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Inhibition of cardiac inward-rectifier K^+ current by terodiline

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Abstract

The antispasmodic agent terodiline has cardiotoxic effects that include QT lengthening. To determine whether inhibition of inwardly-rectifying K^+ current (I_{K1}) might be a factor in the cardiotoxicity, we measured I_{K1} in guinea pig ventricular myocytes. Terodiline reduced outward I_{K1} with an IC_{50} of 7 μM; maximal reduction was 60% with 100–300 μM concentration. Inhibition was independent of current direction, and persisted after removal of the drug. Terodiline (3–5 μM) lengthened action potentials in guinea pig papillary muscles by ca. 10%, primarily by slowing phase 3 repolarization; higher concentrations abbreviated the plateau and markedly slowed late repolarization. Terodiline washout provoked an extra lengthening, consistent with persistent inhibition of I_{K1} and rapid recovery of net inward plateau current. The results suggest that inhibition of I_{K1} is a likely factor in the cardiotoxicity of the drug.

Keywords: Papillary muscle; Action potential; Ventricular myocyte guinea pig; K^+ current; Ca^{2+} current

1. Introduction

Terodiline is an antispasmodic agent (Husted et al., 1980; Noronha-Blob et al., 1991) that was the drug of choice in Europe for the treatment of unstable bladder disorders prior to its withdrawal in 1991 (Langtry and McTavish, 1990). The withdrawal was provoked by reports of cardiotoxic effects that included QT prolongation and torsades de pointes ventricular tachycardia (Connolly et al., 1991; Stewart et al., 1992; Thomas et al., 1995). However, terodiline is still authorized for clinical investigation, and derivatives are under development for possible clinical use (e.g., Take et al., 1996).

The cause of the lengthening of the QT interval by terodiline is unknown, but is most likely prolongation of the ventricular action potential following inhibition of the K^+ currents that govern termination of the action potential plateau and subsequent repolarization (Thomas, 1994). In this regard, two types of K^+ current that need to be considered are the delayed-rectifier (I_K) and the inward-rectifier (I_{K1}) (Carmeliet, 1993). A recent study on guinea pig ventricular myocytes indicates that I_K is reduced by ca. 20% by 10 μM terodiline (Hayashi et al., 1997), but there is no information on possible inhibition of outward I_{K1} by the drug. Outward I_{K1} drives repolarization at potentials negative to −10 mV, and inhibition of the current lengthens the cardiac action potential (Isenberg, 1976; Giles and Imaizumi, 1988; Carmeliet, 1993) and may dispose towards ventricular arrhythmia (Hart, 1994; Tomaselli et al., 1994).

The present study was performed to determine the effects of terodiline on outward I_{K1} in guinea pig ventricular myocytes. The results indicate that terodiline has a concentration-dependent long-lasting inhibitory effect that promotes action potential lengthening.

2. Materials and methods

Adult guinea pigs (ca. 250 g) of either sex were killed by cervical dislocation in accord with Canadian and University regulations on animal experimentation. Hearts were

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rapidly removed, and ventricular myocytes and papillary muscles prepared as described below.

2.1. Ventricular myocytes

Single ventricular myocytes were enzymatically isolated from the hearts of guinea pigs 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 0.08–0.12 mg/ml collagenase (Yakult, 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. Cells were placed in a 0.3-ml perfusion chamber mounted on an inverted microscope stage, and the chamber was perfused (~2 ml/min) 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 HEPES 5 (pH 7.4 with NaOH). In some experiments, KCl was omitted and 0.2 mM Cd^{2+} added (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, Bedfordshire, UK) and filled with K^+ pipette solution that contained (in mM): KCl 40, potassium aspartate 106, Mg–ATP 1, K_2–ATP 4, EGTA 5, and HEPES 5 (pH 7.2 with KOH). In some experiments, K^+ was replaced by Cs^+. 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, USA) and pCLAMP software (Axon Instruments) at a sampling rate of 8 kHz prior to analysis. The voltage clamp protocols used to measure membrane currents are fully explained in the Results. Experiments were conducted at 36°C.

2.2. Papillary muscles

Excised hearts were placed in oxygenated (95% O_2–5% CO_2) Krebs' solution that contained (in 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 (pH 7.4). Papillary muscles were mounted in a bath perfused with warmed (36 ± 0.5°C) Krebs' solution, stimulated at 1 Hz, 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, USA) using conventional microelectrodes filled with 3 M KCl (resistance 8–15 MΩ). Contraction was measured in some muscles as previously described (Ogura et al., 1995).

