

Action Potentials, Contraction, and Membrane Currents in Guinea Pig Ventricular Preparations Treated with the Antispasmodic Agent Terodiline¹

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ABSTRACT

Terodiline was widely prescribed for urinary incontinence before reports of adverse cardiac effects that included bradycardia, QT lengthening, and ventricular tachyarrhythmia. The present study on guinea pig papillary muscles and ventricular myocytes was undertaken to gain insight into the cardioactive properties of the drug. Clinically relevant concentrations (<10 μM) of terodiline lengthened the action potential duration by up to 12%; higher concentrations shortened the duration in a concentration-dependent manner. The drug depressed maximal upstroke velocity in a use-dependent manner; the IC_{50} value was near 150 μM in muscles driven at 1 Hz, 60 μM at 3 Hz, 38 μM at 5 Hz, and 3 μM at 1 Hz in muscles depolarized

with 14 mM K^+ . Submicromolar terodiline frequently had a small positive inotropic effect, whereas micromolar concentrations depressed force in a frequency-dependent manner. Voltage-clamp results on myocytes indicate that terodiline inhibits three membrane currents that govern repolarization: 1) E4031-sensitive, rapidly activating K^+ current with an IC_{50} value near 0.7 μM as previously reported; 2) slowly activating, delayed-rectifier K^+ current with an IC_{50} value of 26 μM ; and 3) L-type Ca^{2+} current with an IC_{50} value of 12 μM . These findings are correlated with the changes in action potential configuration and developed tension and discussed in relation to the cardiotoxic effects of the drug.

Terodiline, a drug with structural similarities to prenylamine and fendiline, was originally marketed in Sweden in the mid-1960s as a prophylactic agent in angina pectoris. The results of studies on isolated bronchial muscle (Irvani and Melville, 1975) and patients with obstructive pulmonary disease (Castenfors et al., 1975) suggested that terodiline had anticholinergic properties. Anticholinergic activity, as well as Ca^{2+} antagonism, was documented in subsequent investigations on isolated bladder (Husted et al., 1980). Based on this spectrum of activity, terodiline was investigated for the treatment of urinary incontinence in adults and of nocturnal enuresis in children (Elmér, 1984; Peters, 1984). The outcome of these trials was withdrawal of the drug as an

antianginal agent in 1985 and reintroduction in more than 20 countries in 1986 as an effective treatment for urinary incontinence. It quickly became the drug of choice for this condition in Europe (Langtry and McTavish, 1990) but was withdrawn in 1991 after reports of serious cardiac side effects, including bradycardia, atrioventricular block, prolongation of the Q-T interval of the electrocardiogram, and ventricular tachyarrhythmia (torsades de pointes) (Connolly et al., 1991; Stewart et al., 1992). The drug is currently available for clinical investigation and is a prototype structure for the development of newer drugs for the treatment of unstable bladder (Take et al., 1996).

Although the specific electrophysiological mechanism or mechanisms that trigger drug-induced torsades are uncertain (Roden et al., 1996; Woosley, 1996), knowledge of the mechanisms of cardiac action of various torsades-inducing drugs contributes to improved understanding of the arrhythmia. In this regard, there have been investigations into the

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ABBREVIATIONS: \dot{V} , upstroke velocity; APA, action potential amplitude; APD, action potential duration; APD_{20} , action potential duration at 20% repolarization level; APD_{90} , action potential duration at 90% repolarization level; DMSO, dimethyl sulfoxide; $I_{\text{Ca,L}}$, L-type Ca^{2+} current; I_{K} , delayed-rectifier K^+ current; I_{Kr} , inward-rectifying K^+ current; I_{Kr} , rapidly activating component of I_{K} ; I_{Ks} , slowly activating component of I_{K} ; \dot{V}_{max} , maximal upstroke velocity.

alterations of electrophysiological parameters, contraction, and membrane ionic currents caused by class Ia antiarrhythmic quinidine (Nawrath, 1981; Imaizumi and Giles, 1987), class III antiarrhythmic sotalol (Campbell, 1987), the antidepressant imipramine (Valenzuela et al., 1994), antihistamines terfenadine and astemizole (Berul and Morad, 1995; Salata et al., 1995), and the antibiotic erythromycin (Nattel et al., 1990; Daleau et al., 1995).

To our knowledge, there have been two previous studies on the direct effects of terodiline on cardiac cell function. Pressler et al. (1995) found that exposure of canine Purkinje fibers to clinically relevant concentrations (1–10 μM) of terodiline shortened the action potential duration (APD; 50% repolarization) by $\sim 40\%$. More recently, we determined that the rapidly activating component of delayed-rectifier K^+ current (I_{Kr}) in guinea pig ventricular myocytes is inhibited by terodiline with IC_{50} values near 0.7 μM and that slowly activating I_{Ks} is inhibited by high concentrations of the drug (Jones et al., 1998). Although the latter actions would have lengthening influences on ventricular action potentials and may therefore be important factors in QT lengthening, it is unclear whether other actions of the drug on ventricular cells might not outweigh the lengthening influences and shorten the action potential as reported for Purkinje fibers. To obtain further information on these and other aspects of terodiline action on ventricular muscle, we recorded action potentials and developed tension in guinea pig papillary muscles treated with 0.1 to 100 μM concentrations of the drug, and we measured Ca^{2+} and K^+ membrane currents in guinea pig ventricular myocytes.

Materials and Methods

Papillary Muscles. Papillary muscles were obtained from the right ventricles of adult guinea pig hearts. The guinea pigs (250–350 g) were sacrificed by cervical dislocation, and the hearts were quickly removed and placed in oxygenated (95% $\text{O}_2/5\% \text{CO}_2$) Krebs' solution that contained 113.1 mM NaCl, 4.6 mM KCl, 2.45 mM CaCl_2 , 1.15 mM MgCl_2 , 21.9 mM NaHCO_3 , 3.48 mM NaH_2PO_4 , and 10.0 mM glucose (pH 7.4). Muscles were excised and mounted in a Perspex bath (0.25 ml volume) perfused with solution ($36 \pm 0.2^\circ\text{C}$) at 4 to 6 ml/min. The mural end of the muscle was fixed by a clamp, and the tendinous end was fixed by a pin or attached to an isometric force transducer (model UC2; Gould Statham, Oxnard, CA). Muscles were adjusted to a length that produced $\sim 75\%$ peak developed tension (resting tension of 50–90 mg).

Muscles 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 to 90 min before data collection. When normal Krebs' solution was replaced by Krebs' solution with higher (8–14 mM) than standard (4.6 mM) K^+ concentration, there was no compensation for the change in osmolality. Action potentials were recorded with a high-input impedance amplifier (model 750; WP Instruments, New Haven, CT) using conventional microelectrodes filled with 3 M KCl (resistance 8–15 M Ω), and the upstroke was electronically differentiated to record upstroke velocity (\dot{V}). The action potentials were displayed on a storage oscilloscope (model 5103N; Tektronix, Beaverton, OR) and recorded on film, with a chart recorder (model RS 3400; Gould, Cleveland, OH) and/or by computer via Axoscope (Axon Instruments, Foster City, CA).

