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Differences in the effects of urinary incontinence agents S-oxybutynin and terodiline on cardiac K^+ currents and action potentials

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1 The cardiac electrophysiological effects of S-oxybutynin, a single-enantiomer drug under evaluation for the management of urinary incontinence, have been investigated and compared with those of terodiline, an incontinence agent withdrawn following reports of QT lengthening and ventricular tachyarrhythmia. Membrane currents were recorded from whole-cell configured guinea-pig and rabbit ventricular myocytes, and action potentials were recorded from guinea-pig and rabbit papillary muscles.

2 L-type Ca^{2+} current ($I_{Ca,L}$), rapidly-activating K^+ current (I_{Kr}) and slowly-activating K^+ current (I_{Ks}) were unaffected by submicromolar S-oxybutynin and inhibited by higher concentrations; IC_{50} values were 17.8 μM for $I_{Ca,L}$, 12 μM for I_{Kr}, and 41 μM for I_{Ks}. Terodiline IC_{50} values were somewhat lower for $I_{Ca,L}$ (15.2 μM) and I_{Ks} (30 μM), but 24 fold lower in the case of I_{Kr} (0.5 μM).

3 The durations of action potentials in guinea-pig and rabbit papillary muscles driven at 1 Hz were unaffected or moderately shortened by 0.1–100 μM S-oxybutynin, but lengthened by terodiline. Terodiline ($\leqslant$10 μM) also depressed maximal upstroke velocity.

4 The action potential plateau shortened by an average of 23% when control rabbit papillary muscles were driven at 0.4 Hz instead of 1 Hz. Plateau shortening was significantly smaller in the presence of drugs (30 μM S-oxybutynin, 3 and 30 μM terodiline), suggesting that they suppress the transient outward current (I_{to}) involved in rate-dependent shortening. In experiments on rabbit ventricular myocytes, 3 and 30 μM S-oxybutynin inhibited I_{to} by 9 ± 2% and 35 ± 3%, respectively, whereas 3 and 30 μM terodiline inhibited the current by 31 ± 3% and 87 ± 3%, respectively.

5 The results indicate that S-oxybutynin has relatively weak non-specific effects on cardiac ion channels, and that clinically relevant submicromolar concentrations are unlikely to have terodiline-like proarrhythmic actions on the myocardium.

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Abbreviations: APA, action potential amplitude; APD_{20}, action potential duration at 20% repolarization level; APD_{90}, action potential duration at 90% repolarization level; APD_{0mV}, action potential duration at 0 mV; DMSO, dimethyl sulphoxide; EGTA, ethylene glycol-bis(b-aminoethyl)-N,N,N,N-tetraacetic acid; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; IC_{50}, concentration that produces 50% of maximal inhibition; $I_{Ca,L}$, L-type Ca^{2+} current; I_K, delayed-rectifier K^+ current; I_{K1}, inward-rectifying K^+ current; I_{Kr}, rapidly-activating component of I_K; I_{Ks}, slowly-activating component of I_K; I_{to}, transient outward current; I-V, current-voltage relationship; P, probability (significance level); s.e.m., standard error of the mean; $\dot{V}$, upstroke velocity; $\dot{V}_{max}$, maximal upstroke velocity

Introduction

Micturation dysfunction is a major medical and social problem that has a particularly high incidence in young children and older adults (Thomas *et al*., 1980; Fonda, 1989; Caione *et al*., 1997; Hjalmas, 1997; Theodorou *et al*., 1998). Management with anticholinergic drugs is a long-standing practice (Yarker *et al*., 1995; Wein, 1998), and it is believed that anticholinergics with Ca^{2+} antagonistic activity are particularly effective (Andersson, 1984; Smith *et al*., 1998). One such dual-action drug, terodiline, secured a prominent share of the market after its introduction in 1986 (Langtry & McTavish, 1990). However, it was withdrawn five years later amid reports of cardiotoxicity (conduction disturbances, QT prolongation, ventricular tachyarrhythmia (torsades de pointes)) (Connolly *et al*., 1991; McLeod *et al*., 1991; Stewart *et al*., 1992). In the interim, oxybutynin has emerged as a drug of choice for relief of symptoms such as urinary urge with or without incontinence, nocturia, enuresis, postsurgical bladder dysfunction, neurogenic spastic bladder, and detrusor muscle instability (Yarker *et al*., 1995; Åmark *et al*., 1998; Theodorou *et al*., 1998). Like terodiline, it is a tertiary amine that has anticholinergic activity and direct relaxant effects on smooth muscle (Lish *et al*., 1965; Fredericks *et al*., 1975; Tonini *et al*., 1987; Noronha-Blob & Kachur, 1991; Smith *et al*., 1998).

A major problem in the management of voiding dysfunction with anticholinergic agents is the high incidence of side-effects (e.g. dry mouth) that may lead patients to discontinue

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treatment (Langtry & McTavish, 1990; Yarker *et al*., 1995; Buyse *et al*., 1998). Compared to the incidence of such side-effects with oxybutynin, a lower incidence is the major advantage claimed for the recently-introduced terodiline derivative, tolterodine (Abrams *et al*., 1998), as well as for S-oxybutynin, a stereoisomer that is currently undergoing evaluation as a urinary incontinence agent (Koch *et al*., 1998; Smith *et al*., 1998). As part of that process, we have investigated the effects of the drug on cardiac membrane currents and action potentials, and compared them with those of terodiline.

Methods

All procedures were carried out in accordance with national and university regulations on the care and treatment of laboratory animals. Male guinea-pigs (250–300 g) were killed by cervical dislocation, whereas male New Zealand white rabbits (ca. 1.5 kg) were anaesthesized by injection of sodium pentobarbitone (40 mg kg^{-1}) into a marginal ear vein, and killed by removal of the heart. Papillary muscles and ventricular myocytes were prepared as described below.

Papillary muscles

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), and papillary muscles were excised from right ventricles. The muscles were mounted in a Perspex bath (0.25 ml volume) perfused with solution (36°C ± 0.2°C) at 4–6 ml/min, 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), 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, U.S.A.) and recorded on film, by a chart recorder (model RS 3400, Gould, Cleveland, OH, U.S.A.), and/or by computer *via* Axoscope (Axon Instruments, Foster City, CA, U.S.A.).

Ventricular myocytes

Whole-cell membrane currents were recorded from guinea-pig and rabbit ventricular myocytes. The myocytes were enzymatically isolated as described previously (Ogura *et al*., 1995). Excised hearts were mounted on a Langendorff column, and retrogradely perfused through the aorta with Ca^{2+}-free Tyrode's solution (37°C) containing collagenase (0.08–0.12 mg/ml: 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. 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 (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 10, and N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid (HEPES) 5 (pH 7.4 with NaOH).

