British Journal of Pharmacology (1999) 127, 1837–1845
http://www.stockton-press.co.uk/bjp

Block and modified gating of cardiac calcium channel currents by terodiline

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1 Terodiline, an anticholinergic/antispasmodic drug effective in the treatment of urinary incontinence, is presently restricted due to adverse side effects on cardiac function. To characterize its effects on cardiac L-type Ca^{2+}-channel current carried by Ca^{2+} ($I_{Ca,L}$) and Ba^{2+} ($I_{Ba,L}$), concentrations ranging from 0.1 to 100 μM were applied to whole-cell-configured guinea-pig ventricular myocytes.

2 Although sub-micromolar concentrations of terodiline had no effect on $I_{Ca,L}$ at 0 mV, 100 μM drug reduced its amplitude to ca. 10% of pre-drug control. The estimated IC_{50} (15.2 μM in K^+-dialysed cells, 12.2 μM in Cs^+-dialysed cells; 0.1 Hz pulsing rate) is eight times higher than reported for $I_{Ca,L}$ in bladder smooth muscle myocytes.

3 Terodiline affected $I_{Ca,L}$ in a use-dependent manner; block increased when the pulsing rate was increased from 0.1 to 2–3 Hz, and when holding potential was lowered from −43 mV. The drug accelerated the decay of $I_{Ca,L}$ at 0 mV in a concentration-dependent manner, and slowed the recovery of channels from inactivation.

4 Terodiline reduced peak $I_{Ba,L}$ more effectively than peak $I_{Ca,L}$, and markedly accelerated the rate of inactivation of the current.

5 The results are discussed in terms of mechanisms of Ca^{2+} channel block and relation to the therapeutic and cardiotoxic effects of the drug.

Keywords: Terodiline; guinea-pig ventricular myocytes; L-type Ca^{2+} current ($I_{Ca,L}$); L-type Ba^{2+} current($I_{Ba,L}$); use-dependent block; enhanced inactivation

Abbreviations: ($I_{Ca,L}$), L-type Ca^{2+} current; ($I_{Ba,L}$), L-type Ba^{2+}current

Introduction

Terodiline is an anticholinergic agent (Husted *et al*., 1980) introduced in 1980 for the treatment of motor urge incontinence (Andersson & Ulmsten, 1980) and subsequently widely prescribed in Europe for a variety of urinary and other smooth muscle disorders (Andersson, 1984; Macfarlane & Tolley, 1984; Hallén *et al*, 1989; Langtry & McTavish, 1990). Terodiline antagonises Ca^{2+}-dependent contraction in smooth muscle tissues (Husted *et al*., 1980; Østergaard *et al*., 1980; Larsson-Backström *et al*., 1985), and this property is a factor in the effectiveness of the drug in urinary incontinence (Andersson, 1984; Langtry & McTavish, 1990). The antagonism probably reflects a block of L-type channels, and Kura *et al*. (1992) has reported that the drug inhibits guinea-pig bladder L-type Ca^{2+} current ($I_{Ca,L}$) with an IC_{50} of 1.7 μM.

Terodiline has adverse effects that include sinus slowing and conduction disturbances in the atrioventricular node (Connolly *et al*., 1991; Davies *et al*, 1991), as well as QT lengthening and polymorphic ventricular tachycardia (McLeod *et al*., 1991; Connolly *et al*., 1991; Stewart *et al*., 1992; Thomas *et al*., 1995). This cardiotoxic profile led to a worldwide withdrawal of terodiline in 1991. However, the drug is still authorized for clinical investigation, and derivatives are either under development for possible therapeutic use (e.g., Taniguchi *et al*., 1994; Take *et al*., 1996) or have already reached the market (Abrams *et al*., 1998).

There have been relatively few studies on the effects of terodiline on isolated cardiac preparations, but they suggest that the drug has a much weaker action on cardiac $I_{Ca,L}$ than on bladder myocyte $I_{Ca,L}$. For example, Larsson-Backström *et al*. (1985) found that terodiline inhibited rat papillary muscle contraction with an IC_{50} of 18 μM, and Hayashi *et al*. (1997) reported that the drug inhibited guinea-pig ventricular $I_{Ca,L}$ with an IC_{50} of 34 μM. We have recorded $I_{Ca,L}$ in guinea-pig ventricular myocytes, and used concentrations of terodiline between 0.1 and 100 μM (therapeutic plasma concentration 1–1.8 μM: Connolly *et al*., 1991) to evaluate concentration-, frequency- and voltage-dependent actions of the drug.

Methods

Ventricular myocytes

Guinea-pigs weighing 250–350 g were killed by cervical dislocation in accord with local and national regulations, and single ventricular myocytes were enzymatically isolated 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 that contained collagenase (0.08–0.12 mg ml^{-1}: Yakult Pharmaceutical Co., Tokyo, Japan) for 10–15 min. The cells were dispersed and stored at

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≈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^{-1}) with Tyrode's solution. The 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, the Tyrode's solution was exchanged for K^+-free Tyrode's or for K^+-free Ca^{2+}-free solution that contained 1.5 mM Ba^{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, U.K.) and filled with (i) K^+ solution that contained (in mM) KCl 40, potassium aspartate 106, Mg-ATP