Ventricular Myocytes. Single ventricular myocytes were enzymatically isolated as described previously (Ogura et al., 1995). The excised hearts were mounted on a Langendorff column and retro-

gradely perfused (37°C) through the aorta with Ca^{2+} -free Tyrode's solution containing collagenase (0.08–0.12 mg/ml; Yakult Pharmaceutical Co., Tokyo, Japan) for 10 to 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. After the cells had settled to the bottom, the chamber was perfused (~ 2 ml/min) with Tyrode's solution at 36°C . The Tyrode's solution contained 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2 , 1 mM MgCl_2 , 10 mM glucose, and 5 mM HEPES, pH 7.4 with NaOH. In some experiments, this solution was replaced by K^+ -free Tyrode's or K^+ , Ca^{2+} -free Tyrode's that included 0.2 mM Cd^{2+} .

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, UK) and filled with 1) K^+ solution that contained 40 mM KCl, 106 mM potassium-aspartate, 1 mM Mg-ATP, 4 mM K_2 -ATP, 5 mM EGTA, and 5 mM HEPES, pH 7.2 with KOH, or 2) Cs^+ solution (K^+ replaced by Cs^+). The pipettes had resistances of 1.5 to 2.5 M Ω when filled with pipette solution, and liquid junction potentials between external and pipette-filling solution were nulled before patch formation. Series resistance ranged between 3 and 7 M Ω and was compensated by 60 to 80%. Membrane current signals were filtered at 3 kHz and digitized with an A/D converter (Digidata 1200A; Axon Instruments) and pCLAMP software (Axon Instruments) at a sampling rate of 8 kHz before analysis.

Drugs. Terodiline was supplied by Sepracor Inc. (Marlborough, MA) and freshly dissolved in dimethyl sulfoxide (DMSO) (Sigma Chemical Co., St. Louis, MO) immediately before use. The highest final concentration of DMSO in the superfusate was 0.1% (for 100 μM terodiline), a DMSO concentration that has no significant effects on electrical and contractile activity in guinea pig papillary muscles or on Ca^{2+} and K^+ currents in guinea pig ventricular myocytes (Ogura et al., 1995). E4031 was obtained from Eisai (Tokyo, Japan) and handled in the same manner as terodiline.

Statistics. Results are expressed as mean \pm S.E., and single comparisons were made using Student's *t* test. Differences were considered to be significant when $p < .05$.

Results

Concentration-Dependent Effects of Terodiline on Action Potential Parameters

Duration and Amplitude. The records in Fig. 1, A–C, illustrate the effects of 0.3, 3, and 15 μM terodiline on the configuration of action potentials in guinea pig papillary muscles. The lower concentrations lengthened the action potential, whereas 15 μM lowered the plateau voltage and slowed phase 3 repolarization.

To quantify the concentration-dependent effects of terodiline, action potential amplitude (APA) and APD at 20 and 90% repolarization levels (APD₂₀ and APD₉₀, respectively) were monitored for 30 min in control and test muscles stimulated at 1 Hz. These parameters were stable in the control muscles and reached quasi-steady state within the observation period when tissues were exposed to terodiline (Fig. 1D). Values at 30 min were expressed as percentages of 0-min values, and the results are shown in Fig. 1E. APD₂₀ was increased by low concentrations of terodiline [peak, $108 \pm 2\%$ ($n = 14$) at 1 μM], slightly reduced by 10 μM , and reduced to $85 \pm 1\%$ ($n = 11$) by 30 μM . The dependence of APD₉₀ on

