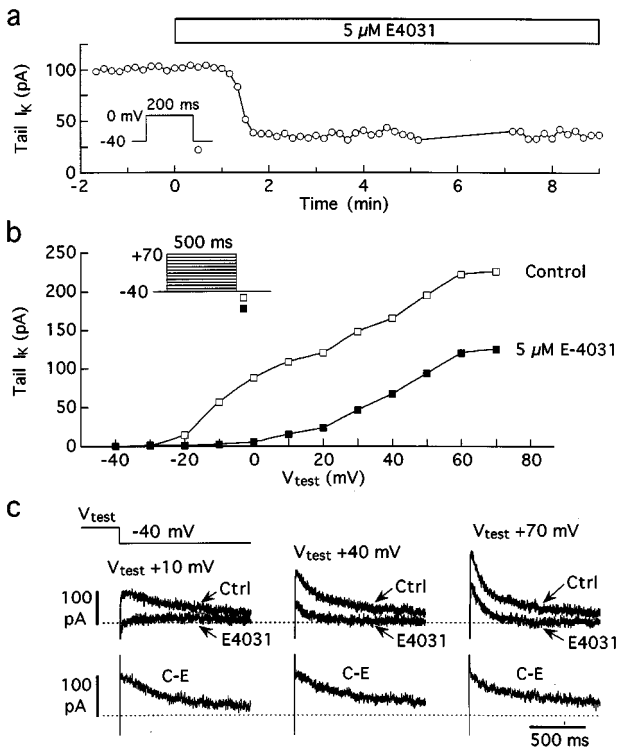

Figure 3 Inhibition of tail I_K by 5 μM E4031. (a) Rapid reduction of tail I_K amplitude during regular pulsing from -40 to 0 mV for 200 ms at 0.2 Hz. (b) Tail I_K-V relationships from 500 ms depolarizations in a different myocyte treated with E4031 for 5 min. (c) Top traces: Records of tail I_K from b. Bottom traces: E4031-sensitive tail I_K (C$-$E) obtained by subtracting the E4031

depressed the tail I_K-V relationship to a greater extent over the test potential range of -30 to $+20$ mV than at higher potentials. Subtraction of tail currents during treatment from corresponding control tails yielded E4031-sensitive tails whose amplitude saturated at lower test potentials.

The voltage dependencies of tail I_K sensitive to 3 μM terodiline and 5 μM E4031 were compared by analysing tail I_K data from myocytes depolarized for 500 ms to potentials up to $+70$ mV. Drug-sensitive tails were computed, and their amplitudes normalized by reference to amplitudes elicited after pulses to $+20$ mV (Figure 4). The normalized E4031 data ($n=12$) are described by the Boltzmann equation with half-activation voltage ($V_{0.5}$) of -12.2 ± 1.6 mV and slope factor (S) of 8.0 ± 0.4 mV, and the terodiline data ($n=10$) are described by $V_{0.5}=-14.5\pm1.7$ mV and $S=8.7\pm1.0$ mV. However, it is important to point out that (unlike E4031 data) the 3 μM terodiline data diverge from the Boltzmann description at potentials above $+20$ mV. This divergence of normalized data was larger with 10 μM terodiline ($n=4$) and absent with 0.3 μM terodiline ($n=4$) (Figure 4). These findings suggest that higher concentrations of terodiline inhibit the I_K activated at potentials above $+20$ mV (i.e. I_{Ks}) (see last section of Results).