2.3. Drugs

Terodiline (Sepracor, Marlborough, MA, USA), daidzein (Calbiochem, LaJolla, CA, USA), and E4031 (Eisai, Tokyo, Japan) were freshly dissolved in dimethyl

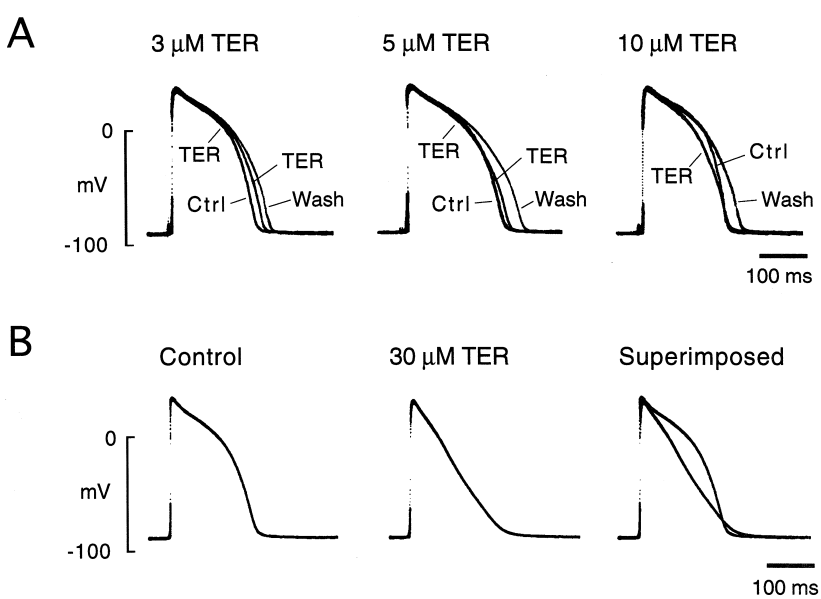

Fig. 1. Action potentials recorded from guinea pig papillary muscles before, during and after treatment with different concentrations of terodiline. Stimulation rate 1 Hz. (A) Superimposed records obtained from muscles exposed to 3, 5 or 10 μM terodiline. Exposures lasted for 40 to 60 min, and wash records were obtained 10 to 20 min later. (B) Action potentials recorded from a muscle treated with 30 μM terodiline for 30 min.

sulfoxide (DMSO) (Sigma, St. Louis, MO, USA); the final concentration of DMSO in the bathing solution was ≤ 0.05%. These concentrations of DMSO have been shown to have no significant effect on membrane currents in guinea pig ventricular myocytes (Ogura et al., 1995).

2.4. Statistics

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

3. Results

3.1. Effects of terodiline on action potentials in guinea pig papillary muscles

The records in Fig. 1 illustrate that terodiline has complex concentration-dependent effects on the action potential in guinea pig papillary muscles. Concentrations below 10 μM reduced the plateau voltage by several millivolts, and slowed phase 3 repolarization; on removal of the drug, the plateau recovered and the action potential lengthened even further (Fig. 1A). The post-drug lengthening was not due to a delayed action of the drug because longer (up to