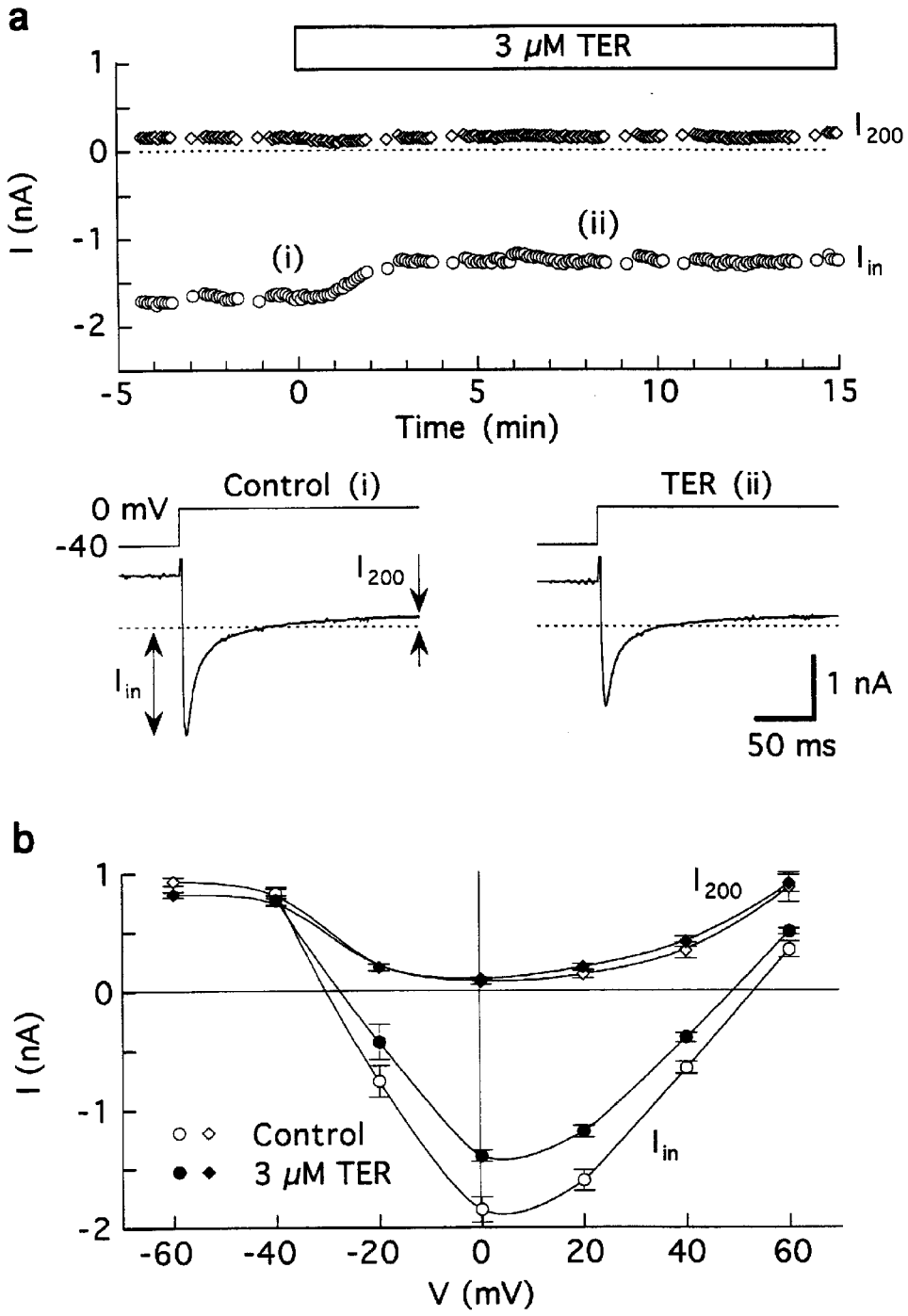

Figure 1 Effects of 3 μM terodiline on membrane currents in guinea-pig ventricular myocytes superfused with Tyrode's solution and dialysed with K^+ pipette solution. The myocytes were depolarised for 100 ms from −80 to −40 mV, and pulsed to 0 mV for 200 ms at 0.2 Hz except for determinations of *I*–*V* relationships. (a) Data from a representative experiment. Top: time course of changes in peak inward (I_{in}) and end-of-pulse current (I_{200}) amplitudes at 0 mV, measured with respect to zero current. Bottom: records obtained at the times indicated in the time plot. (b) Average of *I*–*V* relationships obtained before (control) and 9–12 min after addition of 3 μM drug; *n* = five myocytes.

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), or (ii) K^+-free solution (K^+ 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, U.S.A.) and pCLAMP software (Axon Instruments) at a sampling rate of 8 kHz prior to analysis.

The myocytes were generally held at −80 or ca. −40 mV, and pulsed to 0 mV for 200 ms at 0.1–0.2 Hz to elicit $I_{Ca,L}$; when held at −80 mV, the pulses to 0 mV were preceded by 100-ms depolarizations to −40 mV to inactivate Na^+ current and any T-type Ca^{2+} current. Experiments were conducted at 36±0.5°C.

Drugs

Terodiline (Sepracor Inc., Marlborough, MA, U.S.A.) was 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 0.01% v v^{-1} (100 μM drug), a concentration that has no significant effect on electrical and contractile activity in guinea-pig papillary muscle, or on membrane currents in guinea-pig ventricular myocytes (Ogura *et al.*, 1995). The vehicle was included in control solutions for the experiments depicted in Figures 4–6.