All of the experiments on guinea-pig ventricular myocytes were conducted with normal or modified Tyrode's solution warmed to 36°C. The modified solutions included K^+-free Tyrode's solution (KCl omitted) and K^+-, Ca^{2+}-free Tyrode's solution (KCl and $CaCl_2$ omitted) that also contained 0.2 mM Cd^{2+} to suppress Ca^{2+} channel current. The experiments on rabbit ventricular myocytes were designed for measurement of transient outward current. To isolate the current, the bathing solution (normal Tyrode's at 24 or 36°C) contained 0.2 mM Cd^{2+}, 0.1 mM Ba^{2+}, 3 μM E4031, and 20 μM tetrodotoxin.

Membrane currents were recorded using an EPC-7 amplifier (List Electronic, Darmstadt, Germany) in the whole-cell patch configuration. 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, $MgCl_2$ 1, K_2-ATP 4, ethylene glycol-bis(b-aminoethyl ether)-N,N,N,N-tetraacetic acid (EGTA) 5, and HEPES 5 (pH 7.2 with KOH), or (ii) Cs^+ 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%. Cell capacitance ranged from 80 to 130 pF. 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 prior to analysis.

Drugs

S-oxybutynin and terodiline were supplied as hydrochloride salts by Sepracor Inc. (Marlborough, MA, U.S.A.), and E4031 was supplied by Eisai (Tokyo, Japan). These agents were dissolved in dimethyl sulphoxide (DMSO) (Sigma Chemical Co., St. Louis MO, U.S.A.) immediately prior to addition to the superfusate. The highest final concentration of DMSO in the superfusate was 0.03% (100 μM drug), a concentration of DMSO that has no significant effect on electrical activity or membrane currents in guinea-pig ventricular cells (Ogura *et al*., 1995). Tetrodotoxin (Calbiochem, La Jolla, CA, U.S.A.) and 4-aminopyridine (Sigma) were dissolved in distilled water.

Statistics

Results are expressed as means ± s.e.mean. Single comparisons were made using Student's *t*-test, and multiple comparisons were made using one-way ANOVA followed by the Bonferroni test. Differences were considered significant when $P < 0.05$.

Results

Effects of S-oxybutynin and terodiline on membrane current in guinea-pig ventricular myocytes

Figure 1a,b shows examples of whole-cell membrane currents recorded when myocytes were depolarized from prepulse −40 mV to more positive potentials for 500 ms before and after addition of S-oxybutynin and terodiline. Identifiable components in the records include inward L-type Ca^{2+} current ($I_{Ca,L}$) that reached maximal amplitude on

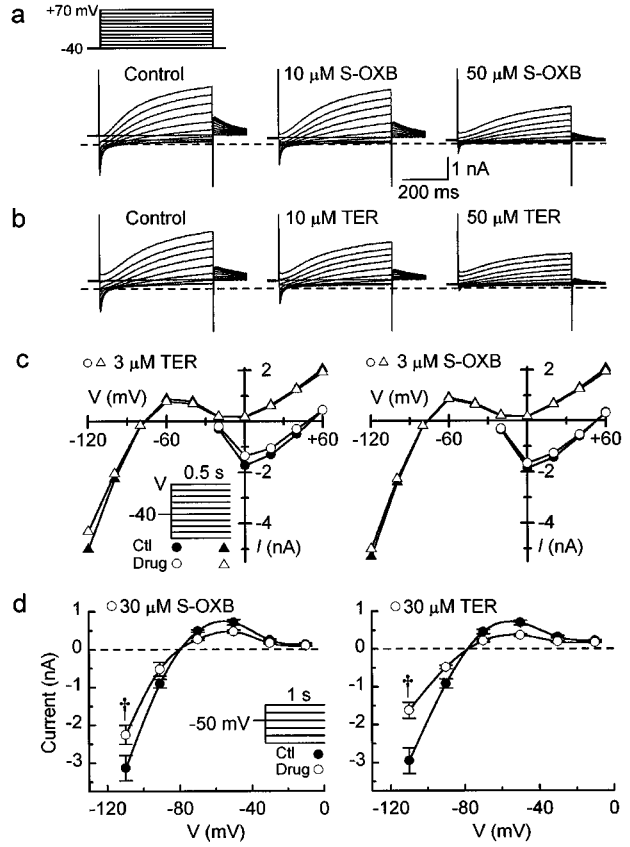

Figure 1 Inhibition of whole-cell membrane currents by S-oxybutynin and terodiline. Guinea-pig ventricular myocytes bathed and dialyzed with K^+-containing solutions were held at −80 mV, and pulsed at 0.1 Hz following 200-ms prepulses to −40 or −50 mV. (a) Records obtained on 500-ms depolarizations from prepulse −40 mV to more positive potentials before (control) and 8–10 min after additions of 10 and 50 μM S-oxybutynin (S-OXB). The dashed line indicates zero-current level. (b) Records from a similar experiment with 10 and 50 μM terodiline (TER). (c) Amplitudes of peak inward current on 500-ms depolarizations, and late (500 ms) current on depolarizations and hyperpolarizations. The representative myocyte was exposed to 3 μM terodiline for 8 min (data in left hand plot), washed for 14 min to obtain new pre-drug control values, and then exposed to 3 μM S-oxybutynin for 8 min (right hand plot). (d) Inhibition of I_{K1} in myocytes pretreated with 0.2 mM Cd^{2+}. Currents were elicited by 1-s pulses from prepulse −50 mV to potentials V, and the end-of-pulse current amplitudes were measured with respect to zero current. Number of myocytes: S-oxybutynin (8), terodiline (7). †$P<0.01$ (one-way ANOVA, Bonferroni test).

depolarizations to ca. 0 mV, and time-dependent outward current that increased with positive potential and deactivated when the membrane was repolarized to −40 mV. Applications of 10 and 50 μM S-oxybutynin (Figure 1a) or of 10 and 50 μM terodiline (Figure 1b) inhibited these current components in a concentration-dependent manner. These drugs also reduced the outward current at prepulse −40 mV (Figure 1a,b), and this was most likely due to concentration-dependent inhibitions of inwardly-rectifying K^+ current (I_{K1}) (Figure 1c,d).

Effects on peak $I_{Ca,L}$ We have previously established that terodiline inhibits peak $I_{Ca,L}$ with an IC_{50} of 15.2 μM (Ogura *et al*., 1999). To determine the effects of S-oxybutynin on peak $I_{Ca,L}$, myocytes were bathed and dialyzed with either K^+ or K^+-free solutions, depolarized for 200 ms from prepulse −40 to 0 mV at 0.1 Hz except for periodic determinations of I-V relationships, and exposed to one or two concentrations of the drug. S-oxybutynin had rapid inhibitory effects that were relatively independent of voltage and reversible on washout of the drug (Figure 2a–c). The Hill equation describing the data has an IC_{50} of 17.8 μM and a coefficient of 0.99 (Figure 2d).