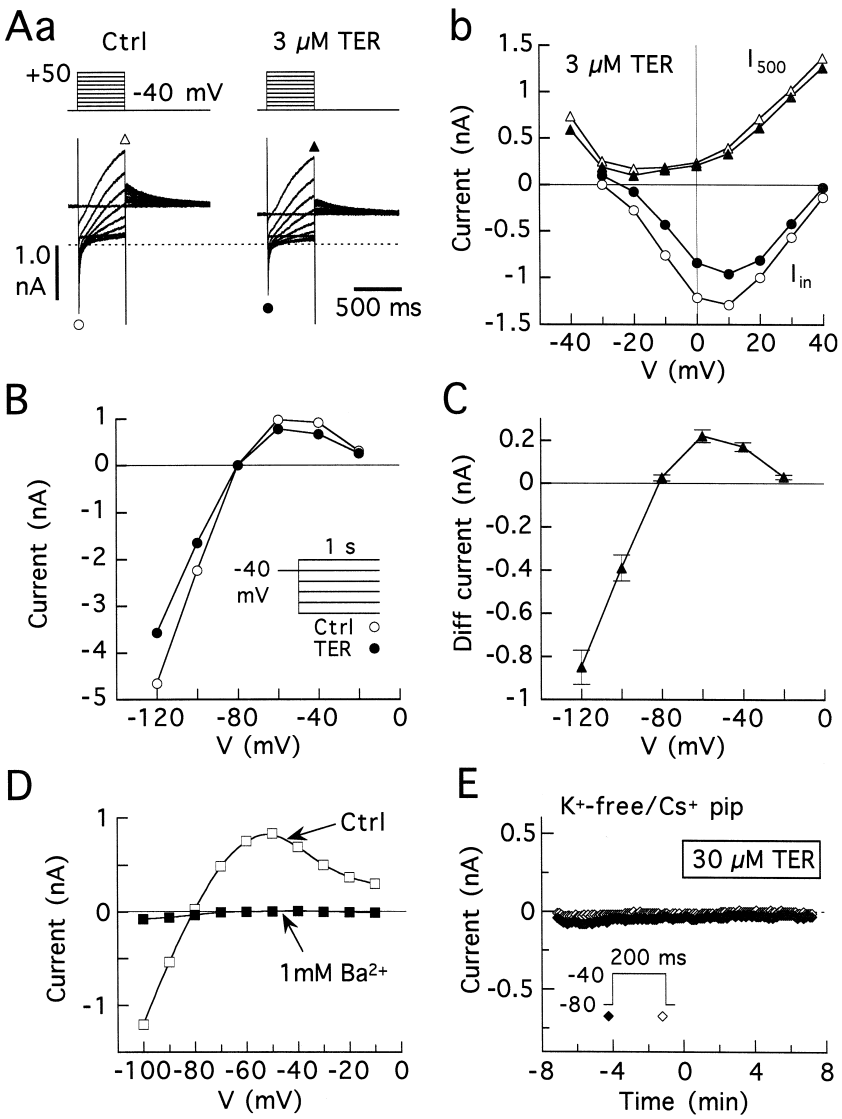

Fig. 2. Membrane currents in guinea pig ventricular myocytes treated with 3 μM terodiline. (A) a: Currents elicited by 500-ms depolarizations from prepulse −40 before (control) and 9 min after addition of the drug (TER). b: *I–V* relationships from a. (B) *I–V* relationships determined from 500-ms depolarizing and hyperpolarizing pulses from prepulse −40 mV before and 12 min after addition of terodiline. (C) Mean *I–V* relationships of the difference current (control minus terodiline) measured from six myocytes investigated as in (B). (D and E) Control experiments on the validity of measuring late outward current at ca. −40 mV to evaluate terodiline action on outward-directed I_{K1}. Late current at ca. −40 mV was almost completely suppressed by 3-min treatment with 1 mM Ba^{2+} (D), and insensitive to terodiline under I_{K1}-inhibitory conditions (K^+-free solution, Cs^+ dialysate) (E).

120 min) exposures than those (40–60 min) shown here were uneventful (5, 10 μM; $n = 5, 3$).

Concentrations of terodiline ≥ 10 μM strongly depressed the plateau, markedly slowed late repolarization, and sometimes reduced the resting potential. For example, records from a representative muscle treated with 30 μM terodiline indicate a 40% shortening at 0 mV and a 5% lengthening at −80 mV, with little change in resting potential. However, depolarizations up to 5 mV were measured in other muscles exposed to 30 μM terodiline, and average resting potential declined from -88.8 ± 0.7 to -85.7 ± 1.0 mV ($n = 9$) ($p < 0.05$). In five muscles treated with 300 μM drug, resting potential declined from -88.9 ± 0.7 to -82.2 ± 1.6 mV ($n = 5$) ($p < 0.05$).

Terodiline had a concentration-dependent inhibitory effect on the force of contraction. In muscles driven at 1 Hz for 45 min, developed force was unaffected by 0.2 μM drug ($97 \pm 3\%$ pre-drug control, $n = 5$), but reduced to $74 \pm 5\%$ control ($n = 4$, $p < 0.01$) by 3 μM drug, and to $34 \pm 6\%$ ($n = 5$, $p < 0.01$) by 30 μM drug.