Statistics

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

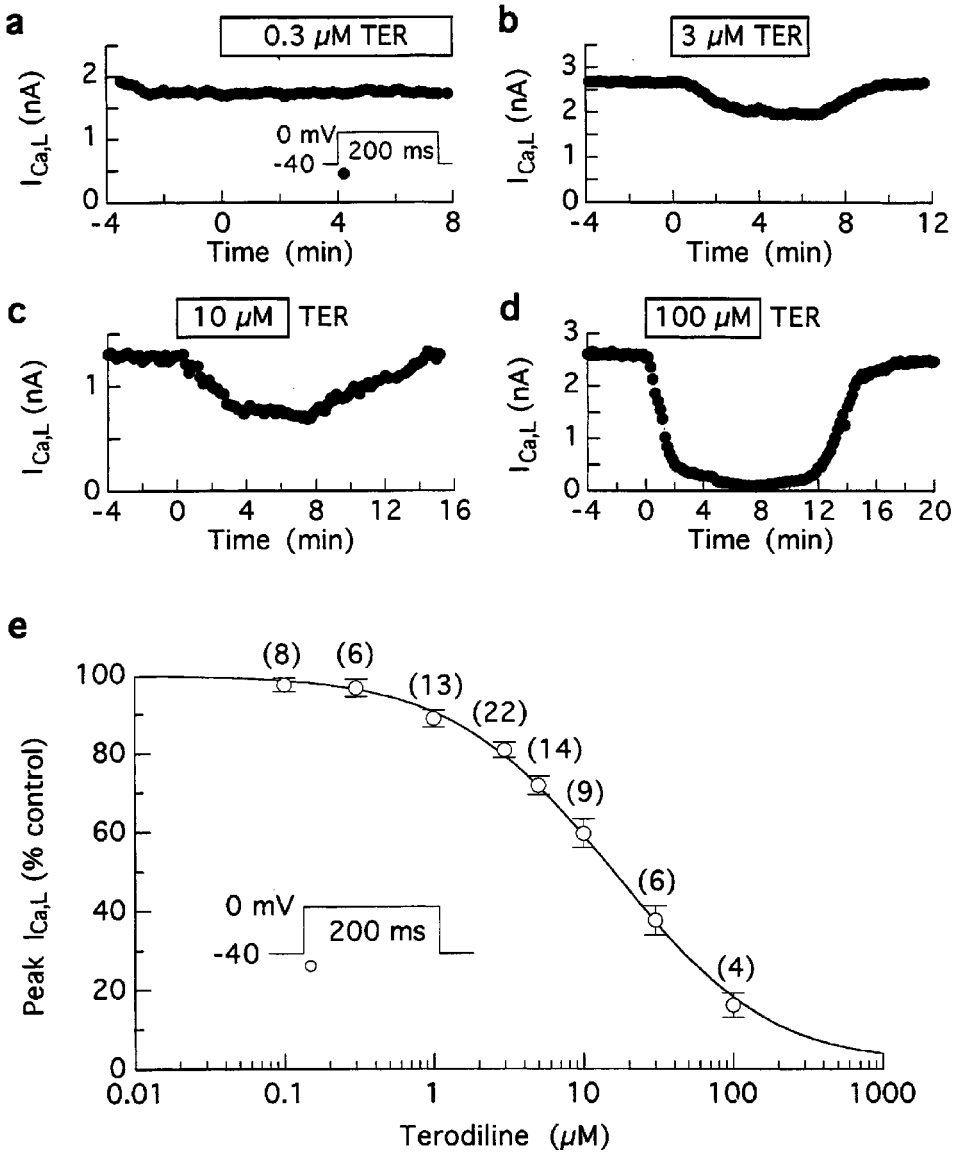

Figure 2 Concentration-dependent inhibition of $I_{Ca,L}$ amplitude by terodiline. $I_{Ca,L}$ at 0 mV was elicited by 200 ms pulses from −40 mV at 0.1 Hz in myocytes bathed and dialysed with K^+ solutions. (a-d) Results from representative experiments. (e) Concentration-response relationship. Most (>85%) of the myocytes were treated with a single concentration for 6–12 min, and $I_{Ca,L}$ amplitude measured as I_{in} was expressed as a percentage of pre-drug control. The data are described by a Hill equation, $I_{Ca,L}$ (% control) $= 100/\{1+([TER]/IC_{50})n_H\}$, with $IC_{50}=15.2$ μM, and Hill coefficient $n_H=0.84$. Number of myocytes in parentheses.

Results

Concentration-dependent inhibition of $I_{Ca,L}$

Figure 1 depicts the results obtained from five myocytes bathed and dialysed with K^+ solutions, depolarized with 200-ms pulses applied at 0.2 Hz, and treated with 3 μM terodiline for 9–12 min. The drug reduced estimated $I_{Ca,L}$ (peak inward current (I_{in})) at 0 mV by 26±4% ($P<0.005$) (Figure 1b), with little effect on the end-of-pulse outward current (I_{200}) level. The inhibition was independent of test potential between −20 and +20 mV; at more positive potentials, I_{in} curves converged on I_{200} curves in a manner suggesting little effect on the reversal potential of $I_{Ca,L}$.

The time plots in Figure 2a–d illustrate that submicromolar terodiline had little effect on $I_{Ca,L}$ whereas 3, 10 and 100 μM drug reversibly inhibited the current by 25, 40 and 95%, respectively. The results obtained from these and similar experiments are well described by a Hill equation, $I_{Ca,L}$ (% control) = $100/\{1+([TER]/IC_{50})n_H\}$, in which the IC_{50} is 15.2 μM, and the Hill coefficient is 0.84 (Figure 2e).

Dependence of inhibition on holding potential and stimulation rate

To investigate whether inhibition of $I_{Ca,L}$ by terodiline is sensitive to depolarization, the holding potential in myocytes pulsed to 0 mV was changed from ca. −43 mV to more positive potentials for short (2- to 3-min) test periods. Although lowering of the holding potential reduced control $I_{Ca,L}$ (as expected from a voltage-dependent reduction in Ca^{2+} channel availability), the effect was much larger after addition of the drug (Figure 3a,b). In trials on 23 myocytes treated with 1 or 5 μM terodiline, the degree of drug-induced inhibition was assessed at holding potentials of −43, −35, −30 and −25 mV. Each reduction in holding potential accentuated