Effects on delayed-rectifier K^+ currents An overview of the effects of S-oxybutynin and terodiline on total delayed-rectifier K^+ current (I_K) was obtained by measuring the amplitudes of the I_K tail ($I_{K,tail}$) that followed 500-ms depolarizations to test potentials (V) between −30 and +70 mV. Based on selective suppression of the rapidly-activating component of I_K (I_{Kr}) by E4031 (3 μM) (Follmer & Colatsky, 1990; Sanguinetti & Jurkiewicz, 1990; Heath & Terrar, 1996), the I-V relationships of $I_{K,tail}$ were comprised of an I_{Kr}-dominated phase at low test voltages and I_{Kr} plus slowly-activating I_K (I_{Ks}) at high test voltages (Figure 3a). S-oxybutynin up to 3 μM had negligible effects, whereas higher

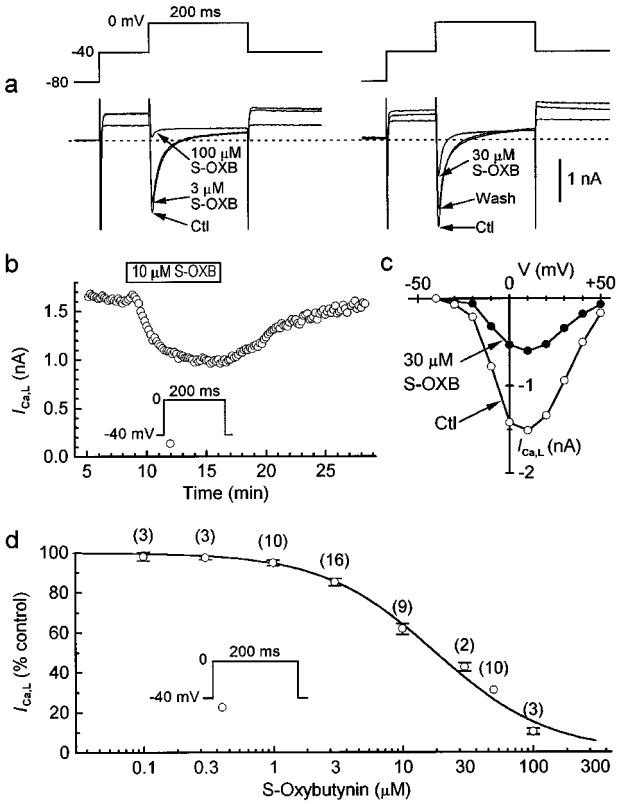

Figure 2 Inhibition of $I_{Ca,L}$ by S-oxybutynin. Myocytes were held at −80 mV, depolarized to prepulse −40 mV for 100 ms, and then pulsed at 0.1 Hz. (a) Records from experiments. Left: effects of sequential applications of 3 μM (10 min) and 100 μM (5 min) drug; the lowest traces at −40 mV were obtained with 100 μM drug. Right: reversible effects of 30 μM drug (drug treatment 12 min; washout 8 min); records at −40 mV were obtained during (top to bottom) control, 30 μM, and washout treatments. The experiments were performed using K^+-containing external and internal solutions. The dashed lines indicate zero-current levels. (b) Time course of reversible inhibition of peak $I_{Ca,L}$ by 10 μM S-oxybutynin in a myocyte bathed and dialyzed with K^+-free solutions. (c) I-V relationships determined before and 6 min after addition of 30 μM S-oxybutynin. The myocyte was bathed and dialyzed with K^+-free solutions. (d) Effect of drug concentration on peak $I_{Ca,L}$ elicited by pulses to 0 mV. Drug exposure times ranged from 6–10 min (high concentrations) up to 15 min (low concentrations). Myocytes were exposed to a single concentration of the drug, and 27 of the 56 experiments were performed with K^+-free solutions. The Hill equation describing the data has an IC_{50} of 17.8 μM and a coefficient 0.99. The numbers of myocytes are given in parentheses.

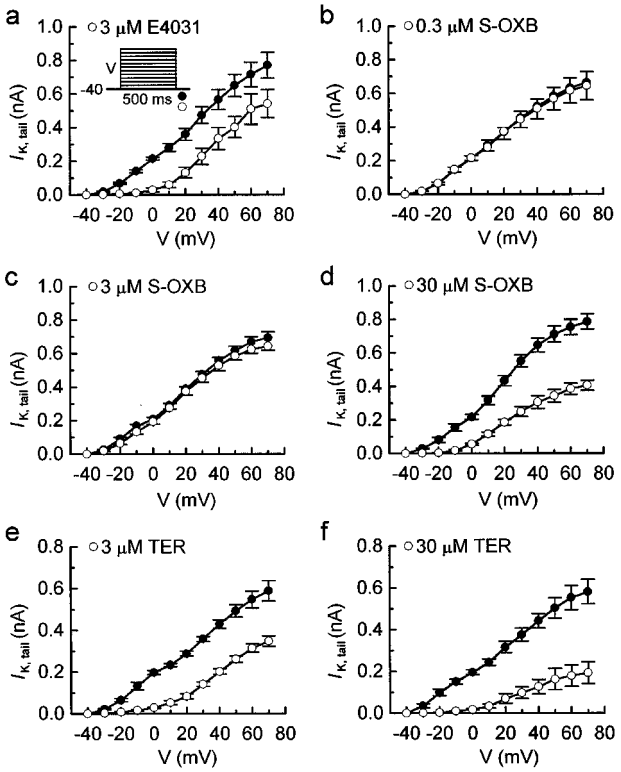

Figure 3 I-V relationships of $I_{K,tail}$ from experiments on myocytes treated with E4031, S-oxybutynin, and terodiline. The amplitudes of $I_{K,tail}$ following 500-ms depolarizations to test potentials (V) up to +70 mV were measured before and after drug treatments that lasted for 5–8 min. (a) Results with 3 μM E4031 ($n=6$). (b–d) Results with 0.3 μM ($n=5$), 3 μM ($n=4$) and 30 μM ($n=5$) S-oxybutynin. (e,f) Results with 3 μM ($n=5$) and 30 μM ($n=6$) terodiline. Note the relative inactivity of 3 μM S-oxybutynin compared to 3 μM E4031 and 3 μM terodiline.

concentrations inhibited both components (Figure 3b–d). Although the effects of terodiline on the I-V relationship of $I_{K,tail}$ were also dependent on drug concentration, they were evident at considerably lower concentrations than observed with S-oxybutynin (Figure 3e,f).