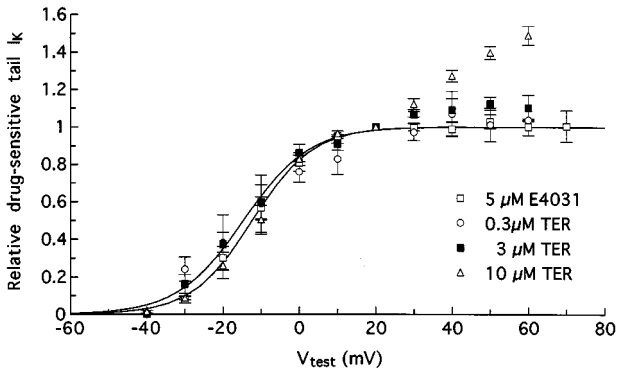

Figure 4 The voltage dependencies of tail I_K sensitive to terodiline and 5 μM E4031. Plot of the differences between control and treatment tail currents measured from myocytes treated with either 3 μM terodiline ($n=10$) or 5 μM E4031 ($n=12$). The data are described by the Boltzmann equation, $I/I_{+20}=1/[1+\exp((V_{0.5}-V_t)/S)]$, where I is the tail amplitude after a 500 ms pulse to V_t (activating test pulse), I_{+20} is the tail amplitude after a 500 ms pulse to $+20$ mV, $V_{0.5}$ is the test voltage eliciting half-activation, and S is the slope factor. The $V_{0.5}$ and S values are -12.2 ± 1.6 and 8.0 ± 0.4 mV, respectively, for the E4031 data, and -14.5 ± 1.7 mV and 8.7 ± 1.0 mV, respectively, for the 3 μM terodiline data. Also shown are normalized data from experiments with 0.3 μM ($n=4$) and 10 μM ($n=4$) terodiline.

Occlusion of E4031 action by terodiline The data in Figure 4 indicate that the voltage dependence of tail I_K sensitive to 3 μM terodiline closely resembles that of tail I_K sensitive to 5 μM E4031, whereas the data in Figure 2 suggest (but do not prove) that 3 μM terodiline almost completely inhibits I_{Kr}. To investigate the latter issue in a more rigorous manner, myocytes were pretreated with 3 μM terodiline and the extent

of I_{Kr} inhibition was assessed from responses to subsequent 5 μM E4031. The tail I_K-V relationships in Figure 5a illustrate that inhibition of tail I_K by 5 μM E4031 was almost completely occluded by 3 μM terodiline. In five experiments of this type, the amplitude of tail I_K elicited by 500 ms pulses to 0 mV was reduced to 19.1$\pm$6.5% by terodiline, while with the addition of E4031 it was reduced to 19.2$\pm$5.1% (not significant). Similarly, with pulses to +60 mV terodiline reduced tail I_K to 57.7$\pm$5.9% control while with addition of E4031 it was reduced to 52.8$\pm$7.3% (not significant).

Inhibition of I_{Kr} by E4031 lengthened the action potential in guinea-pig papillary muscle by 24% (Sanguinetti & Jurkiewicz, 1990), and similar-sized lengthenings have been measured in myocytes isolated from guinea-pig (Sanguinetti *et al.*, 1991) and human (Li *et al.*, 1996) ventricular tissue. We found that pretreatment of guinea-pig papillary muscles with 5 μM terodiline reduced action potential lengthening by 5 μM E4031 from 29$\pm$3% (n=4) to 3$\pm$2% (n=4) (P<0.001) (e.g. Figure 5b).

Dependence of I_{Kr} *inhibition on terodiline concentration*

Figure 6 relates terodiline concentration to inhibition of the amplitudes of I_K tails measured after 500 ms depolarizations to 0 mV. There was no inhibition with 0.03 μM terodiline, and 80–90% inhibition with 3, 5 and 10 μM drug. These data were converted into inhibition of I_{Kr} by using the reduction of tail amplitude by 5 μM E4031 (to 17.7$\pm$2.4% control, n=17) as an indicator of full inhibition of I_{Kr}, i.e. I_{Kr} (% control)=(100−17.7)/{1+([terodiline]/IC_{50})}^{n_H}+17.7. The curve in Figure 6 has IC_{50}=0.68 μM, and Hill coefficient n_H=1.63.

Inhibition of I_{Ks} *by terodiline*

The most likely explanation for the lack of saturation of the inhibition of tail I_K with 3 and 10 μM terodiline (see Figure 4) is that these concentrations inhibit the I_{Ks} that is activated at higher positive potentials. To examine this possibility, myocytes were pretreated with 5 μM E4031 and then exposed to 3 μM terodiline. I_K tail after 500 ms pulses to 0 mV was reduced to 16.2$\pm$1.9% control by E4031, and to a similar 18.0$\pm$3.0% by E4031+terodiline (n=4). Tail I_K after 500 ms pulses to +60 mV was reduced to 52.7$\pm$4.3% control by E4031 alone, and to 42.5$\pm$6.6% by E4031+terodiline. The latter difference is not significant, but the trend suggests that 3 μM terodiline may have a minor inhibitory effect on I_{Ks}.