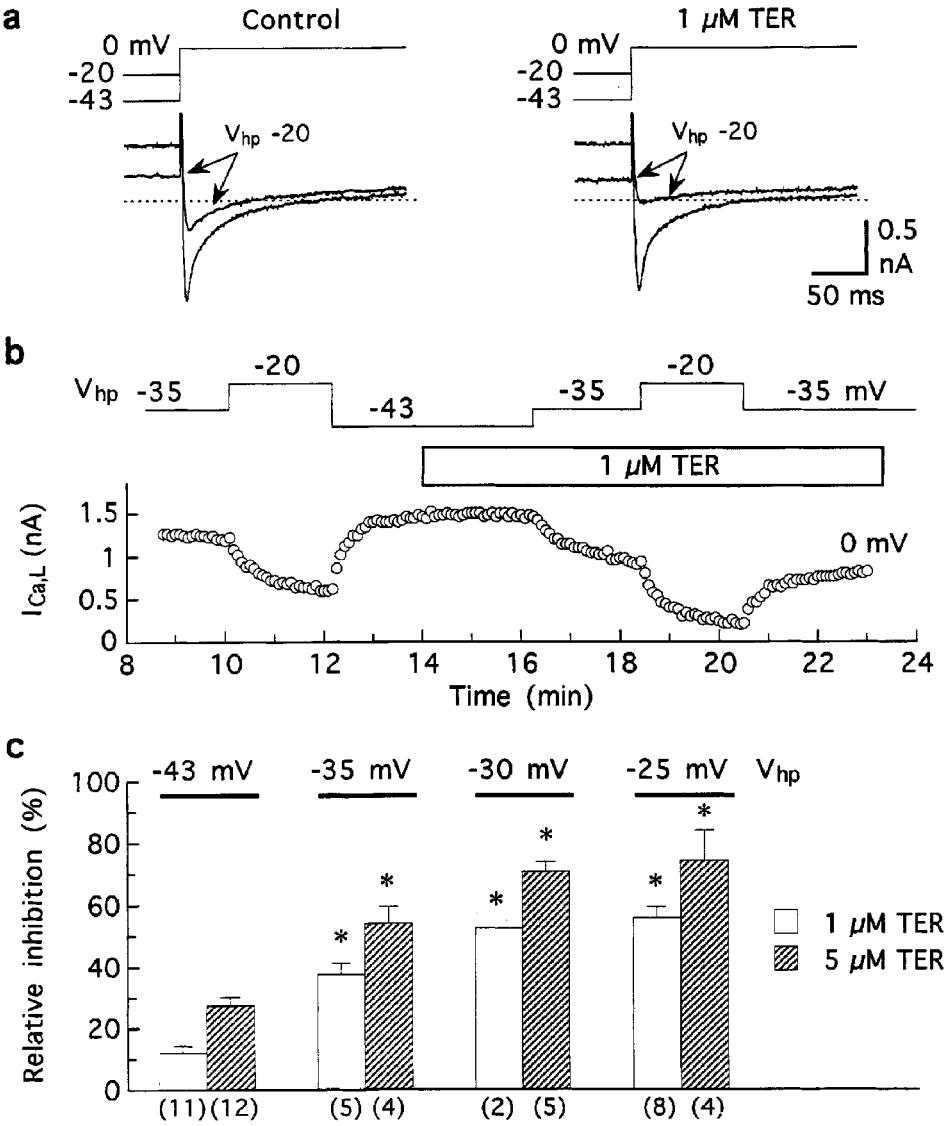

Figure 3 Effect of holding potential on the inhibition of $I_{Ca,L}$ by terodiline. Myocytes bathed and dialysed with K^+ solutions were held at different potentials and pulsed to 0 mV at 0.2 Hz. (a) Records showing the steady-state effects of changing the holding potential (V_{hp}) from −43 to −20 mV for 3 min before (left), and 6 min after addition of 1 μM drug. (b) Time course of changes in $I_{Ca,L}$ amplitude induced by changes in holding potential before and during exposure to 1 μM drug. To minimize the effects of the shift in late current on $I_{Ca,L}$ measurements, the amplitude was measured as $I_{200}-I_{in}$ (see Figure 1). (c) Summary of data obtained from myocytes treated with 1 or 5 μM drug. Inhibition at each holding potential is expressed relative to pre-drug current elicited at 0 mV from that holding potential. Numbers of myocytes in parentheses. *$P<0.001$ versus relative inhibition at −43 mV.

inhibition by the drug (Figure 3c). For example, relative inhibition by 1 μM terodiline was $12\pm2\%$ ($n=11$ observations) at -43 mV holding potential, $33\pm5\%$ ($n=5$) at -35 mV, and $56\pm4\%$ ($n=8$) at -25 mV.

To investigate whether inhibition is sensitive to stimulation rate, myocytes were regularly pulsed for 200 ms from -40 to 0 mV at 0.1 Hz, and periodically tested with trains of 25 pulses at 3 Hz. Since the 133-ms period between pulses in a train was too short for full recovery of $I_{Ca,L}$ from inactivation, these trains provoked significant reductions in $I_{Ca,L}$ under pre-drug control conditions (*c.f.*, Boyett *et al.*, 1994; Asai *et al.*, 1996) (Figure 4a). Relative to $I_{Ca,L}$ amplitude at 0.1 Hz just prior to a 3-Hz train, the reductions caused by the trains were more severe in the presence of the drug (Figure 4a). In seven myocytes, $I_{Ca,L}$ at 3 Hz declined to $32\pm5\%$ of its 0.1-Hz amplitude after treatment with 5 μM terodiline, compared to pretreatment decline to $68\pm5\%$ ($P<0.001$). Smaller effects were observed with lower terodiline and lower test frequency. For example, an increase from 0.1 to 2 Hz in the presence of 1 μM terodiline reduced $I_{Ca,L}$ to $74\pm5\%$ ($n=4$) *versus* pretreatment $87\pm5\%$ ($P<0.01$).

