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Inhibitory effects of (±)-propranolol on excitation-contraction coupling in isolated soleus muscles of the rat

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1 The effect of a β-adrenoceptor antagonist, propranolol, was investigated on excitation-contraction coupling in small, intact bundles of soleus muscle fibres from the rat.

2 (±)-Propranolol significantly inhibited twitch and tetanic tension with IC_{50} values of 6.7 μM and 3.5 μM, respectively.

3 (+)-Propranolol (which has 100 times less β-blocking activity than the (±) form) was approximately one third as effective as the (±) form at inhibiting isometric tension.

4 (±)-Propranolol (20 μM) had no significant effect on the amplitude of caffeine contractures, suggesting that it did not directly inhibit Ca^{2+} release from the sarcoplasmic reticulum.

5 The resting membrane potential measured after 15 min perfusion with 20 μM (±)-propranolol was not significantly different from control. However, this concentration of (±)-propranolol significantly reduced both the peak amplitude and the maximum rate of rise of the action potential. Both effects were only partially reversible after extensive washing.

6 (±)-Propranolol perfusion caused a modest reduction in the amplitude of sub-maximal K^+ contractures at concentrations (5 μM) that markedly depressed tetanic tension.

7 The results indicate that (±)-propranolol can decrease isometric tension independently of β-receptor occupation by (i) reducing the amplitude and rate of rise of the action potential and (ii) by directly inhibiting excitation-contraction coupling. The relatively low IC_{50} for the 'membrane-stabilizing' action of propranolol on tetanic tension (3.5 μM), combined with the ability of the drug to accumulate gradually in biological membranes, may contribute to a peripheral component of the tremorolytic and fatigue-inducing actions of propranolol on skeletal muscle.

Keywords: β-Adrenoceptors; excitation-contraction coupling; (±)-propranolol; (+)-propranolol; skeletal muscle; membrane-stabilizing effect

Introduction

Propranolol is a lipophilic, non-selective β-adrenoceptor antagonist that has been used for the management of hypertension, angina pectoris and cardiac arrhythmias, and has also proven to be highly effective in the treatment of various types of skeletal muscle tremor (Cleeves & Findley, 1984). At therapeutic plasma concentrations (0.1 to 3 μM; Benet *et al*., 1996) propranolol acts primarily by blocking β-adrenoceptors in the central nervous system and the periphery. However, receptor-independent actions of the drug may occur in both cardiac (Morales-Aguilerá & Vaughan-Williams, 1965) and skeletal muscles (Oota & Nagai, 1977; Chiarandini, 1980) at higher concentrations.

At high concentrations (10 to 1000 μM) it has been suggested that propranolol directly inhibits skeletal muscle contraction by affecting the nicotinic cholinoceptors (Chiarandini, 1980), the action potential and excitation-contraction coupling steps (Oota & Nagai, 1977), the sarcoplasmic reticulum (SR) Ca^{2+} uptake pump (Noack *et al*., 1978; Su & Malencik, 1985), and the ryanodine receptor/SR Ca^{2+} release channel (Zchut *et al*., 1996). It has been difficult to assess the exact contribution of any one site of action to the depression of contraction seen in intact skeletal muscles, because these studies have used widely varying types of muscle, differing degrees of muscle integrity and different propranolol concentration ranges.

In preliminary studies we noted that (±)-propranolol can significantly inhibit tetanic tension in intact bundles of rat soleus muscle fibres at somewhat lower concentrations (1 to 10 μM) than those used previously (Ha & Fryer, 1995; 1996). The present study was therefore designed to determine the mechanism of action of propranolol in rat soleus fibres by systematically investigating its effect on some of the key sites involved in excitation-contraction coupling.

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Methods

Male Wistar Rats (200–300 g) were killed by halothane overdose. The soleus muscle was quickly removed and a small rectangular bundle of 10 to 50 fibres dissected free. The muscle bundle was then transferred to a small, temperature controlled (23±0.2°C) bath where it was perfused at 1.4 ml min^{-1} with normal Krebs-bicarbonate solution (equilibrated with 95% O_2, 5% CO_2 to pH 7.4) and stimulated directly (0.5 ms duration at supramaximal voltage) by platinum electrodes set 5 mm apart. Muscle length was adjusted to give maximum twitch tension. The preparation was then allowed to equilibrate for at least 1 h before the start of the experiment. Tension was measured isometrically by means of a loaded cell tension transducer (BG-10, Kulite, U.K.) and was simultaneously recorded on both chart paper and on a computer through a data acquisition programme (Voltsamp, University of New South Wales, Australia). Twitches were typically elicited at 0.033 Hz, while tetani were produced by 2 s of stimulation at 40 to 50 Hz.