The amplitude of $I_{K,tail}$ after short (200 ms) depolarizations from −40 to 0 mV was reduced to 11±2% control ($n=18$) by 3 μM E4031 (e.g. Figure 4a), and was therefore selected as a useful indicator of drug action on I_{Kr}. $I_{Kr,tail}$ was inhibited by both terodiline and S-oxybutynin, but there was a marked difference in the potency of the two compounds; the estimated IC_{50} values were 0.5 μM for terodiline and 12 μM for S-oxybutynin (Figure 4b–d).

The magnitude of I_{Ks} was estimated in two ways: (i) as the time-dependent current during 500-ms depolarizations to ca. +70 mV (where time-dependent $I_{Ca,L}$ and inwardly-rectifying I_{Kr} are expected to be relatively small), and (ii) as the time-dependent current during 500-ms depolarizations to +30 mV in myocytes superfused with K^+-, Ca^{2+}-free Cd^{2+} solution to minimise I_{Kr} and enhance I_{Ks} (see Sanguinetti & Jurkiewicz, 1992; Jones *et al*., 1998). The results obtained with the two methods were similar, and since I-V relations obtained under the K^+-free conditions revealed little dependence of block on voltage (tests with 30 μM drug; data not shown), the data were pooled. The representative results and summary of pooled-data in Figure 5 indicate that S-oxybutynin was a somewhat weaker inhibitor of I_{Ks} than was terodiline. The Hill fits to the data have an IC_{50} of 43 μM for S-oxybutynin, and a significantly lower ($P<0.01$) 30 μM for terodiline. These results are consistent with the effects of the drugs on the high-voltage component of tail $I_{K\text{-}V}$ relationships (Figure 3).

Effects of S-oxybutynin and terodiline on action potentials in guinea-pig papillary muscles

Action potentials were recorded in control and test groups of guinea-pig papillary muscles for measurement of action potential duration at 20 and 90% repolarization levels (APD_{20}, APD_{90}), action potential amplitude (APA), and maximal upstroke velocity ($\dot{V}_{max}$). The observation periods lasted for 30 min, and data were normalized to the values measured at 0 min. APD_{20} and APD_{90} were stable in control muscles (30-min values of 100±1% and 99±1% ($n=8$), respectively), and not significantly affected by ⩽3 μM S-oxybutynin (Figure 6a,c). Higher concentrations shortened the action potential in a concentration-dependent manner, with the largest changes occurring at the plateau level (e.g., APD_{20} 81±3% ($P<0.001$) and APD_{90} 94±2% ($P<0.05$) ($n=10$, *t*-tests) with 30 μM drug) (Figure 6a,c). $\dot{V}_{max}$ was stable in the control muscles, and unaffected by S-oxybutynin (Figure 6c).

The effects of terodiline on the action potential in guinea-pig papillary muscles differed from those of S-oxybutynin in two major respects: (i) concentrations ⩽10 μM lengthened the action potential (e.g., peak APD_{90} of 115±3% ($n=7$) with 3 μM drug, $P<0.01$), and (ii) concentrations >10 μM markedly depressed $\dot{V}_{max}$ (Figure 6b,c). These terodiline data are in agreement with findings in a previous study (Shuba *et al*., 1999).

Effects of S-oxybutynin and terodiline on action potentials in rabbit papillary muscles

Further information on the cardiac actions of S-oxybutynin and terodiline were sought by examining the effects of the drugs on the action potential in rabbit papillary muscles. This tissue was chosen because it has a different blend of membrane currents governing repolarization than guinea-pig papillary muscle; in particular, a transient outward current (I_{to}) that promotes early repolarization is prominent in rabbit but not guinea-pig ventricular cells (Hiraoka & Kawano, 1987; Carmeliet, 1993).

Concentration-dependent effects After 60–90 min equilibration, muscles were driven at 1 Hz and monitored for 30 min. Action potential parameters were unaffected by ⩽3 μM S-oxybutynin, and the only significant effect of the highest concentration tested (30 μM) was a reduction in APD_{20} to 95±2% of pre-drug control ($n=9$) ($P<0.05$, *t*-test) (Figure 7a,c). In marked contrast, both low and high concentrations of terodiline lengthened the APD_{20} and APD_{90}, with peak lengthening (APD_{90} 128±5%, $n=6$; $P<0.01$, *t*-test) observed at 10 μM (Figure 7b,c). High concentrations of terodiline also depressed $\dot{V}_{max}$ (e.g., to 69±6% ($n=8$; $P<0.01$) at 30 μM), whereas high concentrations of S-oxybutynin had little effect (Figure 7c).

Rate-dependent effects After the 30-min monitoring period (1-Hz stimulation), control and drug-treated muscles were driven at 0.4 Hz for 5 min and then 3 Hz for 3 min to evaluate whether drug treatment affected the response of the action potential plateau to changes in driving rate. Records from a control muscle (Figure 8a, top row) indicate that slowing the stimulation rate from 1 to 0.4 Hz lowered plateau

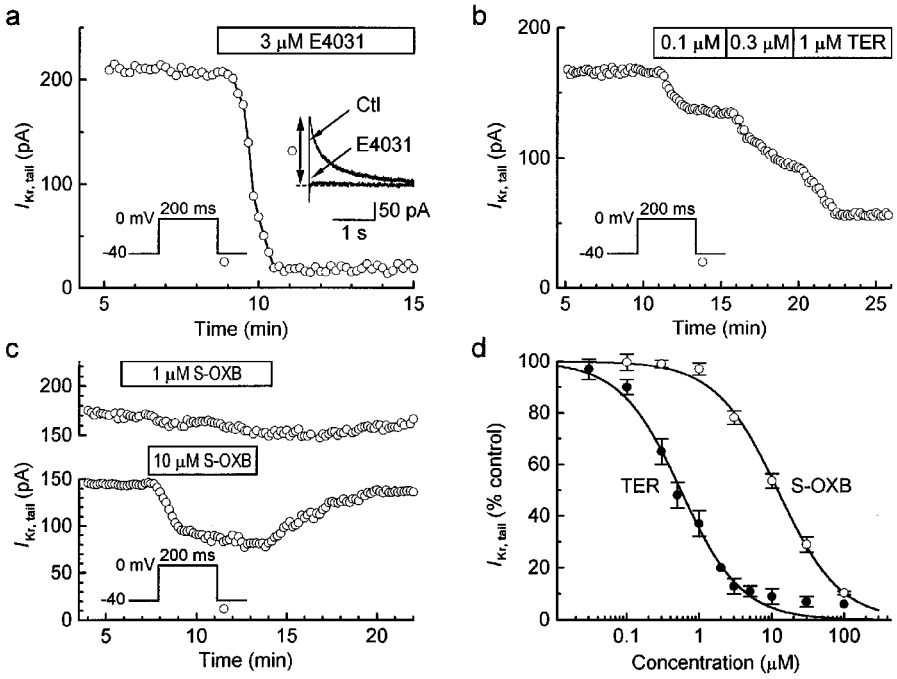

Figure 4 Inhibition of I_{Kr} by terodiline and S-oxybutynin. (a) Identification of $I_{K,tail}$ measured after 200-ms depolarizations to 0 mV as predominantly $I_{Kr,tail}$ based on its sensitivity to 3 μM E4031. (b) Marked inhibition of $I_{Kr,tail}$ by 0.1, 0.3 and 1 μM terodiline. (c) Data from representative experiments with 1 μM (upper panel) and 10 μM (lower panel) S-oxybutynin. (d) Concentration-response relationships. The Hill equation fitting the S-oxybutynin data ($n=3-16$) has an IC_{50} of 12 μM and a Hill-coefficient of 1.01, and that fitting the terodiline data ($n=1$ at 2 μM and 100 μM; otherwise $n=3-18$) has an IC_{50} of 0.5 μM and a Hill-coefficient of 1.04.