A plausible explanation for the frequency-dependent component of terodiline action is that the drug slows the recovery of $I_{Ca,L}$ from inactivation. To determine whether this is the case, 200-ms double pulses to 0 mV were applied to ten myocytes held at -45 mV, with recovery time (separation between the two pulses) ranging from 10 ms to 5000 ms. The time course of recovery prior to drug treatment was adequately

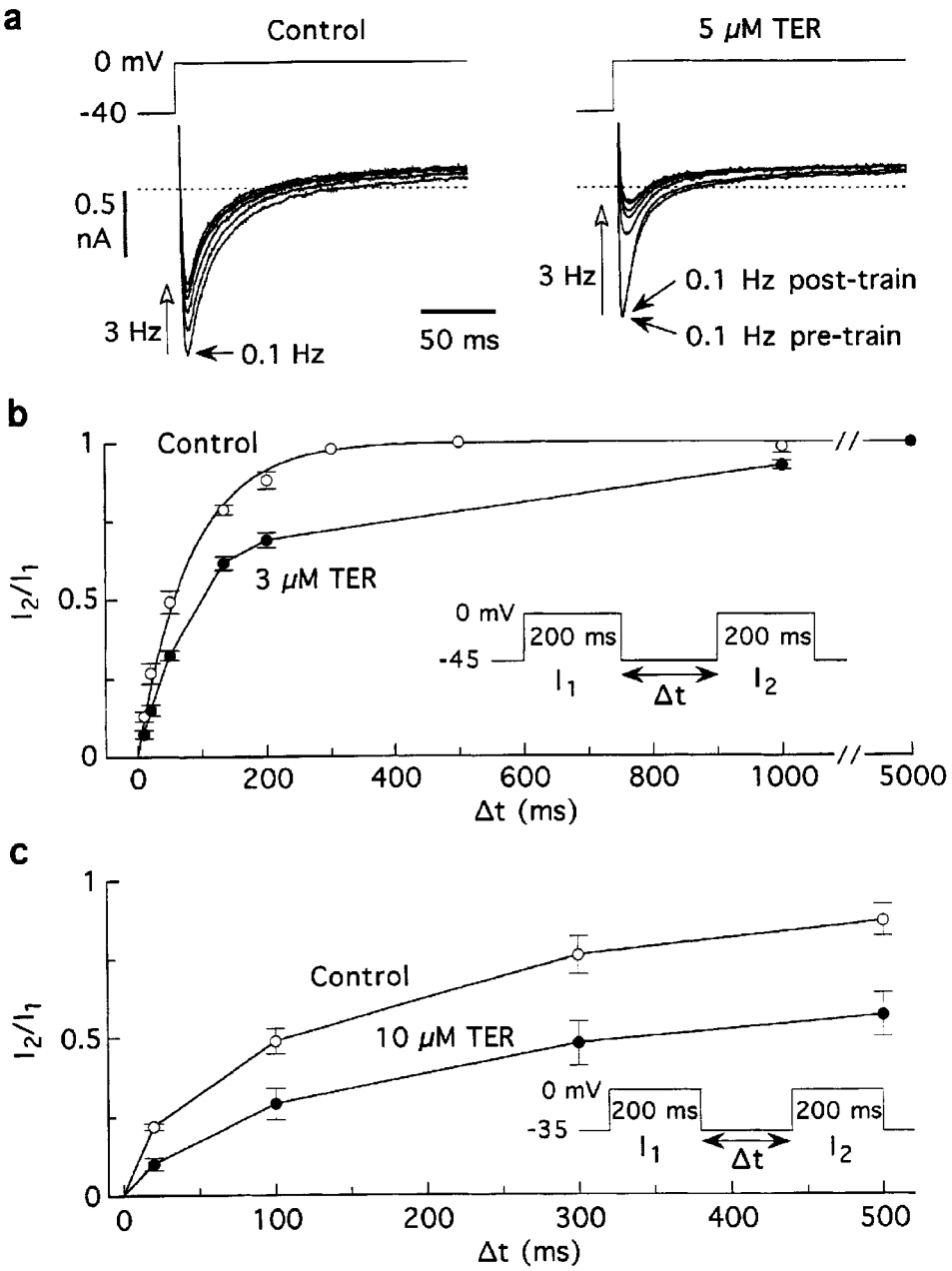

Figure 4 Terodiline enhances frequency-dependent reduction of $I_{Ca,L}$ and slows recovery of the current from inactivation. Myocytes were bathed and dialysed with K^+ solutions, and pulsed at a basic rate of 0.1 Hz. (a) Records illustrating the reduction of $I_{Ca,L}$ induced by 3-Hz trains of 25 pulses before and 6 min after addition of 5 μM terodiline. (b) Time courses of recovery of $I_{Ca,L}$ from 200-ms inactivating pulses before and *ca.* 5 min after addition of 3 μM terodiline. The exponential fitting the control data ($n=10$ myocytes) has $\tau=82$ ms. A point-to-point line is used to connect the terodiline data since they are poorly described by a single exponential (c) Recovery from inactivation in four myocytes held at -35 mV and treated with 10 μM terodiline.

described by an exponential with time constant 82 ms, whereas that determined after *ca.* 5 min treatment with 3 μM terodiline was slower and no longer monoexponential (Figure 4b). When the time course of recovery was characterised in terms of halftime, the drug slowed the process by a factor of 1.9. Four other myocytes held at -35 mV were tested with recovery intervals of 20, 100, 300 and 500 ms. The results in Figure 4c indicate that 10 μM terodiline slowed the halftime of recovery from 105 to 340 ms.

Acceleration of current decay

Terodiline accelerated the decay of Ca^{2+} channel currents observed during 200-ms depolarisations to 0 mV and this effect was more pronounced at higher drug concentrations (Figure 5a). Using $I_{200}-I_{in}$ as an estimate of the time course of $I_{Ca,L}$ in myocytes investigated under standard conditions (Tyrode's solution; K^+ dialysate) (Figure 5a), the time to half-decay declined from control 10.2 ± 0.7 ms to 8.0 ± 0.7 ms ($n=8$) ($P<0.005$) after exposure to 5 μM terodiline.