A more definitive answer on whether high concentrations of terodiline affect I_{Ks} was sought by applying 10 μM terodiline to myocytes in which I_{Kr} was suppressed, and I_{Ks} enhanced, by removal of external K^+ (Sanguinetti & Jurkiewicz, 1992; Liu & Antzelevitch, 1995; Gintant, 1996), addition of 0.4 mM Cd^{2+} (Daleau *et al.*, 1997), and lengthening of test depolarizations to

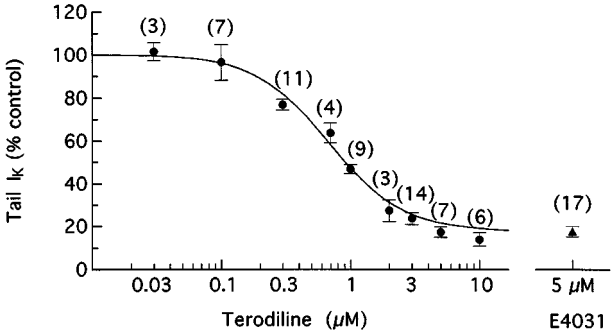

Figure 6 Dependence of I_{Kr} inhibition on terodiline concentration. Myocytes were depolarized to 0 mV for 500 ms and tail currents were measured on repolarization to −40 mV before and during treatment with a single concentration of the drug. The Hill equation fitting the data is I_{Kr} (%control)=(100−17.7)/{1+([terodiline]/IC_{50})}^{n_H}+17.7, where 17.7 (the average size (% control) of tail I_K recorded from 17 myocytes treated with 5 μM E4031 (triangle)) defines the maximal inhibitory response. The IC_{50} for terodiline is 0.68 μM, and the Hill coefficient n_H is 1.63. Number of myocytes in parentheses.

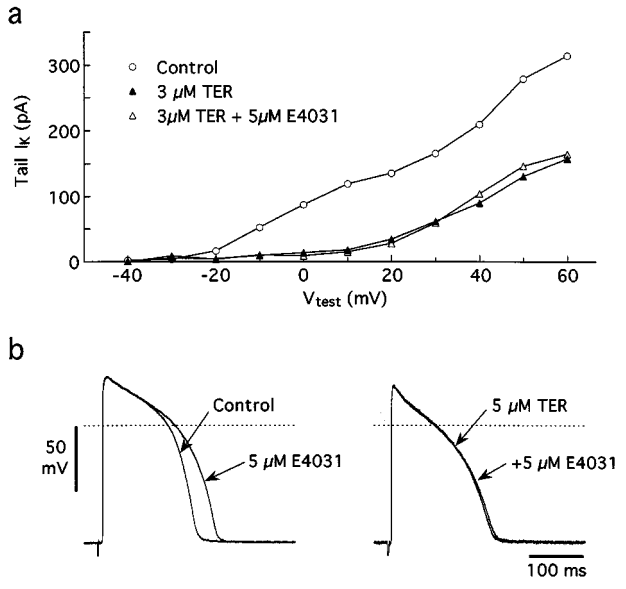

Figure 5 Occlusion of E4031 action by terodiline. (a) Tail I_K-V relationships (500 ms depolarizations) obtained from a myocyte before, 6 min after addition of 3 μM terodiline, and 5 min after further addition of 5 μM E4031. (b) (i) Lengthening of the action potential by 20 min treatment with 5 μM E4031, and (ii) occlusion of this action after a 45 min pretreatment with 5 μM terodiline. The action potentials were recorded from guinea-pig papillary muscles stimulated at 1 Hz.