amplitude by a few millivolts and shortened plateau duration by about 25%. This rate-dependent abbreviation (Gibbs & Johnson, 1961) is generally attributed to an increase in the availability of transient outward current (I_{to}) following a lengthening of the diastolic interval (Kukushkin *et al*., 1983; Hiraoka & Kawano, 1987; Giles & Imaizumi, 1988). Consistent with this interpretation, the action potential plateau recovered when the stimulation rate was subsequently increased to 3 Hz. A similar pattern of plateau abbreviation and recovery was evident in a muscle treated with 3 μM S-oxybutynin, but less so in a muscle treated with 3 μM terodiline (Figure 8a).

Since there were definite (though small) changes in action potential amplitude at different stimulation rates, the effects of rate on plateau duration were assessed by measuring duration at a fixed (0 mV) repolarization level (APD_{0mV}) (Figure 8b,c). When referenced to the duration at the start of the 30-min period at 1 Hz, the APD_{0mV} at the end of each frequency trial in control muscles ($n=16$) was $99\pm2\%$, $77\pm2\%$ and $97\pm2\%$ at 1, 0.4 and 3 Hz, respectively. The durations in muscles treated with S-oxybutynin followed the same pattern: $100\pm3\%$, $75\pm4\%$ and $98\pm3\%$, respectively, at 0.3 μM ($n=4$) (not shown), $99\pm2\%$, $75\pm2\%$ and $98\pm2\%$, respectively, at 3 μM ($n=15$), and $95\pm1\%$, $77\pm2\%$ and $92\pm2\%$, respectively, at 30 μM ($n=15$). However, APD_{0mV} in muscles treated with 3 and 30 μM terodiline was less likely to shorten when the rate was lowered from 1 to 0.4 Hz (e.g., $110\pm3\%$ (1 Hz) and $102\pm2\%$ (0.4 Hz) ($n=9$) at 30 μM), and more likely to shorten (e.g. to $83\pm2\%$ at 30 μM) rather than lengthen when the stimulation rate was increased from 0.4 to 3 Hz. Analysis of the data (one-way ANOVA followed by the Bonferroni test) indicates that (APD_{0mV}) (0.4 Hz) was significantly longer ($P<0.001$) in muscles treated with 3 and 30 μM terodiline than in control muscles, whereas APD_{0mV} (3 Hz) was significantly shorter ($P<0.001$) in muscles treated with 30 μM terodiline than in control muscles.

Effects of S-oxybutynin and terodiline on transient outward current in rabbit ventricular myocytes

Differences in the responses of the action potential in rabbit and guinea-pig papillary muscles to drug treatments (see Discussion) suggested that the drugs might have concentration-dependent inhibitory effects on I_{to} in rabbit papillary muscles. This possibility was examined by measuring the whole-cell I_{to} in rabbit ventricular myocytes. The myocytes were bathed in a modified Tyrode's solution that contained 20 μM tetrodotoxin to block Na^+ current, 0.2 mM Cd^{2+} to block $I_{Ca,L}$, 0.1 mM Ba^{2+} to block I_{K1}, and 3 μM E4031 to block I_{Kr}. In a series of experiments at 36°C, myocytes were held at -80 mV, depolarized to potentials up to $+50$ mV, and exposed to 3 and/or 30 μM drug for times (5–7 min) long enough to reach new steady-state I_{to}. Figure 9a indicates that 3 μM S-oxybutynin reduced I_{to} by $9\pm2\%$ ($n=6$) ($P<0.05$, *t*-test), and 30 μM reduced it by $35\pm3\%$ ($n=4$) ($P<0.001$). Terodiline had a more potent inhibitory action, with 3 μM reducing the current by $31\pm3\%$ ($n=11$) and 30 μM reducing it by $87\pm3\%$ ($n=8$) (both $P<0.001$) (Figure 9b).

In the foregoing series, I_{to} was measured as the time-dependent current (10 ms to end-of-pulse) at $+50$ mV. For improved resolution, experiments were conducted at 24°C (to slow I_{to} kinetics) and 5 mM 4-aminopyridine was added at the end of the experiment to allow measurement of I_{to} on pulses to $+30$ mV (0.1 Hz) as 4-aminopyridine-sensitive current. Using this protocol, the results with 3 and 30 μM terodiline (Figure 9c) were virtually identical to those obtained at 36°C ($35\pm3\%$ inhibition with 3 μM ($n=8$), and $88\pm3\%$ inhibition with 30 μM ($n=8$) (both $P<0.001$)).