Concentration-response curves

Only a single concentration of either (±)-propranolol or (+)-propranolol was applied to the muscle bundle in each experiment. The effect of this fixed concentration of propranolol on isometric tension was recorded after 30 min equilibration with the drug and was compared with pre-drug controls. Cumulative concentration-response experiments were not done because the time course of the effects of propranolol was very slow (particularly at concentrations < 10 μM; Figure 1), prob-

ably due to its slow accumulation in membranous compartments (Hellenbrecht *et al*., 1973).

Electrophysiological recordings

Intracellular recordings of the resting membrane potential and action potential were made in superficial fibres of whole soleus muscles. Microelectrodes were filled with 3 M KCl and had resistances of 10–20 MΩ.

The resting membrane potential (RMP) was recorded from a population of 10 to 15 individual fibres in each muscle during normal Krebs perfusion (control) and after 15 min perfusion with 20 μM propranolol. Recordings under control conditions were accepted if (i) the RMP was more negative than −60 mV and (ii) the RMP changed by <2 mV during the first 2 min following impalement.

Action potentials were recorded by an intracellular electrode placed 3 to 5 mm away from a fire-polished extracellular stimulating electrode. Extracellular stimuli of 20 μs duration (10–20 V) were provided by an isolated stimulator (Model DS2, Digitimer, U.K.). The resulting action potentials were recorded on a digital storage oscilloscope (TDS 310, Tektronix, OR, U.S.A.) and analysed by use of associated software. Control recordings were accepted if (i) the RMP criteria described above were fulfilled and (ii) a second action potential elicited 5 min after the first had not changed in amplitude or shape. Further recordings were made 15 min after perfusion with 20 μM propranolol and then 30 to 60 min after washing the preparation in drug-free Krebs.

Caffeine contractures

Caffeine contractures were elicited at 30 min intervals by rapid perfusion (2.4 ml min^{-1}) with a Krebs solution containing 7 mM caffeine. These contractures typically took 4 to 6 min to reach a plateau, at which point the muscle bundle was perfused with caffeine-free Krebs in order to allow relaxation (Figure 4). The muscle was not stimulated electrically in the interval between caffeine contractures.

K^+ contractures

K^+contractures were obtained by rapid perfusion of the muscle bundle with modified Krebs solutions containing either 60 mM or 124 mM total K^+. After each K^+ contracture the muscle was stimulated at 0.033 Hz to assess the time course of recovery of the isometric twitch. When twitch recovery was complete (typically 45 to 60 min) electrical stimulation was ceased and the next K^+ contracture was elicited. Two control K^+ contractures were usually obtained, followed by another after 30 min equilibration in (±)-propranolol and a final contracture after 60 min of perfusion in drug-free Krebs. It should be noted that these K^+ contractures were considerably slower and smaller than those previously obtained in the same preparation (Dulhunty & Gage, 1985), because the concentration of extracellular chloride ions was not lowered in the present experiments.

Data analysis

The isometric twitch response was analysed by measuring the peak amplitude, the 20 to 80% rise time and the 80 to 20% relaxation time. The peak amplitude, the 20 to 60% rise time and the 80 to 20% relaxation time following the last stimulus pulse were measured for isometric tetani. Effects of propranolol on these parameters were expressed as the percentage change from the control response i.e. ((value after propranolol − control value)/control value × 100%).

Concentration-response curves were computer-fitted (GraphPad Prism v 2.0 software, San Diego, U.S.A.) to the mean concentration-response data by least squares regression analysis by use of a modified form of the Hill equation. Both the Hill coefficient (n_H) and the concentration of propranolol inhibiting 50% of the maximum response (IC_{50}) were calculated from these curves.