3.2. Multiple effects of 3 μM terodiline on membrane currents in guinea pig ventricular myocytes

Fig. 2A depicts the effects of an 8-min exposure to 3 μM terodiline on membrane currents elicited by 500-ms depolarizations applied from prepulse −40 mV to more

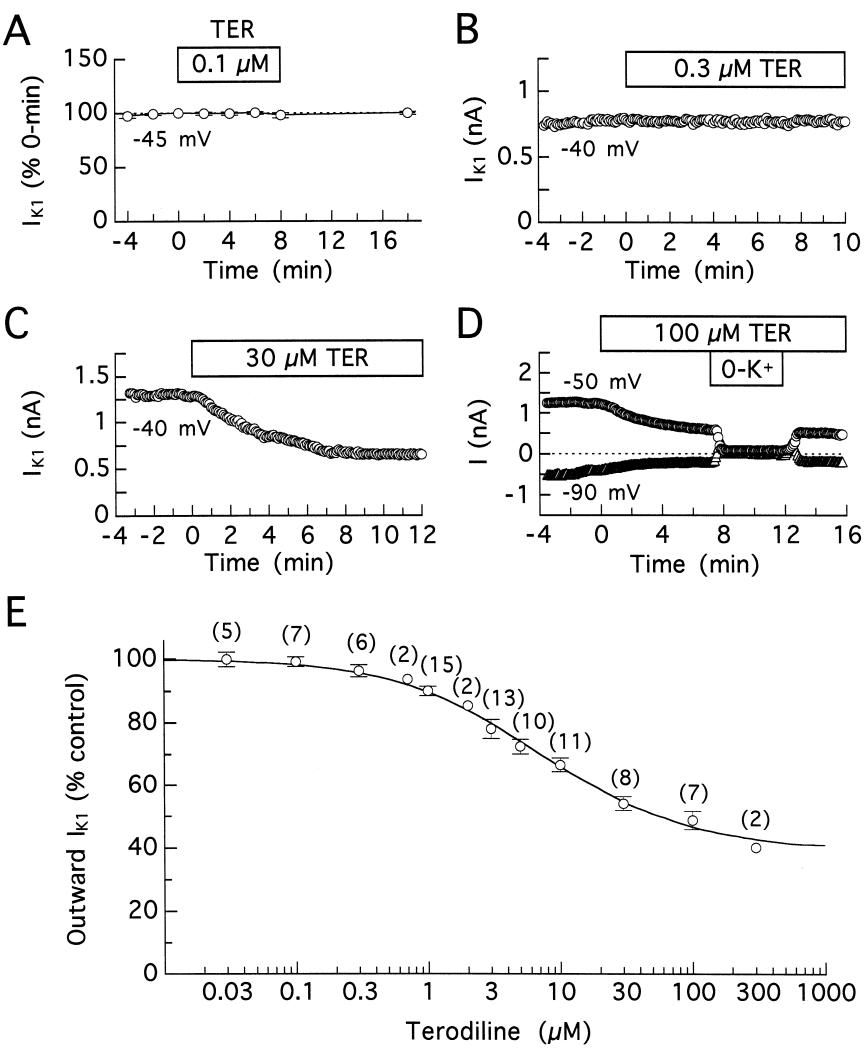

Fig. 3. Time course and concentration dependence of terodiline-induced inhibition of I_{K1}. (A) Stable outward I_{K1} at −45 mV before, during, and after treatment with 0.1 μM terodiline. Current amplitudes were normalized to the current measured at 0 min. Number of myocytes = 7 up to 8 min, and 4 thereafter. (B) Lack of effect of 0.3 μM drug. (C) Inhibition by 30 μM drug in a myocyte pretreated for 7 min with 1 μM E4031. (D) Effects of 100 μM terodiline on outward I_{K1} at −50 mV and inward I_{K1} at −90 mV. K^+-free Tyrode's solution reversibly suppressed terodiline-insensitive current. (E) Concentration–response relationship. Myocytes were equilibrated for 8–15 min after patch breakthrough, and then exposed to a single concentration of the drug for at least 7 min (mean exposure 12.2 min). Steady-state I_{K1} (ca. −40 mV) in the presence of the drug was expressed as a percentage of pre-drug control value. The data are fitted with a Hill equation, I_{K1} (% control) = $(\text{Max}/(1 + (\text{TER}/\text{IC}_{50})^{n_H})) + (100 - \text{Max})$, where Max (maximal percentage inhibition) is 60%, IC_{50} (the concentration of terodiline (TER) causing 50% of maximal inhibition) is 7 μM, and the Hill coefficient is 0.82. Number of myocytes in parentheses.

positive potentials. The drug reduced the amplitude of four membrane current components: the outward current at −40 mV, the peak inward current, late outward current at positive potentials, and decaying outward tail currents at −40 mV. Based on their relative amplitudes, time courses and current–voltage (I–V) relationships (Fig. 2B), the latter three currents are identified as L-type Ca^{2+} ($I_{Ca,L}$), delayed-rectifier K^+ (I_K), and decaying I_K tail, respectively. In six myocytes treated with 3 μM drug, $I_{Ca,L}$ at 0 mV declined from 1.9 ± 0.1 to 1.4 ± 0.1 nA ($p < 0.01$), and the amplitude of the late current at +40 mV declined by 0.1 ± 0.03 nA ($p < 0.05$). These myocytes were also hyperpolarized from prepulse −40 mV for 500 ms, and the example I–V in Fig. 2B indicates that the late current turned inward negative to −80 mV, and that the drug reduced both outward- and inward-directed currents by approximately 20%.