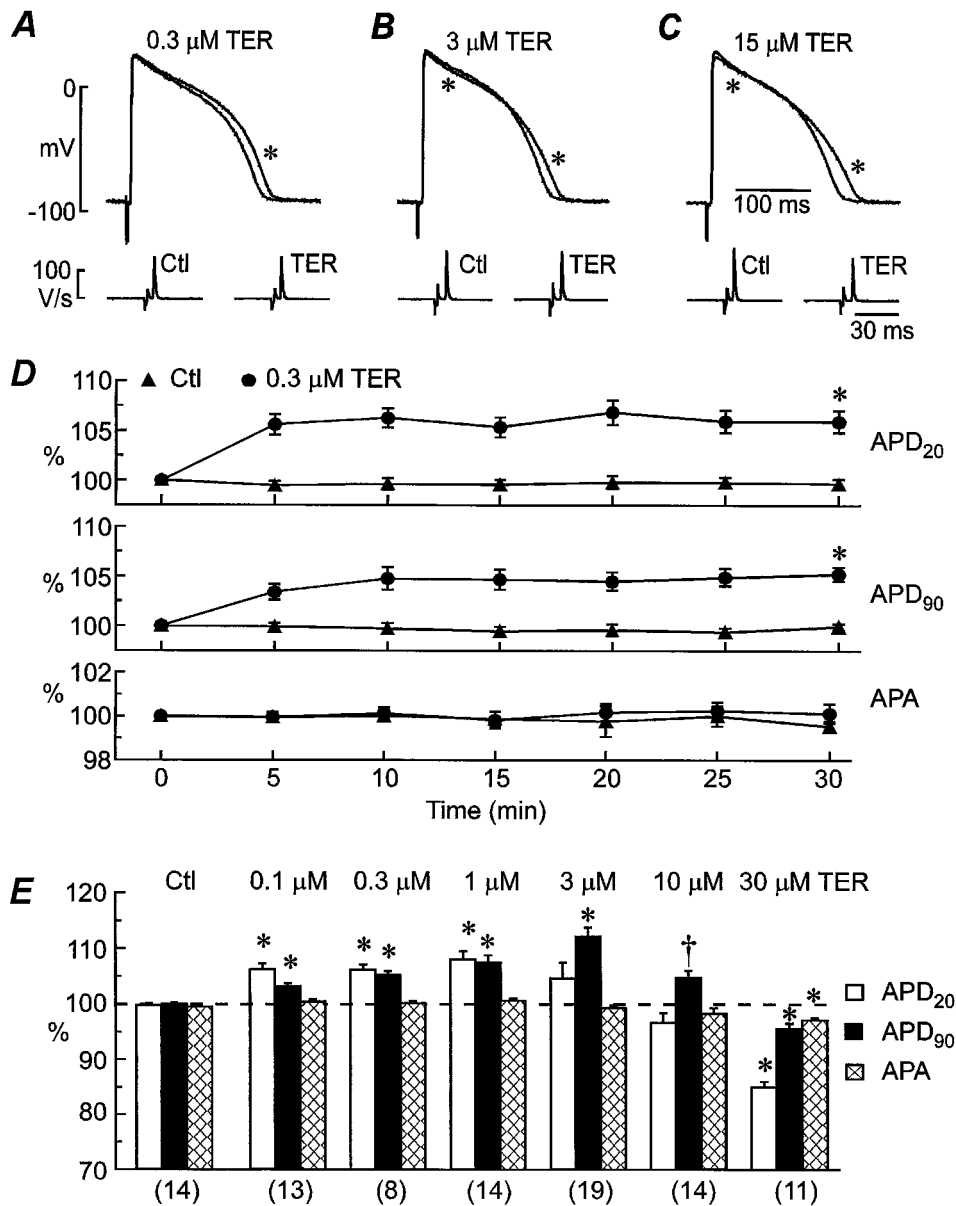


Fig. 1. Effects of terodiline on action potential parameters in guinea pig papillary muscles stimulated at 1 Hz. A–C, action potentials (top) and \dot{V}_{\max} (bottom) recorded just before (Ctl) and 30 min after the addition of 0.3, 3, or 15 μM drug (TER). \dot{V}_{\max} traces have been shifted for presentation purposes. D, time courses of changes in action potential parameters measured from control muscles (Ctl; \blacktriangle , $n = 14$) and from muscles treated with 0.3 μM terodiline (TER; \bullet , $n = 8$). The data are expressed as percentages of values measured at the start (0 min) of the 30-min trials. E, summary of concentration-dependent effects of terodiline on APD₂₀, APD₉₀, and APA. Test muscles were exposed to a single concentration of the drug for 30 min, and measurements at this time were expressed as percentages of 0-min values. Number of muscles in parentheses. $\dagger p < .05$, $*p < .001$ versus control.

drug concentration was similar but shifted to the right; it increased to a peak of $112 \pm 2\%$ ($n = 19$) at 3 μM and declined to $95 \pm 1\%$ ($n = 11$) at 30 μM . APA was unaffected by ≤ 10 μM terodiline and slightly depressed by higher concentrations.

Control and drug-treated muscles were subsequently stimulated at 3 Hz for 5 min and at 5 Hz for an additional 3 min. In the control muscles, APD₂₀ declined to $74 \pm 2\%$ (3 Hz) and $57 \pm 2\%$ (5 Hz) of the 0-min 1-Hz duration, APD₉₀ declined to $79 \pm 1\%$ (3 Hz) and $62 \pm 2\%$ (5 Hz), and APA declined by 2 to 4 mV (Fig. 2). APD₂₀ and APD₉₀ also declined when terodiline-treated muscles were driven at 3 and 5 Hz, with the overall outcome that the increases in rate had little effect on the percentage changes induced by the drug (Fig. 2A). APA in drug-treated tissues was also affected by rate; reductions at

high concentrations were up to 35 mV larger at 5 Hz than at 1 Hz, due primarily to reductions in overshoot (Fig. 2C).

Maximal Upstroke Velocity. Terodiline reduced maximal upstroke velocity (\dot{V}_{\max}) to $97 \pm 1\%$ (3 μM), $78 \pm 4\%$ (30 μM), and $57 \pm 6\%$ (100 μM) in muscles driven at 1 Hz (Fig. 3A). Depolarization of the resting membrane was not a factor in the reductions caused by <30 μM drug but may have been at 100 μM (depolarization of 2–8 mV, $n = 6$). For reference, Pressler et al. (1995) found that ≥ 25 μM terodiline caused marked depolarization and inexcitability of canine Purkinje fibers.

Increases in stimulation rate had a marked effect on \dot{V}_{\max} in terodiline-treated muscles, with estimated IC₅₀ values declining from 150 μM (1 Hz) to 60 and 38 μM at 3 and 5 Hz, respectively (Fig. 3A). Lowering the resting potential by ele-

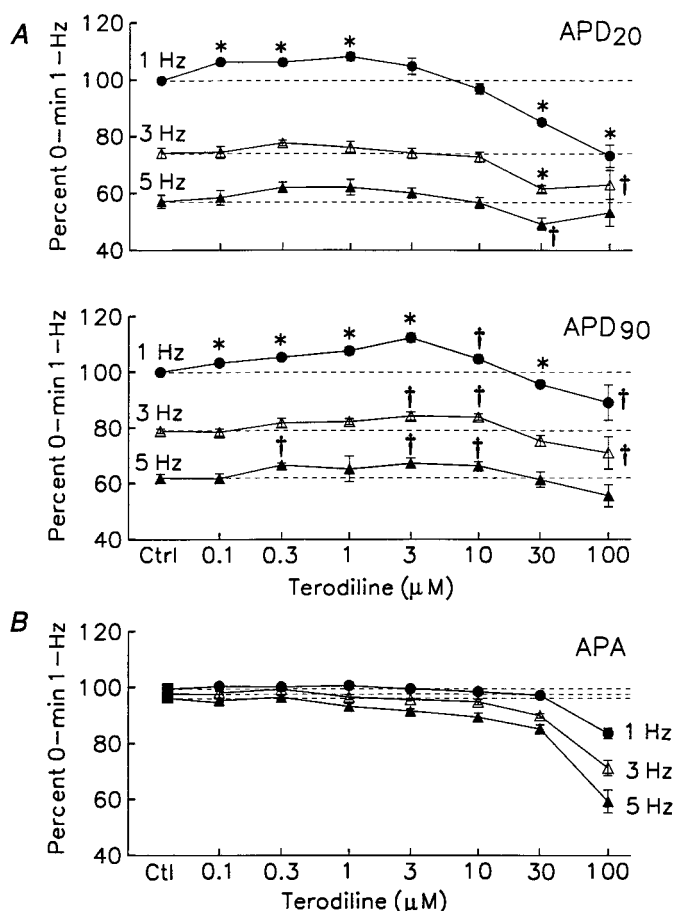


Fig. 2. Rate-dependent effects of terodiline on APD and amplitude. Muscles stimulated at 1 Hz for 30 min (see Fig. 1) were then stimulated at 3 Hz for 5 min and at 5 Hz for a further 3 min. Data are from measurements taken at the end of each of these stimulation periods. For 0.1 to 30 μM , the number of muscles is as in Fig. 1E; for 100 μM , six muscles. A–C, effects on APD₂₀, APD₉₀, and APA expressed as percentages of pretrial (0-min) 1-Hz values. For reference, the durations recorded in control muscles ($n = 14$) were as follows: APD₂₀ (98 ± 1 ms at 1 Hz, 73 ± 2 ms at 3 Hz, and 56 ± 2 ms at 5 Hz); APD₉₀ (194 ± 1 ms at 1 Hz, 153 ± 2 ms at 3 Hz, and 120 ± 3 ms at 5 Hz). $\dagger p < .05$, $*p < .001$.

vating external K^+ concentration from the standard 4.6 mM also affected the inhibition of \dot{V}_{max} . For example, depolarization from ~ -89 to -62 mV with 14 mM K^+ resulted in marked inhibition of (reduced) \dot{V}_{max} by normally ineffective 0.3 μM terodiline (Fig. 3C) and abnormal beat-to-beat variation in plateau generation with ≥ 3 μM drug (Fig. 3D). \dot{V}_{max} data obtained in experiments with 4.6, 8, 11.6, and 14 mM solutions indicate that the IC_{50} values declined from ≈ 150 μM in normally polarized muscles to ≈ 3 μM in muscles depolarized with 14 mM K^+ (Fig. 3E).