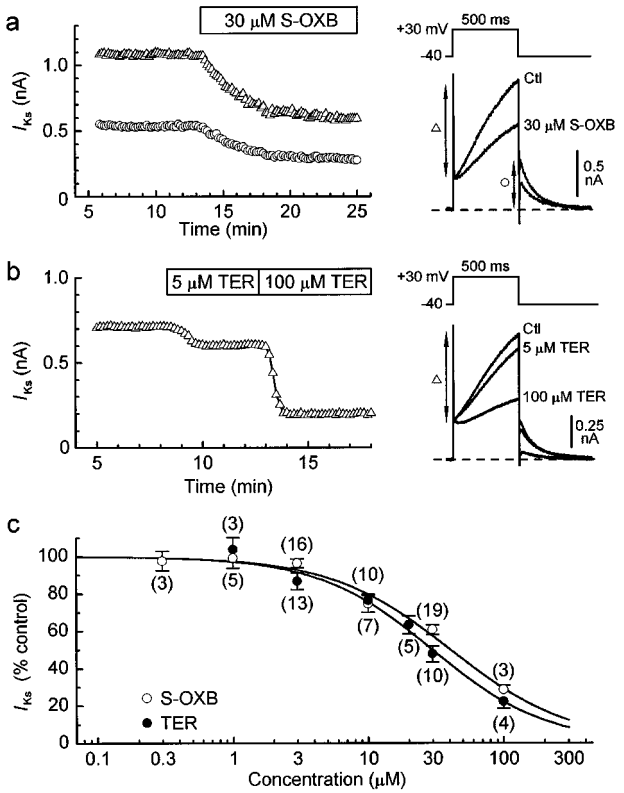

Figure 5 Inhibition of I_{Ks} by S-oxybutynin and terodiline. (a,b) Effects of S-oxybutynin and terodiline on I_{Ks} in myocytes superfused with K^+-, Ca^{2+}-free Cd^{2+} solution. The measurements taken are indicated by the vertical arrows on the records. (c) Concentration-response relationships. I_{Ks} was measured as the time-dependent outward current during 500-ms pulses to +30 mV (as in a,b) (37 of 98 myocytes), or as the time-dependent current during 500-ms pulses to +70 mV under standard conditions (see records in Figure 1a,b). Fits to the pooled data indicate an IC_{50} of 43 μM and a Hill-coefficient of 0.98 for S-oxybutynin ($n=3-16$), and an IC_{50} of 30 μM and Hill-coefficient of 1.11 for terodiline ($n=3-10$).

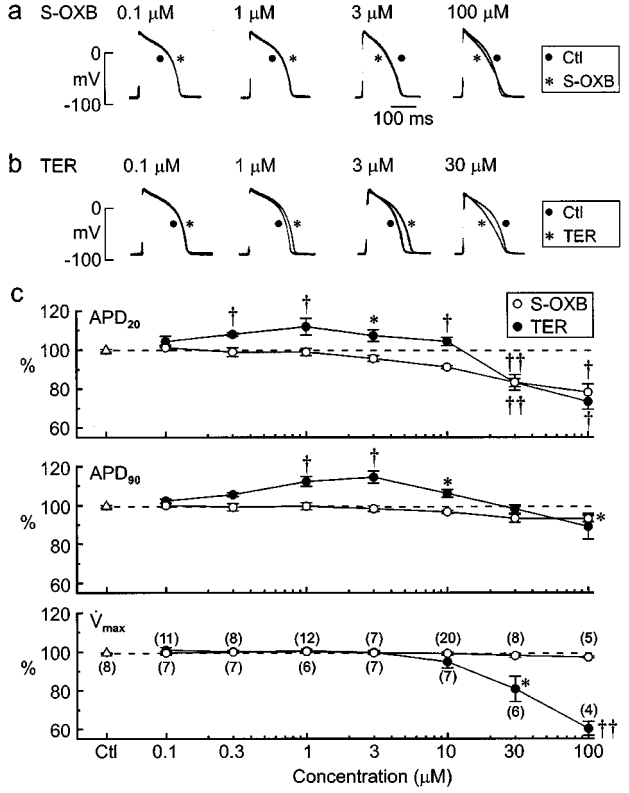

Figure 6 Effects of S-oxybutynin and terodiline on action potentials in guinea-pig papillary muscles. The muscles were stimulated at 1 Hz and treated with a single concentration of the drug. (a,b) Superimposed sets of action potentials; the traces were recorded from muscles before and 30 min after addition of drug. (c) APD_{20}, APD_{90} and $\dot{V}_{max}$ of action potentials from control muscles (no drug treatment) and muscles treated with single concentrations of S-oxybutynin and terodiline. Values measured after 30-min observation periods are expressed as percentages of pre-drug values. Numbers of muscles are shown in parentheses on the bottom plot. Significance was assessed using *t*-tests: $*P<0.05$, $\dagger P<0.01$, $\dagger\dagger P<0.001$.

Discussion

We have compared the effects of S-oxybutynin and terodiline on membrane currents in guinea-pig and rabbit ventricular myocytes and on action potential configuration in guinea-pig and rabbit papillary muscles. Although both S-oxybutynin and terodiline inhibited Ca^{2+} and K^+ currents, terodiline was the more potent of the two, especially in regard to I_{Kr} and I_{to}. Action potentials in guinea-pig and rabbit papillary muscles were unaffected by $\leqslant 10$ μM S-oxybutynin and moderately shortened by higher concentrations, whereas action potentials were lengthened by $\leqslant 10$ μM terodiline and either shortened (guinea-pig) or lengthened (rabbit) by higher concentrations. An additional finding was that rate-dependent changes in rabbit ventricular action potentials were strongly modified by terodiline. We discuss these findings, and consider their implications in relation to clinical safety of the drugs.

Effects on membrane currents and action potentials in guinea-pig ventricular preparations

The results of the voltage-clamp experiments on guinea-pig ventricular myocytes indicate that S-oxybutynin is a relatively weak, non-specific inhibitor of cardiac membrane currents (IC_{50} values of 17.8 μM for $I_{Ca,L}$, and 12, 41 and ca. 50 μM for I_{Kr}, I_{Ks}, and I_{K1}, respectively). The relatively high IC_{50} values explain why concentrations of the drug $\leqslant 3$ μM had little effect on the configuration of action potentials in guinea-pig papillary muscles. Higher concentrations reduced the APD_{20} by up to 22%, suggesting that a plateau shortening influence related to inhibition of $I_{Ca,L}$ outweighed plateau lengthening influences related to inhibitions of I_{Kr} and I_{Ks}. The APD_{90} was less affected, suggesting that reductions in I_{K1} at high concentrations of the drug contributed to a slowing of phase 3 repolarization.

The IC_{50} values for terodiline were moderately lower than those for S-oxybutynin in regard to $I_{Ca,L}$ and I_{Ks}, but 24 fold lower (0.5 μM) in regard to I_{Kr}. The consequence of this selective inhibition was a lengthening of the action potential at terodiline concentrations $\leqslant 10$ μM, with the largest lengthening (10–15%) occurring with 1–3 μM drug. It seems likely that the lengthening was restricted by the concomitant inhibition of $I_{Ca,L}$ since specific inhibition of I_{Kr} by 3 μM E4031 lengthens the duration by $27\pm2\%$ ($n=5$) (Shuba *et al*., 1999).