The amplitude of a caffeine or potassium contracture in the presence of (±)-propranolol (designated C2) was assessed relative to the amplitude of the contractures recorded immediately before propranolol perfusion (C1) and that following washout of propranolol (C3). Control experiments revealed that the amplitude of contractures C1 through C3 rarely stayed constant, but instead tended to run-up or run-down at a constant rate. Thus, the calculated control value for C2 was calculated as the mean of the peak responses in C1 and C3.

Values presented in the text are expressed as the mean ± s.e.mean from *n* preparations. Statistical significance between means was assessed by Student's *t* test for unpaired and paired data where appropriate. *P* values <0.05 were considered significant.

Solutions and drugs

The Krebs-bicarbonate solution contained (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 0.6, $NaHCO_3$ 25, glucose 11, $CaCl_2$ 2.5 and (+)-tubocurarine 0.015. High K^+ solutions containing 60 and 124 mM total K^+ were obtained by replacing NaCl with KCl on an equimolar basis.

Di-sodium ethylenediaminetetraacetic acid 50 μM was routinely added to all solutions in order to retard propranolol oxidation. Caffeine, (+)-propranolol HCl and (±)-propranolol HCl were each dissolved into the Krebs-bicarbonate solution and the pH adjusted to 7.4. All drugs were obtained from Sigma Australia except for (±)-propranolol HCl, which was obtained from RBI Chemical Co. (U.S.A.).

Results

Effects of (±)-propranolol on twitch and tetanic tension

Figure 1 summarizes the mean data from 43 experiments illustrating the time- and concentration-dependence of the inhibition of twitch tension by (±)-propranolol. At lower concentrations (0.1–3 μM) the drug produced a very slow inhibition of peak twitch tension, an effect that had still not reached steady-state after 30 min. Higher concentrations of (±)-propranolol (10–200 μM) produced a faster inhibition of twitch tension that appeared to reach steady-state within the same time period. At a fixed time interval of 30 min after application of the drug, twitch tension was significantly less than equivalent controls at all concentrations ≥0.1 μM. This effect was slowly and only partially reversible after extensive washing of the preparation in drug-free Krebs, as shown by the recovery of twitch tension to only 60 to 70% of the control amplitude after a 60 min wash (not shown). For this reason only a single concentration of propranolol was applied to a muscle bundle in each experiment.

Representative traces of the effect of (±)-propranolol on isometric twitch and tetanic responses are shown in Figures 2a and 3a, respectively, while effects on rise and decay kinetics in the 0.1 to 10 μM range are summarized in Table 1. (±)-Propranolol started to have considerable effects on both peak amplitude and contraction-relaxation kinetics in the 1 to 10 μM range. The rise time of both twitch and tetanic tension was significantly shortened at (±)-propranolol concentrations ≥3 μM, as was the decay time of the twitch ($P<0.05$; Table 1). However, tetanic relaxation was markedly slowed at these concentrations, as indicated by the large increase in the 80 to 20% decay time (Table 1). Concentration-response curves were constructed for the effect of (±)-propranolol on tension amplitude after a fixed time period of 30 min (Figures 2b and 3b). Curves fitted to the mean data ($n=3$ to 8 for each concentration point) yielded the following parameters for twitches (Figure 2b, solid symbols) and tetani (Figure 3b, solid symbols), respectively: IC_{50}: 6.7 ± 1.2 μM and 3.5 ± 1.1 μM; n_H: 1.19 ± 0.22 and 0.96 ± 0.11; r^2: 0.993 and 0.997.