Effects on Force of Contraction

Terodiline had concentration-dependent effects on developed tension in muscles stimulated at 1 Hz for 30 min. At the lowest concentration tested (0.1 μM), the drug induced a 5 to 15% increase in 6 of 11 muscles. Higher concentrations were purely inhibitory (IC_{50} near 15 μM), in part due to an abbreviation of the time to peak tension (Fig. 4, A–C). Increasing the stimulation rate to 3 and 5 Hz increased developed ten-

sion in muscles treated with ≤ 1 μM terodiline to the same degree as in control muscles (Fig. 4C). However, the frequency-dependent inotropy was blunted in muscles treated with 3 to 5 μM terodiline and “reversed” in some muscles treated with 10 to 100 μM drug (Fig. 4C).

Effects on Membrane Currents

Overview. The records and analysis shown in Fig. 5 provide an overview of the concentration-dependent inhibitory effects of terodiline on membrane currents in guinea pig ventricular myocytes superfused with Tyrode’s solution and dialyzed with K^+ solution. The myocytes were depolarized for 500 ms from prepulse -40 mV to elicit inward L-type Ca^{2+} current ($I_{\text{Ca,L}}$) and outward delayed-rectifier K^+ current (I_{K}) and repolarized to -40 mV to elicit outward tail I_{K} . Exposure to 0.3 μM terodiline for 8 min had little effect on either peak $I_{\text{Ca,L}}$ or time-dependent I_{K} at positive potentials, but it reduced tail I_{K} at -40 mV (Fig. 5A). However, exposures to higher concentrations of the drug (10 and 100 μM) caused reductions in $I_{\text{Ca,L}}$ and time-dependent I_{K} , as well as further reductions in tail I_{K} (Fig. 5, B–D).

Effects on I_{K} . Guinea pig ventricular I_{K} is composed of I_{Kr} (a component that is rapidly activated at potentials up to ~ 10 mV and specifically inhibited by E4031) and I_{Ks} (a component that is slowly activated at positive potentials) (Sanguinetti and Jurkiewicz, 1990). Figure 6A, a–c, illustrates that tail I_{K} measured after 500-ms depolarizations can be resolved into tail I_{Kr} and tail I_{Ks} by the application of 3 μM E4031, that low concentrations of terodiline suppress the E4031-sensitive tail, and that 30 μM terodiline reduces the amplitude of the E4031-insensitive tail by $\sim 50\%$. A more detailed analysis of terodiline action on tail I_{Kr} indicates that the IC_{50} value is near 0.7 μM (Jones et al., 1998).

To quantify the effects of terodiline on I_{Ks} , myocytes were bathed in K^+ , Ca^{2+} -free Cd^{2+} solution (conditions that minimize the amplitude of I_{Kr} relative to I_{Ks} ; see Sanguinetti and Jurkiewicz, 1992) and depolarized for 2 s to positive potentials for measurement of time-dependent outward currents. Records from representative myocytes exposed to 5 and 100 μM terodiline indicate that the drug had concentration-dependent inhibitory effects on I_{Ks} elicited by depolarizations up to 80 mV (Fig. 6B, a and b). The effects on current amplitude at 40 mV were used to determine the concentration-response relationship, and the data are well described by a Hill equation with an IC_{50} value of 26 μM and a coefficient of 0.85 (Fig. 6B, c).

Effects on Peak $I_{\text{Ca,L}}$. To evaluate the effects of terodiline on peak $I_{\text{Ca,L}}$, myocytes that were superfused and dialyzed with K^+ -free solutions (to minimize K^+ currents) were pulsed at 0.1 Hz with 200-ms steps from -40 to 0 mV, treated with terodiline, and then exposed to 0.4 mM Cd^{2+} to establish the zero- $I_{\text{Ca,L}}$ background current level. The drug reduced the amplitude of $I_{\text{Ca,L}}$ in a concentration-dependent manner (Fig. 7, A and B), and this action was sensitive to pulsing rate: in three myocytes, trains of 25 pulses at 3 Hz depressed $I_{\text{Ca,L}}$ (0.1 Hz) to a much larger extent in the presence of the drug than in its absence (Fig. 6C).

The data obtained from myocytes pulsed at 0.1 Hz are summarized in Fig. 7D. They are well described by the Hill equation with an IC_{50} value of 12 μM and a coefficient of 0.9.