Effects on action potentials in rabbit papillary muscles

The shape of the action potential in rabbit papillary muscles was almost insensitive to 0.3–30 μM S-oxybutynin. This finding suggests that the action potential shortening and lengthening influence caused by inhibitions of Ca^{2+} and K^+ currents, respectively, were almost in balance over this range

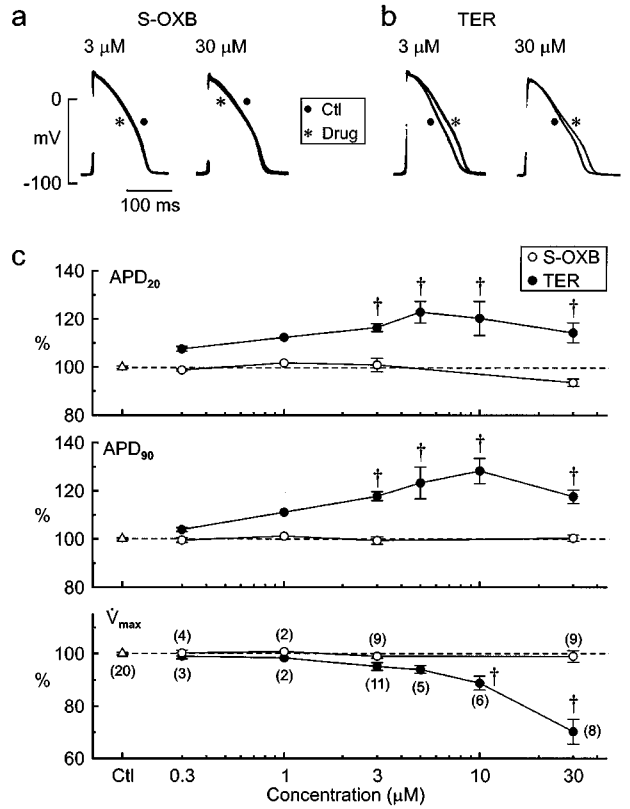

Figure 7 Concentration-dependent effects of S-oxybutynin and terodiline on action potentials in rabbit papillary muscles stimulated at 1 Hz. (a,b) Superimposed sets of action potentials recorded before and 30 min after addition of drug. (c) APD_{20}, APD_{90} and $\dot{V}_{max}$ of action potentials from control muscles and muscles treated with single concentrations of S-oxybutynin and terodiline. Values measured after 30-min observation periods are expressed as percentages of pre-drug values. The numbers of muscles are indicated in parentheses on the bottom plot. Significance was assessed with the *t*-test: $*P<0.05$, $\dagger P<0.01$.

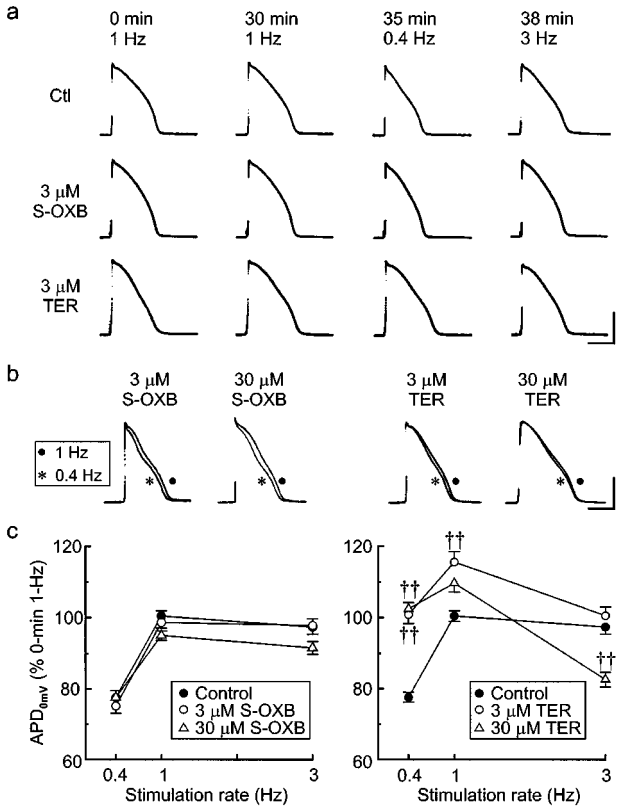

Figure 8 Effects of stimulation rate on the configuration of action potentials in control and drug-treated rabbit papillary muscles. (a) Top to bottom rows: Records from control, S-oxybutynin and terodiline treated muscles. Left to right: the records were obtained just before (0 min) and at the end of successive stimulation periods (30 min at 1 Hz, 5 min at 0.4 Hz, and 3 min at 3 Hz). Time (top) is continuous from 0 min. The calibration bars indicate 50 mV and 100 ms. (b) Superimposed sets of action potentials; the traces were recorded after 30 min drug treatment at 1 Hz stimulation rate and 5 min after reducing the rate from 1 to 0.4 Hz. The calibration bars indicate 50 mV and 100 ms. (c) Summary of the percentage changes in plateau duration at 0 mV (APD_{0mV}) induced by changing the driving rate from 1 to 0.4 Hz for 5 min and then to 3 Hz for 3 min. All data are referenced to APD_{0mV} values measured before the 30-min (control or drug) 1-Hz trial periods. Numbers of muscles: control (16), S-oxybutynin 3 and 30 μM (15 each), terodiline 3 and 30 μM (9,10). Significance was assessed using one-way ANOVA, Bonferroni test: $\dagger\dagger P<0.001$.

of drug concentration. By contrast, the action potential was prolonged by 0.3–30 μM terodiline, suggesting relatively larger inhibition of K^+ currents by this drug.

The terodiline-induced lengthening of the action potential was larger and peaked at a higher drug concentration in rabbit (10 μM) than in guinea-pig (1–3 μM) papillary muscles. The most likely reason for this species difference is that rabbit ventricular cells have a prominent I_{to}, and the shortening influence related to concentration-dependent inhibition of $I_{Ca,L}$ in rabbit muscles is counterbalanced by the lengthening influence related to concentration-dependent inhibition of I_{to}.

Inhibition of I_{to} by terodiline also provides a basis for understanding the behaviour of the action potential plateau when rabbit papillary muscles were driven at different rates. In control muscles, slowing of the driving rate from 1 to 0.4 Hz shortened APD_{0mV} by an average of 23%, i.e., net outward plateau current was enhanced at the slower rate. This enhancement of net outward plateau current was almost certainly due to an increase in I_{to} that outweighed other possible changes induced by the slowing of the driving rate (e.g., increase in $I_{Ca,L}$, reduction in I_K: Hiraoka & Kawano, 1987; Boyett *et al*., 1994; Ogura *et al*., 1999). In drug-treated muscles, the rate-related abbreviation of the plateau was unaffected by 3 μM S-oxybutynin, moderately depressed by 30 μM S-oxybutynin and 3 μM terodiline, and strongly depressed by 30 μM terodiline. This set of results is consistent with the concentration-dependent effects of the drugs on I_{to} (see Figure 9a,b, right hand panels).

When control and S-oxybutynin-treated muscles were driven at 3 Hz, the duration of the plateau was only slightly shorter than at 1 Hz, indicating that likely rate-induced shortening influences (smaller $I_{Ca,L}$, larger I_K) were almost fully offset by the lengthening influence of smaller I_{to} (weaker recovery during the shorter diastolic intervals). In marked contrast, the plateau duration in terodiline-treated muscles was 15% (3 μM) to 26% (30 μM) shorter at 3 Hz than at 1 Hz, suggesting a concentration-dependent removal of the lengthening influence of rate-dependent attenuation of I_{to}. A rough indication of the normal magnitude of this lengthening influence is that the APD_{20} in I_{to}-deficient guinea-pig papillary muscles shortens by 27% when the stimulation rate is increased from 1 to 3 Hz (Shuba *et al*., 1999). In summary (and independent of whether inhibition of I_{to} is the sole factor at play), terodiline induces a 'reverse' rate dependence in rabbit papillary muscles, i.e. the degree of action potential lengthening is accentuated at lower stimulation rates.