3.3. Inhibition of inward-rectifier K^+ current

Both the control and the 3-μM terodiline I–V relationships depicted in Fig. 2B have a shape (large and inward-directed current at potentials negative to the K^+ equilibrium potential, bell-shaped dependence of smaller outward current up to −20 mV) that is typical of I_{K1} in ventricular myocytes (Giles and Imaizumi, 1988). Similarly, the mean I–V relationship of the difference (control minus terodiline) current ($n = 6$ myocytes) shown in Fig. 2C has a typical inwardly-rectifying shape.

To evaluate the extent of inhibition of outward-directed I_{K1} induced by terodiline, myocytes were held at ca. −40 mV, or pulsed to that potential from ca. −80 mV, for measurement of changes in steady (or late) current levels at the depolarized potential. The validity of this approach was examined in two sets of control experiments. Data

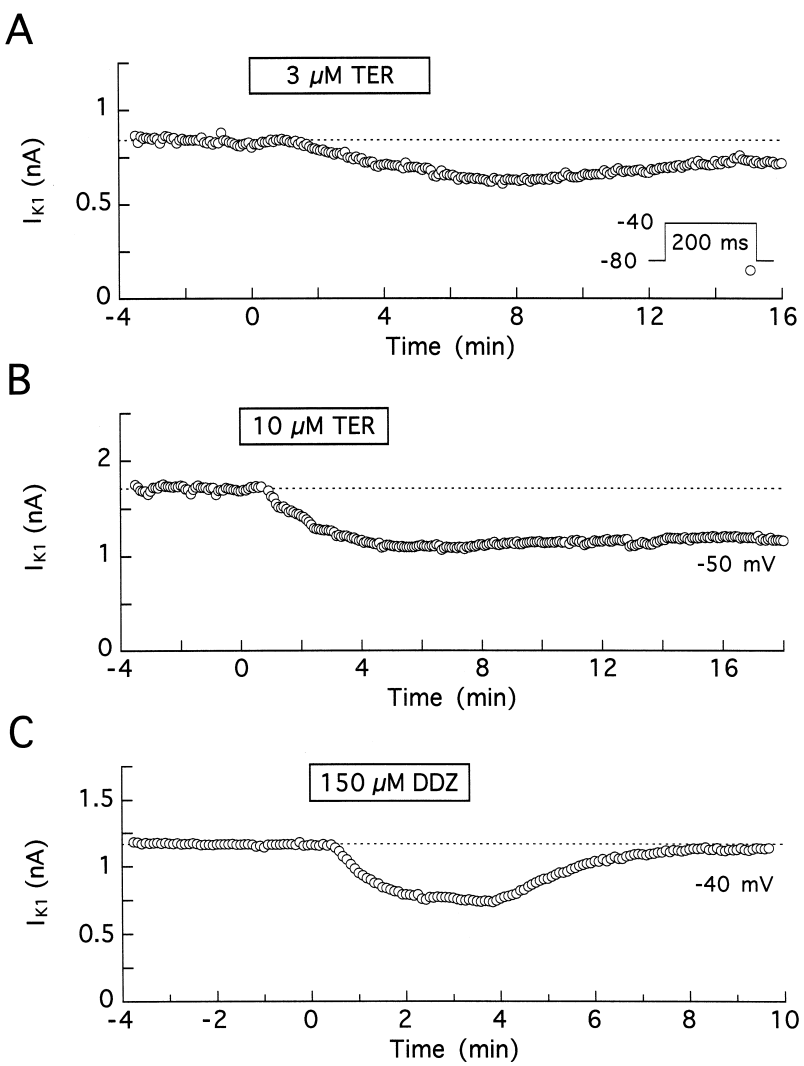

Fig. 4. Recovery of outward I_{K1} after drug treatment. (A and B) Examples of slow recovery after removal of terodiline. (C) Prompt recovery on washout of a reference inhibitory drug (daidzein; DDZ).