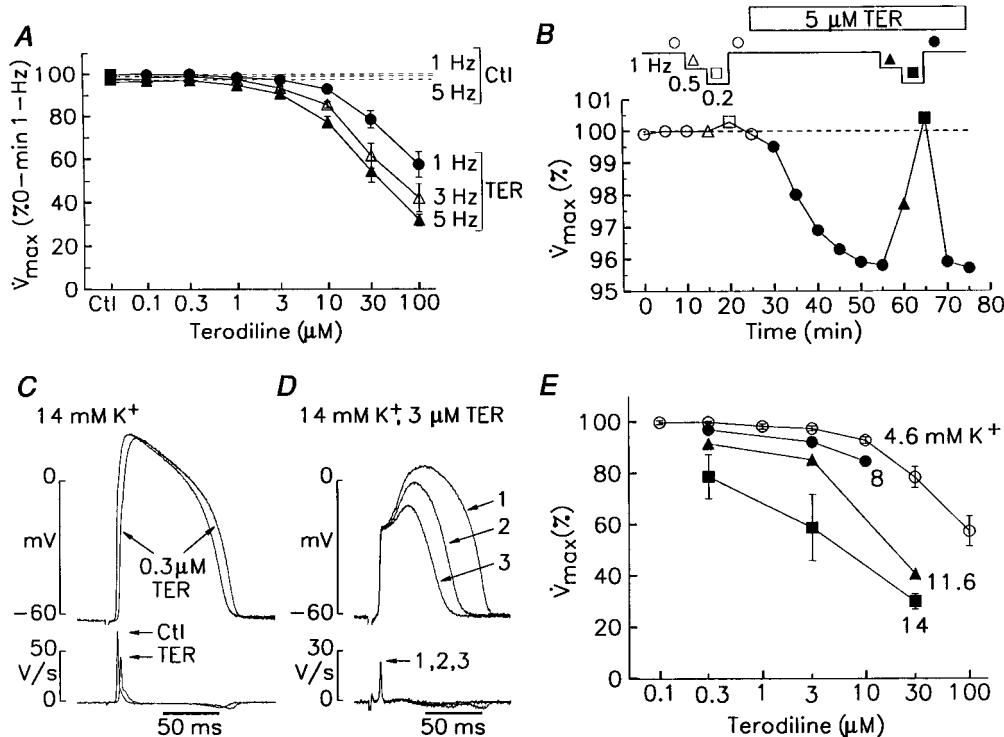


Fig. 3. Concentration-, frequency-, and voltage-dependent inhibition of \dot{V}_{\max} by terodiline. **A**, concentration- and frequency-dependent inhibition. \dot{V}_{\max} data (percent of 1-Hz 0-min values) are from the same muscles and under the same conditions as in Fig. 2. \dot{V}_{\max} was significantly depressed by ≥ 3 μM terodiline (TER) during 1-Hz stimulation and by ≥ 1 μM during 5-Hz stimulation. The dashed lines (1, 3, and 5 Hz, top to bottom) are reference lines extended from the mean values measured in control (Ctl) muscles. **B**, relief of terodiline (TER)-induced inhibition of \dot{V}_{\max} on slowing the stimulation rate from 1 Hz to 0.5 and 0.2 Hz in a muscle treated with 5 μM terodiline. Dashed line, reference 100% (0 min). **C–E**, terodiline-induced inhibition of \dot{V}_{\max} in partially depolarized muscles. Muscles were stimulated at 1 Hz and depolarized with a high- K^+ solution for 20 to 30 min before 30-min treatment with a single concentration of terodiline (TER). The records are from muscles bathed in 14 mM K^+ solution and treated for 10 min with 0.3 μM (C) or 3 μM (D) terodiline. The labels in D indicate the order of three successive action potentials. In plot E, the \dot{V}_{\max} data after 30-min treatment are expressed as percentages of predrug (high- K^+) values. The number of muscles at each concentration of the drug were as follows: 4.6 mM K^+ (see Fig. 2), 8 mM K^+ (four or five), 11.6 mM K^+ (one), and 14 mM K^+ (three or four).

Relation to Action Potential and Contraction. The relevance of the voltage-clamp findings to muscle activity was examined by measuring the effects of 3 μM terodiline on papillary muscles that were pretreated with 5 μM E4031. E4031 slowed repolarization at potentials below ~ 15 mV (Fig. 7E), and the stable APD₉₀ after 15 min was $127 \pm 2\%$ of control duration ($n = 5$). Subsequent exposure to 3 μM terodiline for 30 min lowered the plateau voltage and reduced the lengthened APD₉₀ by $9 \pm 2\%$ ($n = 5$). It seems likely that the plateau reduction and APD₉₀ shortening were due to inhibition of $I_{\text{Ca,L}}$ and that this inhibition was primarily responsible for the accompanying negative inotropy [after 15-min E4031, developed tension was $126 \pm 3\%$ of 0-min control ($n = 5$); after an additional 30-min terodiline, developed tension was $71 \pm 6\%$].

Discussion

APD, Contraction, and Membrane Currents. The findings on APD, contraction, and membrane currents are best correlated by considering them under three terodiline concentration ranges: submicromolar (< 1 μM), micromolar (1–5 μM), and high micromolar (≥ 10 μM). Submicromolar concentrations induced small but significant lengthenings of the

action potential that were almost certainly due to partial inhibition of I_{Kr} ($\text{IC}_{50} \sim 0.7$ μM), and micromolar concentrations induced additional lengthening as expected from more complete inhibition of I_{Kr} . However, the degree of lengthening (e.g., 12% with 3 μM) was smaller than the 27% lengthening in muscles treated with I_{Kr} -inhibiting E4031. The most likely explanation for this shortfall is that the lengthening influence related to I_{Kr} inhibition was partially offset by the shortening influence related to reduced peak $I_{\text{Ca,L}}$ ($\text{IC}_{50} = 12$ μM). In accord with this interpretation, 3 μM terodiline shortened the APD in muscles pretreated with E4031. High micromolar terodiline depressed \dot{V}_{\max} and the plateau, and this was most likely due to inhibition of Na^+ current and additional inhibition of $I_{\text{Ca,L}}$. The plateau-abbreviating influence related to these actions is likely to have been at least partially counteracted by a lengthening influence related to inhibition of I_{Ks} ($\text{IC}_{50} = 26$ μM). Finally, there were indications (lower resting potential; lower outward holding current at -40 mV) that inhibition of inward-rectifying K^+ current may have slowed phase 3 repolarization in muscles treated with high concentrations of the drug.

A small positive inotropy was detected in many of the papillary muscles treated with submicromolar terodiline, and it seems likely that this was a consequence of the partial

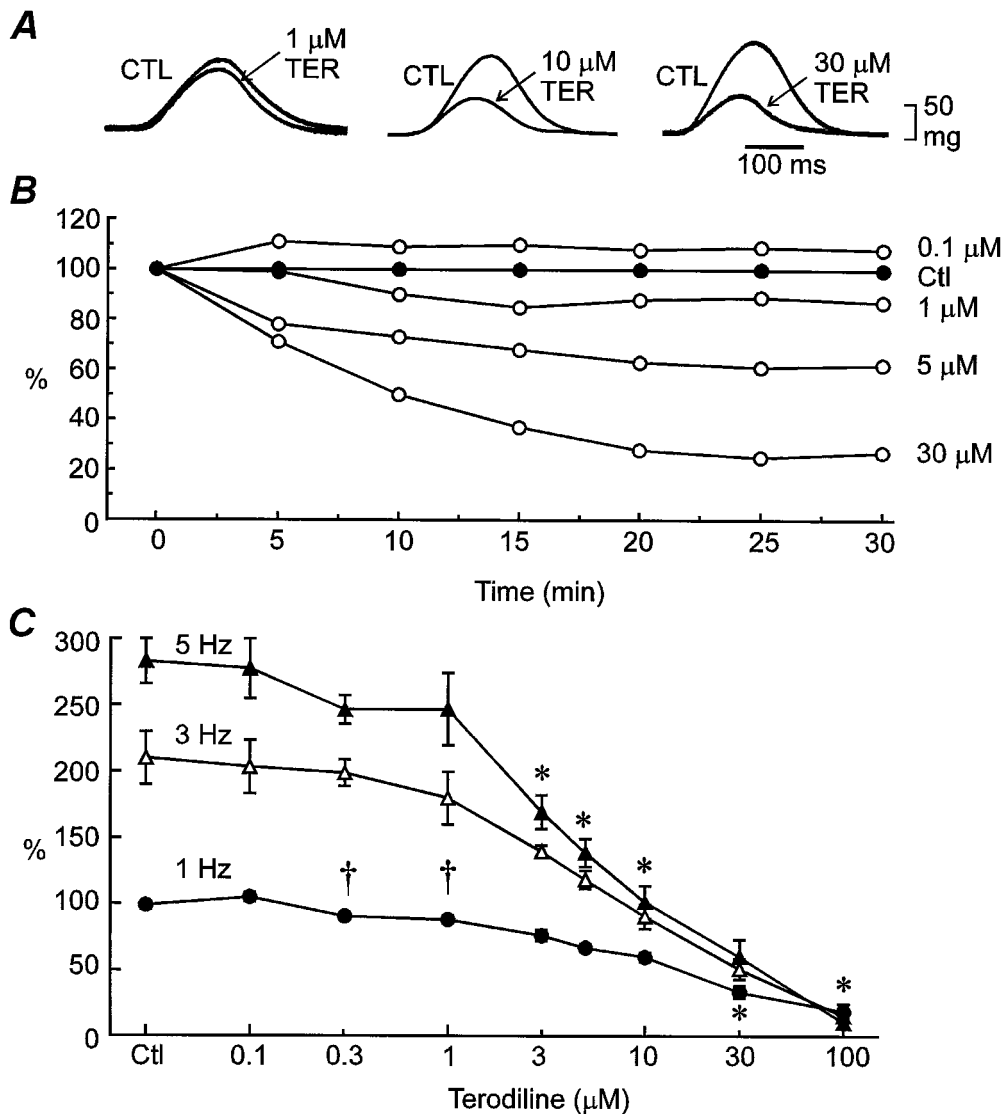


Fig. 4. Concentration- and frequency-dependent effects of terodiline (TER) on developed tension in papillary muscles. A, superimposed traces of contractions recorded from muscles stimulated at 1 Hz and treated with 1, 10, or 30 μM drug for 30 min. B, time courses of changes in representative control and drug-treated muscles. C, summary of data from muscles driven at 1 Hz for 30 min, 3 Hz for 5 min, and then 5 Hz for 3 min. Number of muscles in parentheses. † $p < .05$, * $p < .001$ versus control (Ctl).