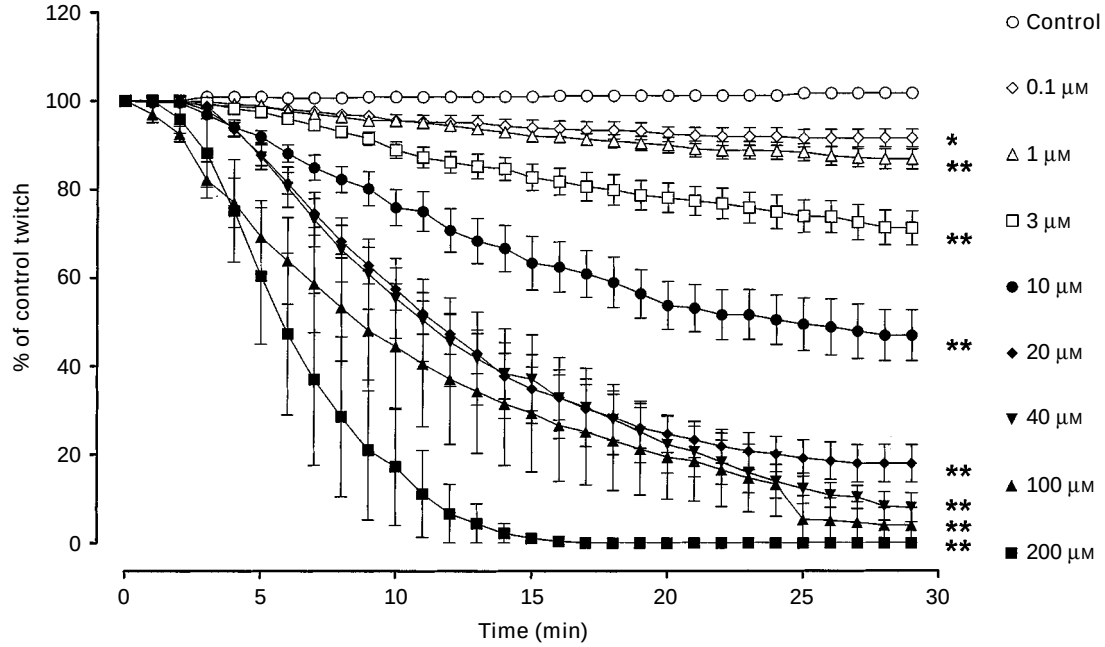

Figure 1 Concentration- and time-dependence of (±)-propranolol effects on twitch amplitude of rat soleus muscle bundles stimulated at 0.033 Hz. Data points represent the mean from 3 to 8 observations at each concentration; vertical lines show s.e.mean. Significant difference from control at 30 min, $*P<0.01$, $**P<0.001$.

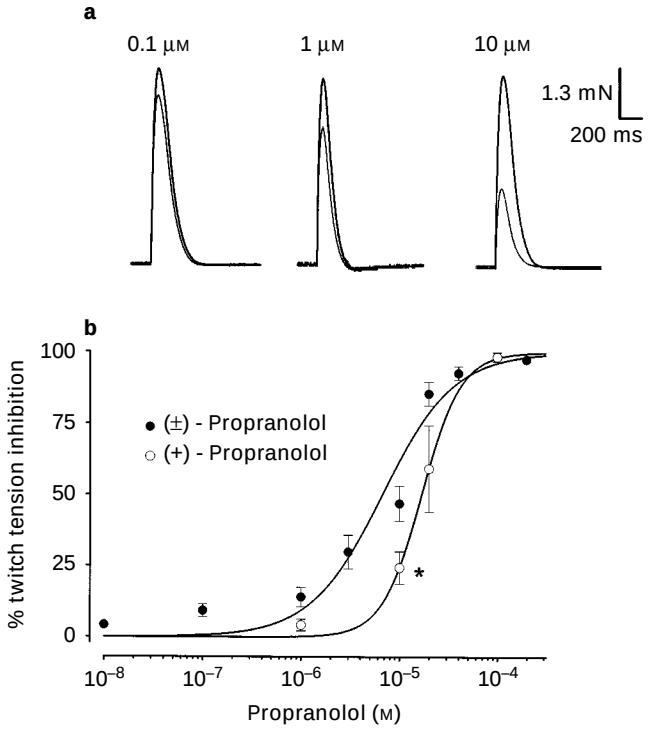

Figure 2 (a) Typical effects of (±)-propranolol on isometric twitches in three separate preparations. Traces were recorded before and after 30 min perfusion with the drug. (b) Concentration-response curves for inhibition of peak twitch tension by (±)-propranolol and (+)-propranolol. Each point represents the mean response for 3 to 8 muscles; vertical lines show s.e.mean. $*P<0.05$ compared to 10 μM (±)-propranolol.

Effects of (+)-propranolol on twitch and tetanic tension

The possibility that (±)-propranolol was acting independently of β-adrenoceptor blockade was probed in a further set of experiments in which (+)-propranolol was used. (+)-Propranolol has 60–100 times less β-blocking activity (Howe & Shanks, 1966) but approximately the same membrane stabilizing properties as the (±) form in cardiac muscle (Coltart & Meldrum, 1971).