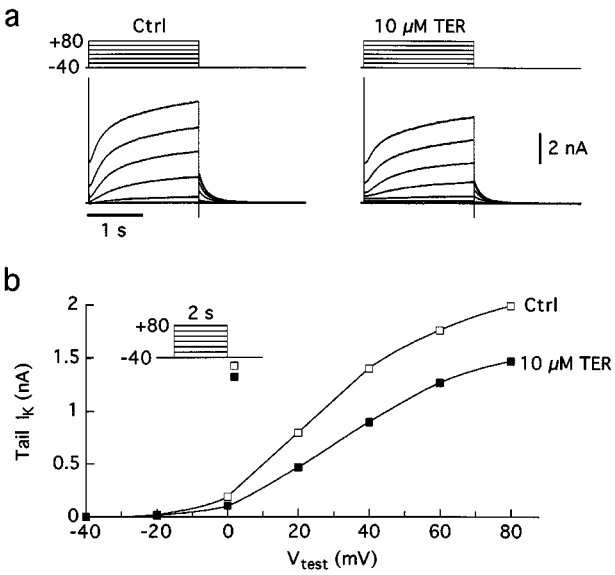

Figure 7 Effects of 10 μM terodiline on activating I_K and tail I_K under conditions designed to minimise I_{Kr} and augment I_{Ks}. Currents were elicited by 2 s depolarizations from −40 mV in a myocyte superfused with K^+-free Tyrode's solution that contained 0.4 mM Cd^{2+}. (a) Records obtained before and 5 min after addition of terodiline. (b) Plot of tail I_{Ks} amplitude (−40 mV) as a function of activating test voltage.

2 s (Sanguinetti & Jurkiewicz, 1990). Under these conditions, large I_{Ks} was activated during pulses from −40 mV to potentials above 0 mV, and large tail I_{Ks} accompanied repolarization (Figure 7a,b). Activating I_{Ks} and tail I_{Ks} were equally inhibited by 10 μM terodiline; on pulses to +60 mV, activating I_{Ks} was reduced to 77±3% control and tail I_{Ks} was reduced to 73±3% ($n=5$ myocytes).

Discussion

The effects of 0.03 to 10 μM terodiline on guinea-pig ventricular I_K have been evaluated, and the results indicate that the drug preferentially inhibits the I_{Kr} component of the current. Identification of this mechanism sheds light on the cardiotoxicity of the compound, and helps clarify recent findings of Hayashi *et al*. (1997) on guinea-pig ventricular myocytes. These investigators measured tail I_K at −40 mV after 500 ms depolarizations to positive potentials, and found that 10 μM terodiline reduced the amplitude by ca. 20%. In the absence of further information, they suggested that the drug might inhibit both I_{Kr} and I_{Ks}. The data presented here indicate that the drug can completely inhibit I_{Kr} with an IC_{50} about 20 times lower than that for I_{Ks}. We discuss the evidence leading to this conclusion, and briefly relate the findings to some of the adverse effects of the drug on cardiac function.

Preferential inhibition of I_{Kr}

E4031 (5 μM) has been widely used as a selective inhibitor of I_{Kr} in guinea-pig ventricular myocytes (Sanguinetti & Jurkiewicz, 1990; Wettwer *et al*., 1992; Heath & Terrar, 1996a,b). Accordingly, we identified I_{Kr} as the tail I_K component sensitive to 5 μM E4031. The normalized E4031-sensitive tail I_K-V relationship had $V_{0.5}=-12.2$ mV and $S=8.0$ mV, in good agreement with recent $V_{0.5}$ and S estimates for I_{Kr} in these myocytes ($V_{0.5}=-14.3$ to −18.7 mV; $S=6.6$ to 8.7 mV) (Heath & Terrar, 1996a) and in human atrial and ventricular myocytes ($V_{0.5}=-14.0$ mV; $S=6.5$ to 7.7 mV) (Wang *et al*., 1994; Li *et al*., 1996).