inhibition of I_{K_r} . Many class III antiarrhythmic agents that inhibit I_{K_r} induce a small positive inotropy (Wallace et al., 1991), and we found that 5 μM E4031 augmented force by 26% in accord with findings on rabbit papillary muscles (Cingolani et al., 1991). The link between inhibition of I_{K_r} and positive inotropy is a lengthening of the action potential that allows a larger influx of Ca^{2+} (compare with Clark et al., 1996). Although action potential lengthening was more pronounced with micromolar than with submicromolar terodiline, this did not result in a larger positive inotropy. On the contrary, ≥ 1 μM terodiline depressed contraction, and this was almost certainly related to the concentration-dependent reduction in $I_{Ca,L}$ (see Shuba and McDonald, 1994; Grantham and Cannell, 1996). In addition, there may well be a correlation between terodiline-induced abbreviation in time to peak tension (Fig. 4A) and terodiline-induced acceleration of the inactivation of $I_{Ca,L}$ (Fig. 7A). A further link between $I_{Ca,L}$ and contraction in drug-treated preparations was that both processes were inhibited by increases in pulsing rate.

Kura et al. (1992) suggested that terodiline block of smooth muscle $I_{Ca,L}$ is rate-dependent.

Frequency- and Voltage-Dependent Inhibition of \dot{V}_{max} . Significant inhibition of \dot{V}_{max} occurred with 3 μM terodiline (reduction to $97 \pm 1\%$) when muscles were driven at 1 Hz, and with 1 μM when they were driven at 5 Hz. Drug-induced inhibition of \dot{V}_{max} was also accentuated when muscles driven at 1 Hz were partially depolarized with elevated K^+ ; in the 14 mM K^+ trials (resting potential lowered from ~ -89 to -62 mV), the IC_{50} value for inhibition by terodiline was near 3 μM (or roughly five times lower than the IC_{50} value for tetrodotoxin in normally polarized guinea pig ventricular preparations; Baer et al., 1976).

A number of the \dot{V}_{max} findings on papillary muscles correlate with those reported by Pressler et al. (1995) in their study on canine cardiac Purkinje fibers. For example, they found that significant ($\sim 11\%$) inhibition required 5 μM terodiline when the mean resting potential was -90 mV (2.7 mM K^+) but only 2 μM when it was -82 mV (5.4 mM K^+) and

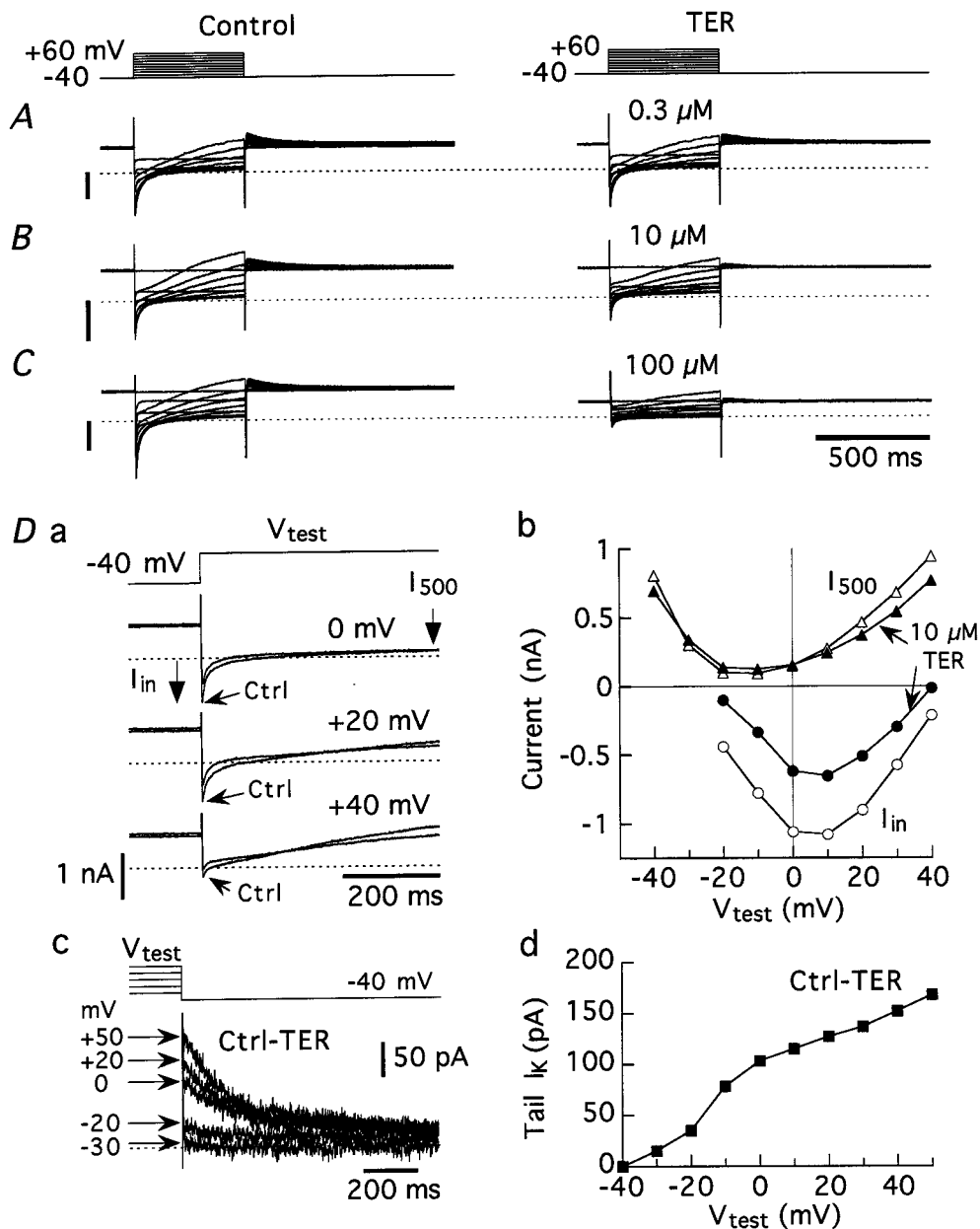


Fig. 5. Concentration-dependent effects of terodiline on membrane currents in guinea pig ventricular myocytes. The myocytes were held at -40 mV and depolarized for 500 ms to more positive potentials at 0.1 Hz before and 6 to 8 min after the addition of the drug. A-C, effects of three different concentrations. The vertical bars indicate 1 nA, and the horizontal dashed lines indicate zero current. D, analysis of the data obtained from the myocyte treated with 10 μM terodiline (B). a, superimposed example records. b, current-voltage relationships. c, difference tail currents obtained by subtracting tails during drug treatment (TER) from corresponding controls (Ctrl). d, current-voltage relationship of the difference tail current.