Sigmoidal curves were fitted to the mean data for the effect of (+)-propranolol on both peak twitch (Figure 2b, open symbols) and tetanic tension (Figure 3b, open symbols) in order to facilitate comparison with the effects of the racemate. The estimated IC_{50} values from the fitted curves for (+)-propranolol (twitch: 16.8±1.0 μM; tetanus: 10.2±1.3 μM) were approximately 2.5 to 3 fold higher than those calculated for (±)-propranolol. At a fixed concentration of 10 μM, (+)-propranolol had a significantly weaker inhibitory effect on peak tension than (±)-propranolol for both twitches ($P<0.05$, 9 *d.f*) and tetani ($P<0.01$, 9 *d.f*).

Effects of (±)-propranolol on caffeine-induced contractures

One possible explanation for the results above is that propranolol (which is highly lipid soluble) crosses the surface membrane and directly inhibits the release of Ca^{2+} from the sarcoplasmic reticulum (SR). To test this possibility, we investigated the effect of (±)-propranolol on caffeine contractures (Figure 4).

Caffeine (7 mM) typically induced contractures that were 15–20% of maximal tetanic tension. After 30 min perfusion with Krebs solution containing 20 μM (±)-propranolol, the amplitude of caffeine contractures was increased by 25±11% when compared with the interpolated control ($n=5$, $P=0.10$).

Effects of (±)-propranolol on K^+ contractures

Results from the caffeine experiments suggested that (±)-propranolol was acting at a step in EC coupling before SR Ca^{2+} release. The effect of (±)-propranolol was next tested on K^+ contractures, which result from a depolarization-induced release of Ca^{2+} from the SR that is independent of the action potential.

Perfusion of soleus muscle bundles with a modified Krebs solution containing 60 mM total K^+ yielded contractures

that were 2.2±1.6% of maximum tetanic tension (T_{max}) and had a time to peak of 59±4 s ($n=10$). These contractures were recorded on the chart recorder at 10 times higher gain than that used for twitch recording, so that the inhibitory effects of propranolol were easily measurable. Contractures elicited with 124 mM K^+ ($n=8$) had a greater peak amplitude (26.0±4.4% of T_{max}) and a faster time to peak (34±5 s).

K^+ contracture amplitude was depressed by (±)-propranolol in a concentration-dependent manner (Figure 5). Concentrations of (±)-propranolol that completely abolished field-stimulated tetanic tension (100 μM) were found to inhibit K^+ contractures by only 25 to 55%. (±)-Propranolol at either 5 or 100 μM had a greater inhibitory effect on 60 mM K^+ contractures than on 124 mM K^+ contractures (Figure 5, $P=0.03$ and 0.02, 8 and 7 *d.f.*, respectively). The drug had no significant effect on the time to peak of K^+ contractures at either activation level.

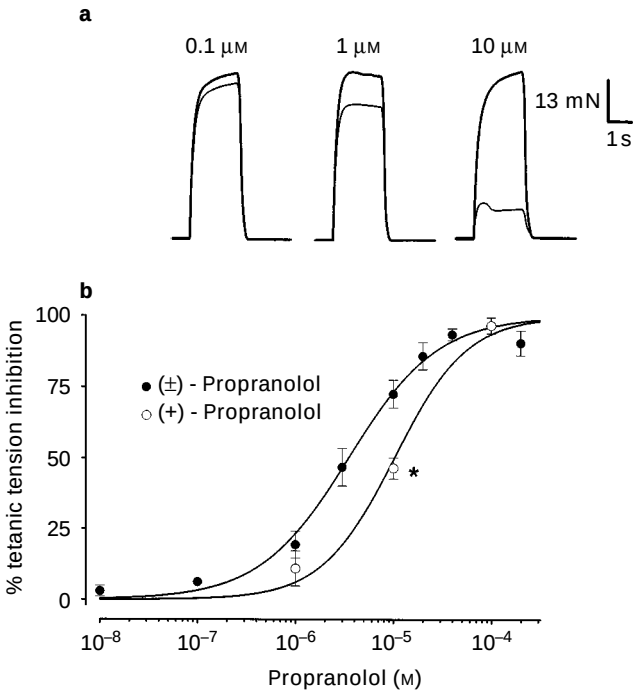

Figure 3 (a) Typical effects of (±)-propranolol on isometric tetani (40 Hz for 2 s) in three separate preparations. Traces were recorded before and after 30 min perfusion with the drug. (b) Concentration-response curves for inhibition of peak tetanic tension by (±)-propranolol and (+)-propranolol. Each point represents the mean response for 3 to 8 muscles; vertical lines show s.e.mean. *$P<0.05$ compared to 10 μM (±)-propranolol.