To investigate the acceleratory effects of terodiline in greater detail, K^+-free internal and external solutions were used to minimise K^+ currents, and 0.4 mM Cd^{2+} was used to establish a background–current reference level for measurement of Ca^{2+}-channel currents carried by either Ca^{2+} or Ba^{2+}. In the first series of experiments, $I_{Ca,L}$ elicited by 200-ms pulses to 0 mV was recorded before and during exposure to 0.3–100 μM terodiline. The representative results in Figure 5b

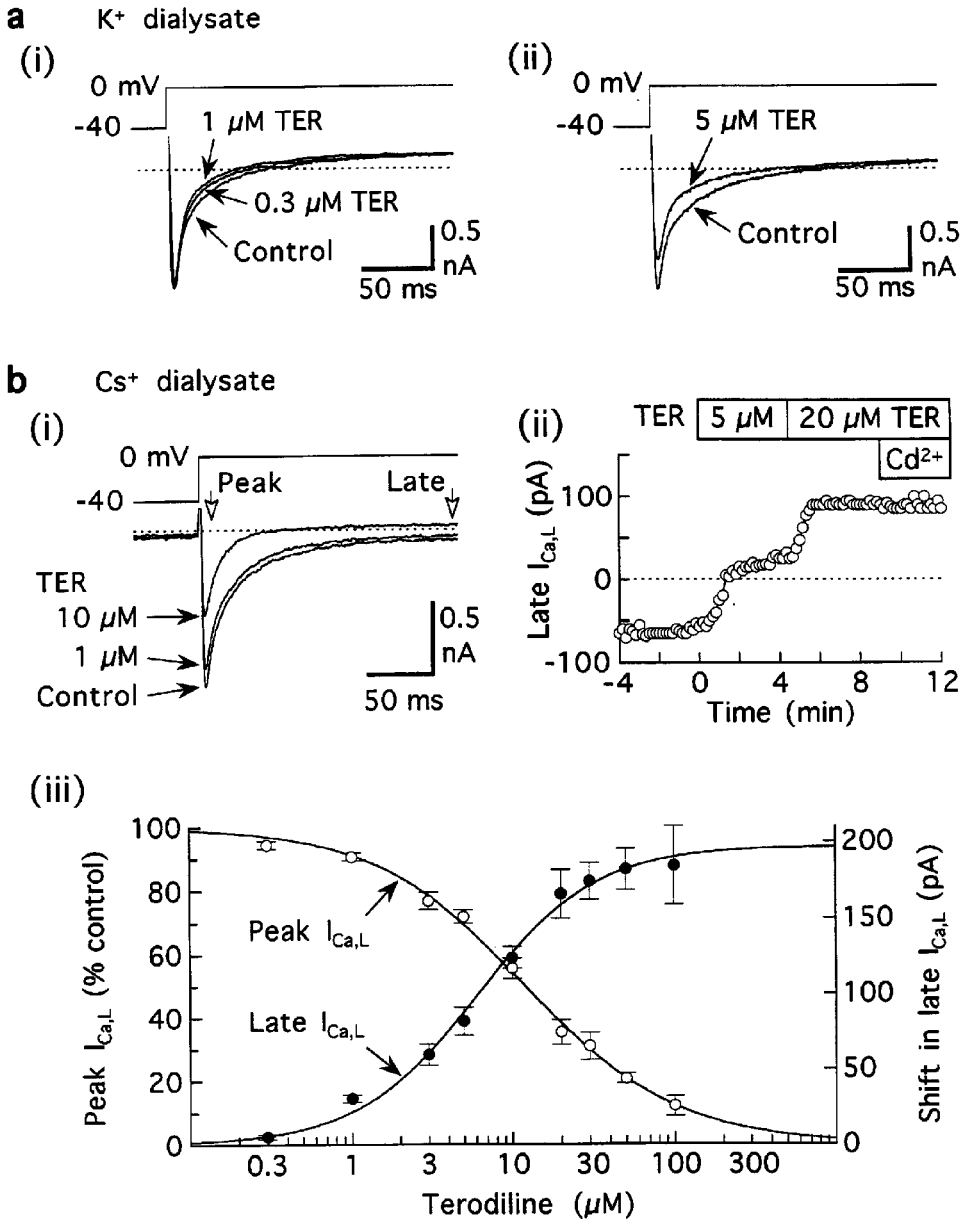

Figure 5 Acceleration of inactivation of $I_{Ca,L}$ by terodiline. (a) Records from myocytes superfused and dialysed with K^+ solutions, and pulsed at 0.1 Hz. (i) Acceleration in the absence of significant reduction of $I_{Ca,L}$ amplitude in a myocyte treated with 0.3 μM terodiline for 5 min and 1 μM terodiline for a further 5 min. (ii) Acceleration in a myocyte treated with 5 μM drug for 3 min. Relatively large outward currents at holding potential -40 mV have been omitted for presentation purposes. (b) Results from experiments in which K^+-free solutions were used to minimize K^+ currents. (i) Records from a representative myocyte treated for 6 min (1 μM) and then 4 min (10 μM). (ii) Time course of shifts in late $I_{Ca,L}$. (iii) Concentration-response relationships for Ca^{2+}-sensitive peak $I_{Ca,L}$ (open circles) and shift in late $I_{Ca,L}$ (closed circles). Pulsing rate 0.1 Hz. Number of myocytes: 4–14 at each concentration.