that inhibition by 10 μM drug was 25% larger at 2 Hz than at 0.67 Hz. These investigators also recorded a 10% lengthening of the His-ventricle interval in anesthetized dogs when plasma terodiline was $\sim 4 \mu\text{M}$ and attributed it to slowed conduction related to Na^+ channel block by the drug.

Adverse Reactions to Terodiline. The adverse cardiac effects of terodiline include slowing of heart rate, atrioventricular conduction disturbances, QT prolongation, and malignant ventricular tachycardia (torsades de pointes) (Connolly et al., 1991; Stewart et al., 1992). Because both Na^+ and Ca^{2+} currents are essential for pacemaking and nodal conduction (Munk et al., 1996; Kodama et al., 1997), it seems likely that inhibition of these currents contributes to the bradycardia and atrioventricular block observed in patients receiving terodiline. In addition, action potential lengthening

and more positive diastolic potential related to inhibition of delayed-rectifier K^+ current may make important contributions to these disturbances (see Ono and Ito, 1995).

The QT lengthening caused by terodiline has been shown to be dependent on the plasma concentration of the drug (Thomas et al., 1995). The therapeutic concentration is $\sim 1.5 \mu\text{M}$, and a concentration of 9.3 μM was measured in one elderly patient (Connolly et al., 1991). Action potentials in guinea pig papillary muscles were lengthened in the presence of 0.1 to 10 μM terodiline, and although this lengthening was moderate (peak 12% at 3 μM), it is in good agreement with the degree of lengthening of the monophasic action potential in dogs (Pressler et al., 1995) and rate-corrected Q-T interval in dogs (Natsukawa et al., 1998) and humans (Stewart et al., 1992; Thomas et al., 1995).

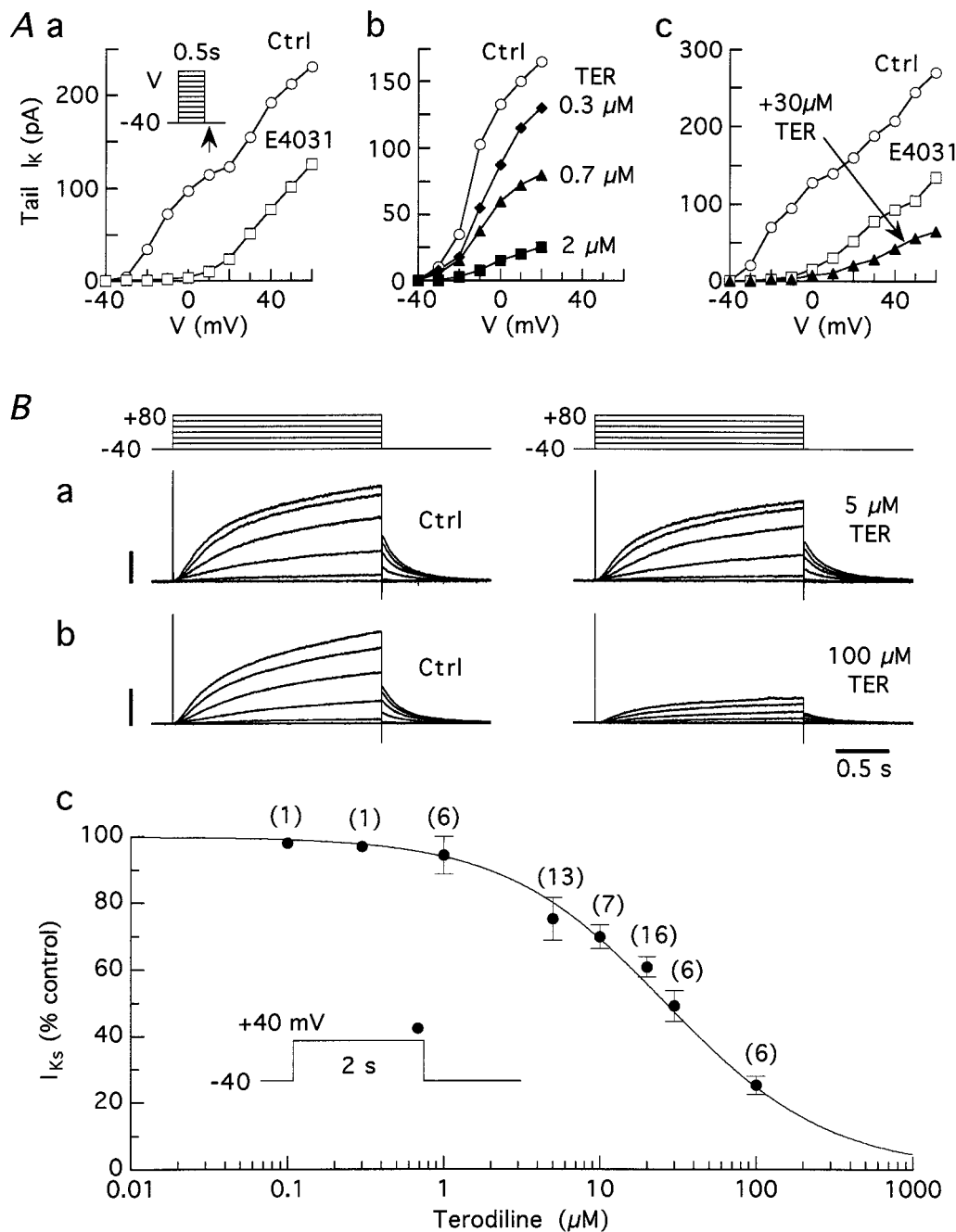


Fig. 6. Inhibition of I_K by terodiline. **A**, tail I_{Ks} -voltage relationships determined in experiments on myocytes bathed and dialyzed with K^+ solutions. **a**, inhibition of low-voltage tail I_{Ks} by 3 μM E4031. **b**, inhibition of low-voltage tail I_{Ks} by low concentrations of terodiline. **c**, inhibition of high-voltage, E4031-insensitive tail I_{Ks} by 30 μM terodiline; the duration of the pretreatment with 3 μM E4031 was 5 min. **B**, inhibition of I_{Ks} in myocytes with I_{Ks} suppressed by superfusion with K^+ -, Ca^{2+} -free Cd^{2+} solution. **a** and **b**, effects of 5 and 100 μM terodiline (5–8 min) on I_{Ks} elicited by 2-s depolarizations from -40 to 80 mV in 20-mV increments. The initial levels of the currents during the pulses have been zeroed for presentation purposes. The vertical bars indicate 0.5 nA. **c**, dependence of I_{Ks} inhibition on the concentration of terodiline. I_{Ks} was measured as the time-dependent current during 2-s pulses to 40 mV in myocytes superfused with the K^+ -free solution. For the Hill equation fitting the data, $IC_{50} = 26 \mu\text{M}$ and slope factor = 0.84. Number of myocytes in parentheses.