from the first of these indicated that Ba^{2+}, a classic blocker of inward-rectifier K^+ (Kir) channels (Giles and Imaizumi, 1988; Kamouchi et al., 1997; Gillis et al., 1998), suppressed nearly 100% of the late outward current at ca. −40 mV ($n = 4$) (Fig. 2D). Results from the second set of experiments established that terodiline does not induce any 'contaminant' current at ca. −40 mV: (i) neither 3 nor 30 μM terodiline affected current at −40 mV in Ba^{2+}-treated myocytes ($n = 4$) (not shown), and (ii) current at −40 mV in myocytes with I_{K1} minimized by ion substitutions (K^+-free Tyrode's superfusate; Cs^+ dialysate) was unaffected by 30 μM terodiline ($n = 4$) (Fig. 2E).

Patch-breakthrough was generally followed by a small run-up of outward-directed I_{K1} at ca. −40 mV; thereafter, there was a period of I_{K1} stability that was suitable for tests with a single concentration of the drug (Fig. 3A). The representative records in Fig. 3B and C illustrate that I_{K1} in myocytes held at ca. −40 mV was insensitive to submicromolar concentrations of terodiline, but markedly inhibited (halftime ca. 3 min) by higher concentrations.

In some experiments, myocytes were held about 5–10 mV negative to the zero-current potential and pulsed to a more positive potential to monitor the effects of the drug on both inward- and outward-directed I_{K1}. Fig. 3D shows that 100 μM terodiline blocked about 65% of both the inward current at −90 mV and the outward current at −50 mV; the remaining current declined to near zero when inward-rectifier channel conductance was suppressed by removal of external K^+. The outward-I_{K1} data from 88 myocytes treated with terodiline are well described by a Hill equation that projects maximal inhibition of 60%, a concentration of 7 μM for half-maximal inhibition (IC_{50}), and a Hill coefficient of 0.82 (Fig. 3E). It was possible, if unlikely, that these data were tainted by a drug-induced inhibition of steady-state delayed-rectifier K^+ current. To ascertain that this was not the case in regard to the most likely candidate (rapidly-activating component of I_K (I_{Kr})), we examined the effects of terodiline on eight myocytes that were pretreated with 1 μM E4031 for ≥5 min to inhibit I_{Kr} (see Sanguinetti and Jurkiewicz, 1990). The inhibition of I_{K1} by terodiline in these myocytes was in good accord with that measured in non-pretreated myocytes: 1 μM (10 ± 2% inhibition, $n = 4$); 10 μM (mean 27% inhibition, $n = 2$); and 30 μM (mean 45% inhibition, $n = 2$, e.g., Fig. 3C).

3.4. Delayed recovery of I_{K1} from inhibition

Terodiline-induced inhibition of I_{K1} was not easily reversed, even when the exposures to the drug were relatively brief (Fig. 4A and B). For example, washout for 13 min only restored the current to 70% control after a 4-min treatment with 10 μM drug had reduced it to 63% control (Fig. 4B). Additional experiments with 3 μM ($n = 4$), 10 μM ($n = 2$), 30 μM ($n = 2$), and 100 μM ($n = 2$) terodiline confirmed this pattern. The slow reversibility was not a consequence of channel lability because inhibition of I_{K1} by a 'reference' K^+ channel blocker (150 μM daidzein:

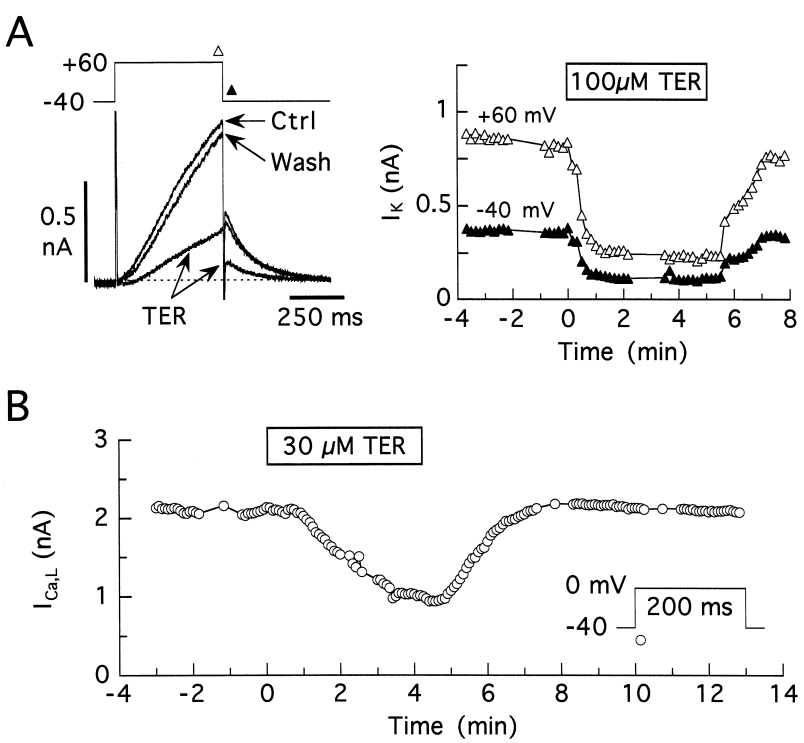

Fig. 5. Time courses of reversal of terodiline-induced inhibition of I_K and $I_{Ca,L}$. (A) Prompt recovery of time-dependent I_K at +60 mV and tail I_K at −40 mV. Time-dependent currents at +60 mV (right) were zeroed for presentation purposes. (B) Prompt recovery of $I_{Ca,L}$ at 0 mV. Pulsing rate 0.1 Hz.

Smirnov and Aaronson, 1995) was promptly reversed upon removal of the drug (Fig. 4C).

To assess whether recovery from *any* inhibition by terodiline is an inherently slow process, we measured other membrane currents before, during, and after exposure of myocytes to high concentrations of terodiline. Although I_K activating at +60 mV and associated tail at −40 mV were rapidly suppressed by 100 μM terodiline, they quickly recovered when the drug was withdrawn (e.g., Fig. 5A). Similarly, there was a quick recovery of $I_{Ca,L}$ after removal of 30 μM terodiline (e.g., Fig. 5B).

4. Discussion

The results indicate that terodiline inhibits up to 60% of outward I_{K1} in guinea pig ventricular myocytes with an IC_{50} of 7 μM. This action is more pronounced than that reported for the drug in a recent study on inward I_{K1} (−100 to −120 mV) by Hayashi et al. (1997) (10–15% reduction with 10 μM). We have no explanation for this discrepancy, especially since we found that terodiline has roughly proportional effects on inward and outward I_{K1} (e.g., Fig. 2BFig. 3D). The proportional inhibition is of interest because it is becoming apparent that I_{K1}-inhibitory compounds can have directional effects on I_{K1}. For example, both RP58866 and its active enantiomer, terikalant, have similar inhibitory effects on inward and outward I_{K1} (Jurkiewicz et al., 1996; also see McLarnon and Xu, 1995), whereas taurine appears to have a selective inhibitory action on inward I_{K1} (Satoh, 1998), and the inhalational anaesthetic sevoflurane inhibited inward I_{K1} but enhanced outward I_{K1} (Stadnicka et al., 1997).

It is possible that the extent of the inhibition produced by terodiline was underestimated if the (mean 12.2 min) exposures to the drug were too short to achieve full steady-state action. Nevertheless, the results indicate that terodiline is a relatively potent inhibitor of inward-rectifier current. It is comparable with RP58866 ($IC_{50} \approx 6$ μM: Jurkiewicz et al., 1996) and terikalant ($IC_{50} \approx 8$ μM: Jurkiewicz et al., 1996; maximal inhibition ≈73%: McLarnon and Xu, 1995), and appears to be more potent than quinidine (30% inhibition at 20–40 μM: Salata and Wasserstrom, 1988).

Terodiline at micromolar concentrations is not a specific inhibitor of I_{K1}. Its spectrum of action on K^+ and Ca^{2+} currents (Figs. 2 and 5) appears to be quite similar to that of micromolar quinidine in cardiac ventricular preparations (Nawrath, 1981; Hiraoka et al., 1986). Depending upon species and experimental conditions, the net effect of quinidine on the action potential duration may be a shortening, lengthening, or little change (Nawrath, 1981; Hiraoka et al., 1986; Imaizumi and Giles, 1987; Salata and Wasserstrom, 1988). In the case of 3–5 μM terodiline, the net effect on action potential duration was a slight shortening at the plateau level and a ca. 10% lengthening at more negative potentials. We attribute the absence of a marked effect of these concentrations on the plateau to offsetting effects of reduction in $I_{Ca,L}$ and reduction in I_K, and the lengthening at more negative potentials to further reduction of repolarizing current due to inhibition of I_{K1}. Higher concentrations of the drug strongly reduced plateau duration due to pronounced inhibition of $I_{Ca,L}$, but still lengthened the action potential at later stages of repolarization (due in part to pronounced inhibition of I_{K1}). The 'triangularization' of the action potential induced by 30 μM terodiline is a configurational change that has also been observed in the action potential of canine Purkinje fibres exposed to high concentrations of the drug (Pressler et al., 1995).