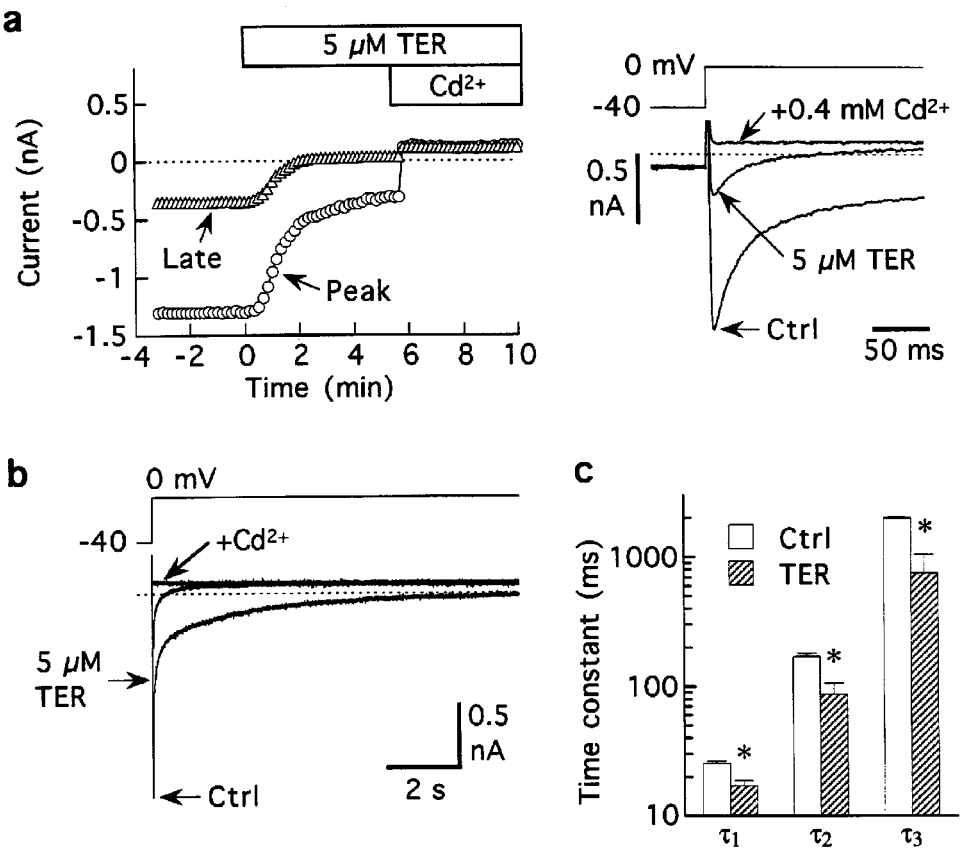

Figure 6 Inhibition of Ba^{2+}-carried current by 5 μM terodiline. (a) Representative results. Note that inhibition of late $I_{Ba,L}$ was larger than inhibition of peak $I_{Ba,L}$. Pulsing rate 0.1 Hz. (b) Superimposed records showing the first 10 s of currents elicited by 20-s depolarizations before, 6 min after addition of 5 μM terodiline, and 3 min after subsequent addition of 0.4 mM Ca^{2+}. (c) Time constants obtained from multiexponential analysis of Cd^{2+}-sensitive $I_{Ba,L}$ recorded from five myocytes investigated as in (b) $^*P<0.05$.

indicate that the drug inhibited both peak $I_{Ca,L}$ and late (end-of pulse) $I_{Ca,L}$. The IC_{50} for inhibition of peak $I_{Ca,L}$ was 12 μM, and the IC_{50} for inhibition of late $I_{Ca,L}$ was 6 μM (Figure 5c). This discrepancy in IC_{50} values suggests that post-peak-$I_{Ca,L}$ decayed more rapidly after addition of terodiline.

For the experiments on Ba^{2+}-carried current ($I_{Ba,L}$), external Ca^{2+} was replaced by 1.5 mM Ba^{2+}, and myocytes were pulsed at 0.1 Hz with 200-ms steps to 0 mV. The results from a representative myocyte indicate that 5 μM terodiline had a pronounced inhibitory effect on peak $I_{Ba,L}$, and an even stronger one on late $I_{Ba,L}$ (Figure 6a). In eleven myocytes, 5 μM drug reduced peak Cd^{2+}-sensitive $I_{Ba,L}$ to 43$\pm$2% control, and reduced late Cd^{2+}-sensitive $I_{Ba,L}$ to 24$\pm$1% control, a difference ($P<0.001$) that is best explained as an acceleration of inactivation. To establish the extent of the acceleration, five myocytes otherwise pulsed to 0 mV for 200 ms at 0.1 Hz, were probed with single 20-s depolarizations to 0 mV on three occasions: before addition of 5 μM terodiline, after steady-state inhibition, and after subsequent nulling of the current with 0.4 mM Cd^{2+}. Although control $I_{Ba,L}$ failed to inactivate completely during 20-s depolarizations prior to addition of terodiline, it decayed to the background current level within 5 s when the drug was present (Figure 6b). As previously reported by Boyett *et al.* (1994), the time course of inactivation of Cd^{2+}-sensitive $I_{Ba,L}$ was best-described by up to four exponential functions. However, the salient features are captured by the first three exponentials (time constants 25$\pm$1, 171$\pm$9, 1982$\pm$38 ms), each of which was significantly ($P<0.05$) shortened by the drug (time constants 17$\pm$2, 87$\pm$19, 749$\pm$290 ms) (Figure 6c).

Discussion

Concentration-dependent inhibition of peak $I_{Ca,L}$

Terodiline induced a concentration-dependent reduction in the amplitude of $I_{Ca,L}$ in guinea-pig ventricular myocytes pulsed to 0 mV at 0.1–0.2 Hz. Data from myocytes bathed and dialysed with K^+ solutions were best described by a Hill equation with IC_{50} of 15.2 μM, whereas data on Cd^{2+}-sensitive peak $I_{Ca,L}$ from myocytes investigated under K^+-free conditions were best described with an IC_{50} of 12.2 μM. The estimate determined under K^+ conditions is considerably lower than the $K_{0.5}$ of 33.5 μM reported by Hayashi *et al.* (1997) in a study on guinea-pig ventricular myocytes under similar (K^+, temperature, pulsing rate) experimental conditions. A difference in methodology is that they measured the effects of short ($\approx$3 min) cumulative exposures to six terodiline concentrations between 1 and 300 μM, and it is possible that this skewed the results to some degree.

The IC_{50} determined for ventricular $I_{Ca,L}$ is about eight times larger than the 1.7-μM value reported by Kura *et al.* (1992) for inhibition of $I_{Ca,L}$ in guinea-pig bladder smooth muscle myocytes. One difference in the behaviour of $I_{Ca,L}$ in the two studies is that pre-drug $I_{Ca,L}$ in the bladder myocytes was strongly affected by relatively low pulsing frequencies; a *ca.* 40% reduction in amplitude occurred during short 0.2-Hz trains of depolarisations to 0 mV, presumably because the recovery of $I_{Ca,L}$ from inactivation was slow at the low (22°C) experimental temperature employed in the study. It may be that measurement difficulties arising from calculations of

terodiline inhibition based on relative control- and terodiline-induced use-dependent inhibition contributed to over-estimates of terodiline action at the two concentrations (1 and 10 μM) whose effects were fitted with a Michaelis-Menten equation to determine the IC_{50}. However, this is speculation, and the difference in IC_{50} values may instead reflect a significant difference in the sensitivity of $I_{Ca,L}$ to terodiline in the two cell types.