Torsades de pointes is a malignant ventricular arrhythmia associated with a wide variety of drugs administered for their antiarrhythmic properties (e.g., quinidine, sotalol, amiodarone) or for entirely different purposes (e.g., terfenadine, erythromycin, terodiline), with the common outcome that they lengthen the Q-T interval (Roden et al., 1996; Woosley, 1996). The electrophysiological alterations responsible for this arrhythmia are not fully characterized, but it has been postulated that bradycardia, action potential prolongation, and early afterdepolarizations are crucial factors (Jackman et al., 1988; Thomas et al., 1995; Roden et al., 1996). We found no indications of either early or delayed afterdepolarizations in terodiline-treated muscles, most likely because development of these arrhythmogenic events is inhibited

when excitatory inward currents are suppressed (compare with Hayashi et al., 1987; January and Shorofsky, 1990; Woosley, 1996). These predicted antiarrhythmic influences of terodiline notwithstanding, concomitant blockade of Na^+ and K^+ channels has proarrhythmic potential related to slowed conduction and increased heterogeneity in repolarization time courses (see Follmer and Colatsky, 1990; Sicouri and Antzelevitch, 1993), especially when the block of the Na^+ channels is use-dependent (Starmer et al., 1991). Unlike guinea pig myocardium, transient outward current contributes to early repolarization in the human myocardium (Beuckelmann et al., 1993), and it is possible that terodiline affects this current in a proarrhythmic manner. Finally, other factors that predispose to acquired ventricular tachy-

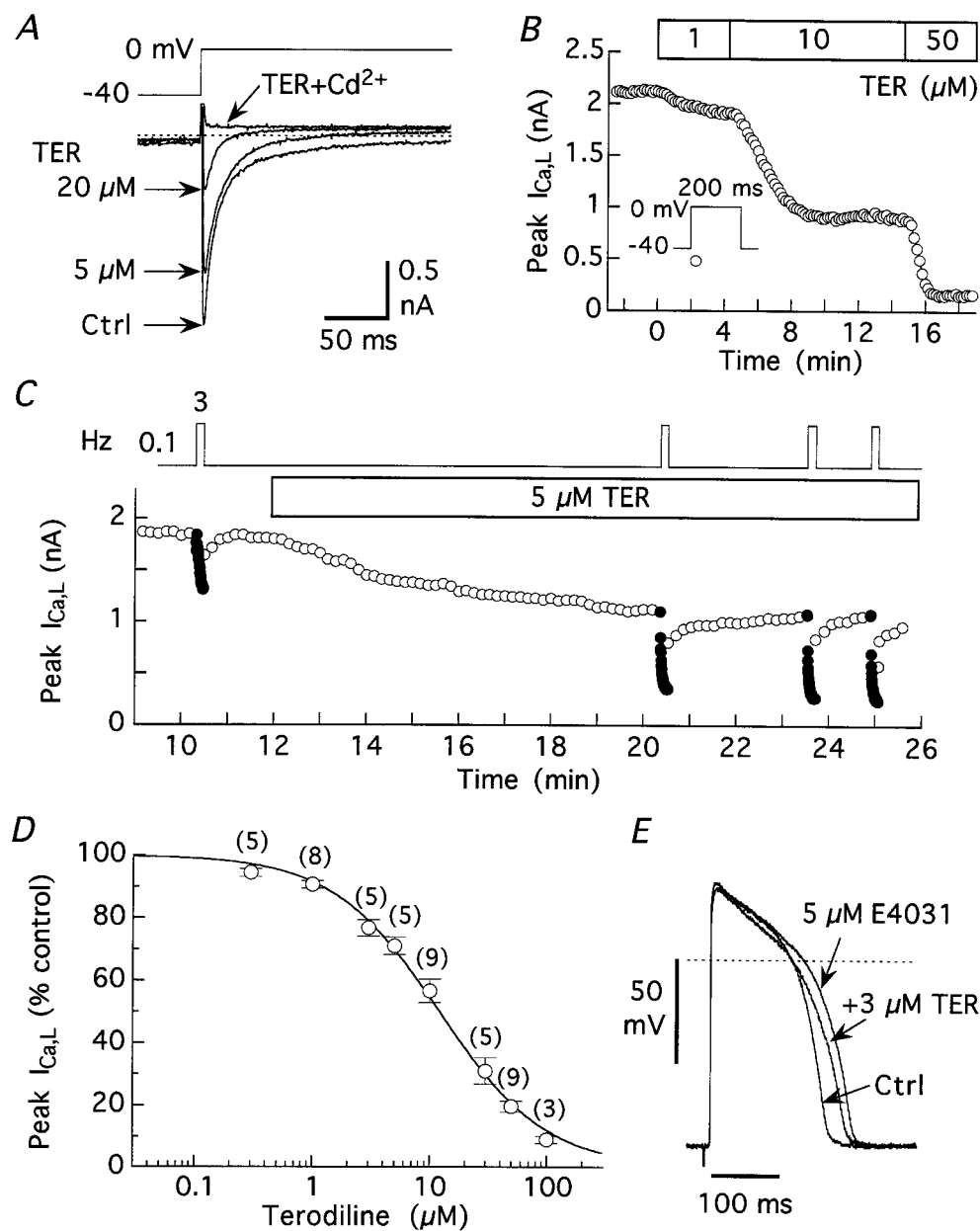


Fig. 7. Inhibition of $I_{\text{Ca,L}}$ by terodiline. Myocytes (A–D) were bathed in K^+ -free solution, dialyzed with Cs^+ solution, and regularly depolarized from -40 to 0 mV for 200 ms at 0.1 Hz. Exposures to terodiline were followed by the additions of 0.4 mM Cd^{2+} to establish background current levels at 0 mV for measurement of peak $I_{\text{Ca,L}}$. A and B, data from representative experiments. C, trains of 25 pulses at 3 Hz (\bullet) inhibited $I_{\text{Ca,L}}$ to a larger degree in the presence of the drug than in its absence. D, concentration-response relationship. For the Hill equation fitting the data, $\text{IC}_{50} = 12$ μM and coefficient = 0.9 . Number of myocytes in parentheses. E, terodiline-induced abbreviation of the action potential in a papillary muscle pretreated for 15 min with I_{Kr} -inhibiting E4031. Stimulation rate, 1 Hz.

arrhythmia (e.g., hypokalemia, myocardial ischemia, antiarrhythmic drugs) may be important in the cardiotoxicity of terodiline (Thomas et al., 1995).

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