Washout of 3–10 μM terodiline resulted in an additional lengthening of the action potential in guinea pig papillary muscles. A similar phenomenon has been observed during washouts of micromolar quinidine from canine Purkinje fibres (Nattel and Bailey, 1983) and millimolar sotalol from guinea pig papillary muscles (Carmeliet, 1985). Salata and Wasserstrom (1988) reported that washout of 10 μM quinidine from canine papillary muscles lengthened the action potential at 90% repolarization to 115% control from 106% at the end of drug treatment. They attributed the extra lengthening to a lack of recovery of I_{K1} from inhibition because voltage ramps (−90 to −10 mV) applied to canine ventricular myocytes elicited outward currents that were smaller after washout than either before or during treatment. Balser et al. (1991) observed that both the onset and washout of single Kir channel block were relatively slow processes, and Hiraoka et al. (1986) found that whereas quinidine-induced inhibition of I_{K1} was 'irreversible', quinidine-induced inhibition of $I_{Ca,L}$ and I_K was quickly reversible. A similar temporal pattern of recovery of I_{K1}, $I_{Ca,L}$ and I_K offers a satisfactory explanation for the lengthening of the action potential upon washout of terodiline from papillary muscles, i.e., the recovery of the plateau currents unmasks the full consequences of inhibited I_{K1}.

Arena and Kass (1988) studied the block of guinea pig ventricular I_{K1} and I_K by externally-applied quaternary clofilium and two of its tertiary nitrogen structural analogs. Clofilium (50–100 μM) blocked I_{K1}, whereas the analogues blocked both currents. The time courses of block of I_K by the analogues (halftimes < 1 min) were three- to six-fold faster than those for block of I_{K1}; recovery of I_K was equally rapid, whereas recovery of I_{K1} was slow and often incomplete after 10-min washes. These aspects of tertiary clofilium action closely resemble those described here for terodiline (Figs. 3–5) suggesting (Arena and Kass, 1988) different off-rates for binding to the drug receptors responsible for inhibition of the two currents.

It may be that different onset and washout time courses for different channel types affected by terodiline have a bearing on clinical findings reported by Hartigan-Go et al.

(1996). They administered single oral doses of terodiline that secured near-therapeutic peak plasma concentrations (ca. 1 μM) in volunteers, and compared changes in QT interval with changes in plasma concentrations. There was a lengthening of the QT interval that lagged behind the rise to peak plasma concentration, and then lagged behind the ensuing fall in plasma concentration. This created a hysteresis in the QT-concentration relationship such that QT prolongation was near 75% of its peak value at a time when drug plasma concentration had fallen to 20% of its peak value.

Administration of antihistamine terfenadine can cause QT lengthening and torsades de pointes (Monahan et al., 1990; Woosley, 1996), and it has been suggested that, aside from block of I_K, block of ventricular I_{K1} may be an important element in this adverse reaction (Berul and Morad, 1995). Acquired QT lengthening is frequently associated with predisposing factors (Jackman et al., 1988; Colatsky et al., 1990; Ben-David and Zipes, 1993), and a number of these can be linked to suppression of I_{K1} (co-prescription of cardiac drugs (e.g., quinidine (Hiraoka et al., 1986)); heart disease (Lue and Boyden, 1992; Beuckelmann et al., 1993; Kääb et al., 1996; McIntosh et al., 1998); hypokalemia (e.g., Harvey and Ten Eick, 1988)). These considerations, possible heterogeneity related to regional differences in I_{K1} density across the ventricular wall (Furukawa et al., 1992), the complex influences of I_{K1} on myocardial excitability (Delmar et al., 1989; Shimoni et al., 1992), and the finding that the 7-μM IC_{50} for inhibition of I_{K1} by terodiline is lower than the 9.3-μM plasma concentration measured in an adversely-affected patient (Connolly et al., 1991), suggest that inhibition of I_{K1} is a factor in the concentration-dependent cardiotoxicity (Thomas et al., 1995) of terodiline.

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