Table 1 Percentage change of twitch and tetanic time course (relative to control) after 30 min perfusion with different concentrations of propranolol

	Propranolol (μM)			
	0.1	*1.0*	*3.0*	*10*
Twitch				
Rise time 20%–80%	0±4	−6±3	−13±3*	−20±6*
Decay time 80%–20%	−3±3	−5±3	−6±1*	−16±4*
Tetanus				
Rise time 20%–60%	1±6	−15±6	−35±4*	−66±5*
Decay time 80%–20%	7±6	15±4*	41±10*	123±23*

*Significant difference from control ($P<0.05$, paired *t* test on raw data).

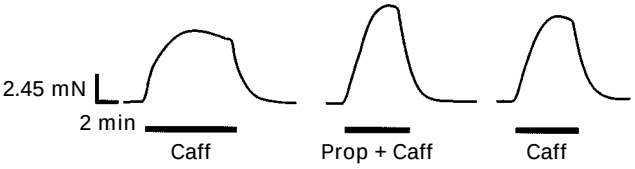

Figure 4 Effect of (±)-propranolol on caffeine contractures. Contractures shown in each panel were elicited by perfusion with 7 mM caffeine under the following conditions: Left, control; centre, after 30 min exposure to 20 μM (±)-propranolol; right, 45 min after washing with drug-free Krebs.

Effects of (±)-propranolol on resting membrane and action potentials

The effect of (±)-propranolol on the resting membrane potential (RMP) was studied in nine soleus muscles. The RMP measured after 15 min perfusion with 20 μM (±)-propranolol (67.9±1.2 mV, $n=152$ fibres) was not significantly different from the control values (69.3±1.3 mV, $n=103$ fibres, $P>0.09$).

The effect of (±)-propranolol on the intracellularly recorded action potential was determined in four experiments where stable recordings were maintained for the full 45 min experimental period. Figure 6 shows action potential recordings made before, during and after application of 20 μM (±)-propranolol in one of these experiments. Fifteen minutes perfusion with 20 μM (±)-propranolol decreased the maximum rate of rise of the action potential from 131±30 V s^{-1} to 23±10 V s^{-1} and decreased its amplitude from 82±5 mV to 42±14 mV. The (±)-propranolol effect was poorly reversible (Figure 6), as demonstrated by the maximum rate of rise (65±13 V s^{-1}) and peak amplitude (52±13 mV) of the action potential measured 30 to 60 min after subsequent perfusion with drug-free Krebs solution.

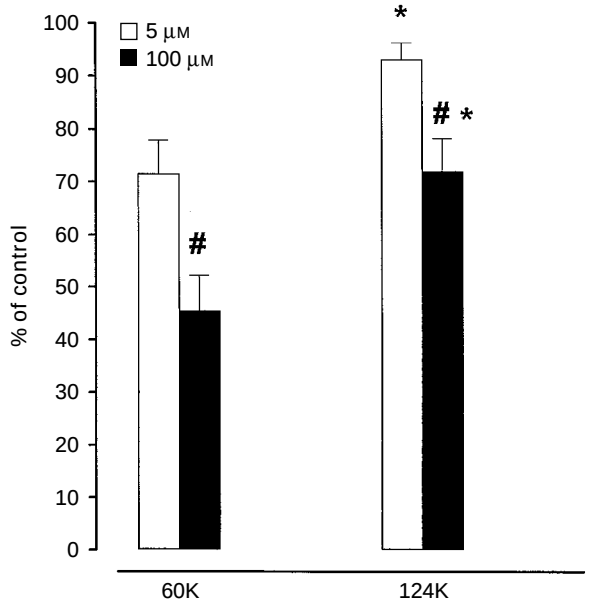

Figure 5 Effect of (±)-propranolol on potassium contractures. Contractures were elicited by perfusion with either 60 mM K^+ (60K) or 124 mM K^+ (124K) in the presence of either 5 μM or 100 μM (±)-propranolol. Each point represents the mean response±s.e.mean for 4 to 6 muscles. *Significantly different from the 60K result at the same propranolol concentration, $P<0.05$. #Significantly different from the 5 μM result, $P<0.05$.