Inhibition of peak $I_{Ba,L}$

$I_{Ba,L}$ was more sensitive than $I_{Ca,L}$ to inhibition by terodiline. Peak $I_{Ba,L}$ was reduced to $43\pm2\%$ ($n=11$) by 5 μM drug, whereas peak $I_{Ca,L}$ in similar (Cs^+-dialysed) myocytes was only reduced to $72\pm2\%$ ($n=7$) by 5 μM drug ($P<0.001$ *versus* $I_{Ba,L}$), and to $56\pm3\%$ ($n=13$) by 10 μM drug ($P<0.01$ *versus* $I_{Ba,L}$). Based on the peak $I_{Ca,L}$ data presented in Figure 5c, terodiline was about 2.7 times more inhibitory on peak $I_{Ba,L}$ than $I_{Ca,L}$. In a recent study on fendiline, a drug with structural similarities to terodiline, Nawrath *et al*. (1998) found that the IC_{50} was 8 μM for $I_{Ba,L}$ and 13 μM for $I_{Ca,L}$. They suggested that the more potent inhibition of $I_{Ba,L}$ was related to the enhanced openness of Ca^{2+} channels when Ba^{2+} is the charge-carrier, and a similar reason could be advanced in regard to terodiline action.

Use-dependent inhibition

In their study on bladder smooth muscle myocytes, Kura *et al*. (1992) observed that inhibition of $I_{Ca,L}$ by 10 μM terodiline developed more quickly when myocytes were pulsed at 1 Hz rather than 0.1 Hz. The present results indicate that terodiline-induced block of $I_{Ca,L}$ in guinea-pig ventricular cells is enhanced by increases in pulsing frequency. They also establish that inhibition by terodiline is favoured by lower holding potentials, and that recovery of $I_{Ca,L}$ from inactivation is slowed in drug-treated myocytes, two properties that are associated with a number of organic compounds that block L-type Ca^{2+} channels (McDonald *et al*., 1994).

The decay of $I_{Ca,L}$ at 0 mV was accelerated when myocytes were exposed to terodiline. The acceleration was evident in myocytes treated with low micromolar concentrations that had little effect on $I_{Ca,L}$ amplitude, and was more pronounced in myocytes treated with higher concentrations of the drug. As a consequence, late $I_{Ca,L}$ flowing at 0 mV declined with increasing drug concentration. Terodiline also accelerated the decay of $I_{Ba,L}$, a long-lasting current that inactivates with a multi-exponential time course (Boyett *et al*., 1994), and analysis of this action indicates that the drug shortens the time constant of the three major phases of the inactivation.

There is evidence that some organic Ca^{2+} channel blockers accelerate the decay of Ca^{2+} channel currents, and that others do not (McDonald *et al*., 1994). Nawrath *et al*. (1998) have recently reported that fendiline illustrates this diversity: 10 μM fendiline accelerated the decay of $I_{Ba,L}$ in guinea-pig ventricular myocytes, whereas 10 μM verapamil did not. They postulated that verapamil does not affect the time course of current decay during a depolarization because it primarily binds to inactivated channels. However, fendiline is postulated to block open channels, and the resultant time-dependent removal of channels from the conducting pool is observed as a speeding up of the decay of the current. To estimate the time course of this block, they analysed the 'fractional block' of $I_{Ba,L}$ elicited by 300-ms pulses to +10 mV, and concluded that block occurred along a monoexponential time course with $\tau\approx$ 100 ms.

Our results with terodiline are not as tidy as those with fendiline because a similar type of fractional block analysis failed to identify a monoexponential process. One could argue that the requirement for a multiexponential description for the fractional block reflects differing rates of binding to the multiple open states of Ca^{2+} channels conducting Ba^{2+}. Alternatively, acceleration of the multiple phases of decay of $I_{Ba,L}$ may reflect actual acceleration of multiple inactivation process (Figure 6). The present results do not warrant any additional speculation, especially since the physical meaning of the multiexponential decay of long-lasting $I_{Ba,L}$ is obscure (*c.f*., Boyett *et al*., 1994).

Relation to clinical observations

Use-dependent block of L-type Ca^{2+} channels may well have been an important factor in the treatment of unstable bladder with terodiline, not only because the bladder muscle cells-themselves are in a hyperactive state (Brading & Turner, 1994), but also because activity-dependent facilitation of prejunctional L-type channels appears to be of clinical significance in unstable bladder conditions (Somogyi *et al*., 1997). Since M_1 muscarinic receptors have an important role in this facilitation (Somogyi *et al*., 1997), and M_1 antagonism by terodiline is as potent as that of the classical M_1 antagonist pirenzipine (Noronha-Blob *et al*., 1991; Gallo *et al*., 1993), there is scope for a synergistic stabilizing action of the drug on bladder function.

The primary cardiotoxic effects of terodiline include prolongation of the QT interval and ventricular tachyarrhythmia. Inhibition of $I_{Ca,L}$ is unlikely to be the cause of these developments, and may even help prevent their occurrence. However, inhibition of the current may well be a contributory factor in the slowing of heart rate and nodal conduction observed in patients with high plasma concentrations of terodiline (up to 9.6 μM: Connolly *et al*., 1991; Stewart *et al*., 1992).

We thank Ms Gina Dickie for laboratory assistance and Mr Brian Hoyt for technical support. L.M. Shuba held a scholarship from the Dalhousie Medical Research Foundation. The study was funded by Sepracor Inc. and the Medical Research Council of Canada.

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(Received March 18, 1999
Accepted May 7, 1999)