The similarity of the voltage dependence of tail I_K sensitive to ⩽3 μM terodiline ($V_{0.5}=-14.5$ mV; $S=8.7$ mV) to that of tail I_{Kr} defined by 5 μM E4031 action, is strong evidence for the view that terodiline inhibits I_{Kr}. Further support was obtained from tests on occlusion of E4031 action by 3–5 μM terodiline. The drug fully occluded inhibition of tail I_K by 5 μM E4031, and also occluded lengthening of muscle action potentials by 5 μM E4031. These findings indicate that 3–5 μM terodiline completely inhibits I_{Kr}, and a fit of these and other data with a Hill equation places the IC_{50} near 0.7 μM.

The results include several sets of measurements that bear on the selectivity of terodiline for I_{Kr} over I_{Ks}. The most convincing evidence for inhibition of I_{Ks} was obtained from myocytes studied under conditions that were designed to minimise I_{Kr} and augment I_{Ks} (K^+-free Cd^{2+} Tyrode's; 2 s long activating pulses). In these cells, 10 μM terodiline inhibited tail I_{Ks} by 27±3% ($n=5$). By comparison, 0.3–0.5 μM terodiline was sufficient to inhibit tail I_{Kr} to a similar extent. Although the measurements on I_{Ks} were performed under conditions where $I_{Ca,L}$ was depressed, this should not invalidate the comparison because inhibition of $I_{Ca,L}$ with nisoldipine has no effect on I_{Kr} in guinea-pig ventricular myocytes (Daleau *et al*., 1997).

Inhibition of I_{Ks} by 10 μM terodiline explains why the voltage dependence of the inhibition of normalized tail I_K (larger after 500 ms pulses to high positive potentials than after pulses to +20 mV) diverged from the saturating-type relationship found with E4031 (Figure 4). A divergence was also evident in the case of 3 μM terodiline; however, it was 2–4 times smaller than with 10 μM, suggesting a rather weak inhibitory effect of this concentration on I_{Ks}. In line with this trend, there was no indication of any inhibition of I_{Ks} by 0.3 μM terodiline, i.e. the Boltzmann description for inhibition of tail I_K was indistinguishable from the E4031 description. This similarity also suggests that, like E4031 (Sanguinetti & Jurkiewicz, 1990), but unlike other I_{Kr} inhibitors such as dofetilide (Carmeliet, 1992) and WAY 123,398 (Spinelli *et al*., 1993), inhibition of I_{Kr} by terodiline is voltage-independent.

Relationship to the cardiotoxicity of terodiline

Adverse effects of terodiline on the heart include bradycardia, atrioventricular disturbances, QT prolongation, and malignant ventricular tachycardia (torsades de pointes) (Connolly *et al*., 1991; McLeod *et al*., 1991; Stewart *et al*., 1992; Pressler *et al*., 1995). Since terodiline inhibits cardiac $I_{Ca,L}$ (Hayashi *et al*., 1997, Ogura *et al*. submitted; Pressler *et al*., 1995), it is possible that this is the sole mechanism by which the drug causes bradycardia and atrioventricular block. However, inhibition of I_{Kr} by terodiline may contribute in both a direct and an indirect manner if, as in rabbit nodal tissues (Shibasaki, 1987; Verheijck *et al*., 1995; Ono & Ito, 1995; Lei & Brown, 1995), partial inhibition of I_{Kr} in human nodal tissues lengthens the action potential, slows the pacemaker rate, and lowers the maximum diastolic potential. Since the inhibition of $I_{Ca,L}$ by terodiline is voltage-dependent (Ogura *et al*. unpublished data), I_{Kr}-related depolarization of diastolic potential could potentiate terodiline-induced inhibition of $I_{Ca,L}$ and consequent disturbance of nodal pacing and conduction. In turn, the bradycardia may be an important element in QT lengthening and ventricular tachyarrhythmias (Stewart *et al*., 1992; Thomas *et al*., 1995). Based on the results presented here, the primary mechanism underlying the QT lengthening is a lengthening of the ventricular action potential due to inhibition of I_{Kr}. QT lengthening increases with increasing plasma concentration of terodiline (Thomas *et al*., 1995), and a concentration of 9.3 μM has been measured in one adversely affected patient (Connolly *et al*., 1991). In such cases, it is likely that concentration-dependent inhibitions of other K^+ currents, such as I_{Ks}, contribute to the QT lengthening.

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