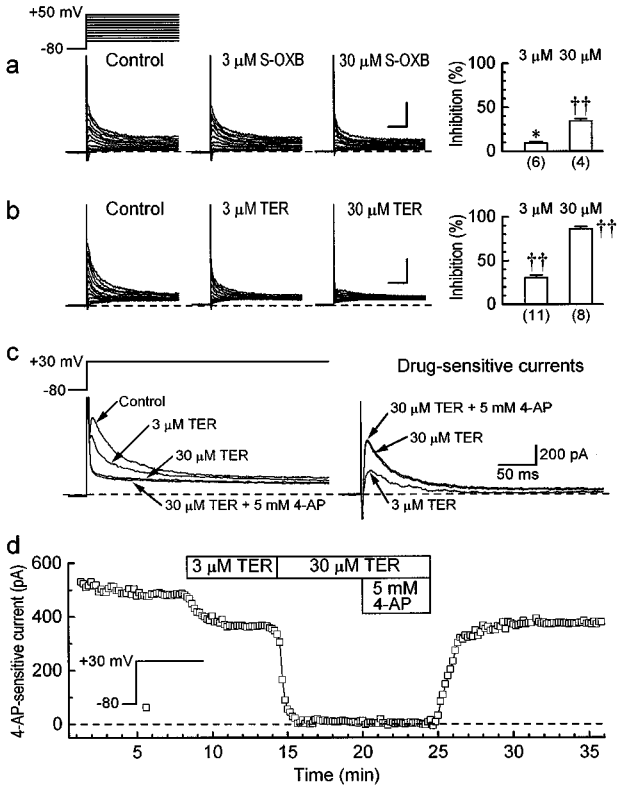

Figure 9 Effects of S-oxybutynin and terodiline on I_{to} in rabbit ventricular myocytes. The myocytes were superinfused with Tyrode's solution that contained 0.2 mM Cd^{2+}, 0.1 mM Ba^{2+}, 3 μM E4031 and 20 μM tetrodotoxin. (a,b) Records from experiments conducted at 36°C. The myocytes were held at −80 mV, and depolarized to more positive potentials for 400 ms at 0.1 Hz before and approximately 6 min after exposures to 3 and/or 30 μM drug. Left: representative results. Right: percentage inhibition of I_{to} amplitude. The amplitude was measured as the time-dependent current (10 ms to 400 ms) elicited on pulses to +50 mV. The calibration bars indicate 0.5 nA and 100 ms. Numbers of myocytes in parentheses. Significance was assessed with the *t*-test: $*P<0.05$, $\dagger P<0.01$, $\dagger\dagger P<0.001$. (c,d) Results from representative experiments conducted at 24°C. The myocytes were held at −80 mV and depolarized to +30 mV for 400 ms at 0.1 Hz. I_{to} at +30 mV was measured as the current sensitive to 5 mM 4-aminopyridine.

Relation to clinical safety

A recent pharmacokinetic study on human volunteers (Koch *et al*., 1998) indicates that the peak plasma concentration C_{max}) of orally-administered S-oxybutynin was similar to that found in earlier studies on oxybutynin. In the case of patients receiving therapeutic doses of oxybutynin, there is considerable inter-patient variability but average C_{max} is approximately 0.01 μM (Hughes *et al*., 1992; Lukkari *et al*., 1997a, b). On that basis, the negligible effects of 1 μM S-oxybutynin on muscle action potential $\dot{V}_{max}$ and myocyte $I_{Ca,L}$ suggest that cardiac function dependent on Na^+ and Ca^{2+} channel activity is highly unlikely to be compromised by the drug. The situation with terodiline is less clearcut because C_{max} is near 1 μM (Connolly *et al*., 1991), $\dot{V}_{max}$ is much more sensitive to terodiline than to S-oxybutynin, and this sensitivity is enhanced by lower resting potential and higher stimulation rates (Shuba *et al*., 1999).

A large number of chemically and therapeutically unrelated drugs are known to cause QT lengthening that can lead to ventricular tachyarrhythmia (torsades de pointes) (Zipes, 1987; Zehender *et al*., 1991; Roden *et al*., 1996). Although electrolyte disturbances, dietary deficiencies and heart disease can be contributing factors to torsades, and the precise events triggering the arrhythmia are still uncertain, it is highly likely that drug-induced inhibition of repolarizing K^+ currents, especially I_{Kr}, play a pivotal role (Roden *et al*., 1996). In that regard, the results of the present study identify a major difference in the cardiac effects of terodiline and S-oxybutynin: terodiline inhibited I_{Kr} with IC_{50} 0.5 μM, whereas S-oxybutynin inhibited it with IC_{50} 12 μM. When this difference is considered in relation to the respective C_{max} values of 1 and 0.01 μM (see above) by taking the ratio of C_{max} to the IC_{50} for I_{Kr}, the outcome is 2.0 for terodiline and ca. 0.001 for S-oxybutynin. For comparison, the ratio for chlorpheniramine, a first-generation antihistamine with a good safety record, appears to be about 0.02 (C_{max} of 0.03 μM (Yasuda *et al*., 1995); IC_{50} for I_{Kr} of 1.6 μM (Salata *et al*., 1995)), whereas the approximate ratio for the second-generation, torsades-inducing terfenadine is a much larger 0.4 (C_{max} near 0.02 μM (Woosley *et al*., 1993) and IC_{50} for I_{Kr} of 0.05 μM (Salata *et al*., 1995)). A final point is that the frequency response of the rabbit ventricular action potential was unaffected by 0.3–3 μM S-oxybutynin, and only moderately affected by a 30 μM concentration, suggesting that the I_{to}-dominated frequency response of human myocardial cells (Shibata *et al*., 1989; Beuckelmann *et al*., 1993; Wettwer *et al*., 1994) is also unlikely to be affected by S-oxybutynin. This is important in view of the link between drug-induced 'reverse' rate-dependence, bradycardia and torsades de pointes (Jackman *et al*., 1988; Stewart *et al*., 1992; Thomas *et al*., 1995; Roden *et al*., 1996), and the likelihood that many elderly patients receiving incontinence drugs have aging-related cardiac hypertrophy (Svanborg, 1997) and lowered density of I_{to} (Beuckelmann *et al*., 1993; McIntosh *et al*., 1998).

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