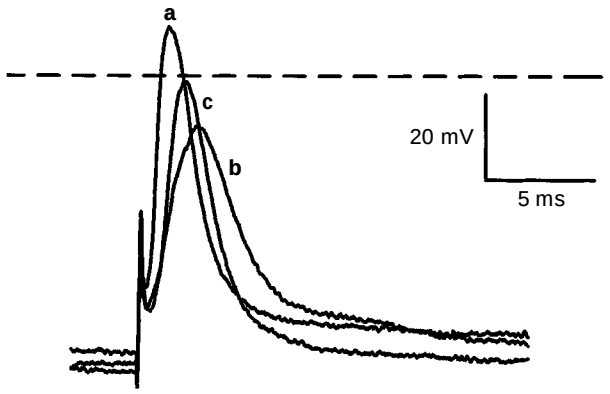

Figure 6 Effect of (±)-propranolol on intracellular action potentials. (a) Control, (b) after 15 min in 20 μM (±)-propranolol, (c) after 60 min wash in drug-free Krebs. Resting membrane potentials for traces (a)–(c) were −63, −66 and −65 mV, respectively.

Discussion

The results obtained in the present study demonstrate that (±)-propranolol depresses twitch and tetanic tension development in mammalian skeletal muscle in a concentration-dependent manner by (i) decreasing the amplitude and rate of rise of the action potential and (ii) directly inhibiting excitation-contraction coupling. These are the first experiments to characterize fully the complete concentration-response relationship for the effects of (±)-propranolol on skeletal muscle, yielding IC_{50} values of ~3.5 μM and ~7 μM for the tetanus and twitch, respectively. Such values are low compared with the 25% inhibition of twitch tension seen with 50 μM (±)-propranolol in rat inferior rectus muscles (Chiarandini, 1980) and are in complete contrast to the potentiation of twitch tension by 10 μM (±)-propranolol seen in single frog skeletal muscle fibres (Oota & Nagai, 1977).

The inhibitory actions of propranolol on contraction appear to be independent of β-adrenoceptor blockade because (+)-propranolol (which has ~100 times less β-blocking ability than the racemate; Howe & Shanks, 1966) was only 2.5 to 3 fold less potent than (±)-propranolol at inhibiting electrically-evoked tension. If the two isomers of propranolol had equal membrane stabilizing properties (Coltart & Meldrum, 1971) then the concentration-response curves for the (+)-isomer and the racemate should have been identical. Our results revealed a rightward shift of the concentration-response relation in the presence of (+)-propranolol, suggesting that it has approximately 2 to 3 fold less membrane stabilizing activity than the (−)-isomer in this preparation.

Effects on sarcoplasmic reticulum

It has recently been shown that high concentrations of (±)-propranolol (200 μM) can completely block the opening of single SR Ca^{2+} release channels incorporated into lipid bilayers (Zchut *et al*., 1996). Such direct effects could potentially account for the depression of tension by propranolol in intact fibres. However, the present results clearly show that concentrations of (±)-propranolol that severely attenuated tetanic tension (20 μM) failed to inhibit caffeine contractures, thus ruling out direct inhibition of SR Ca^{2+} release channel opening as a primary mechanism for the inhibition of tension in the intact muscle preparation.

Previous experiments in skinned muscle fibres have also led to the proposal that propranolol decreases contraction by inhibiting Ca^{2+} uptake by the SR Ca^{2+} pump (Su & Malencik, 1985). This action is unlikely to explain our results, because specific inhibition of the SR Ca^{2+} pump by other drugs leads to enhanced and prolonged tetanic tension responses in mammalian skeletal muscle (Westerblad & Allen, 1994). Furthermore, direct inhibitory effects of (±)-propranolol on SR Ca^{2+} uptake have only been shown at much higher concentrations (300–1000 μM) than those used in the present study (Noack *et al*., 1978; Su & Malencik, 1985). Nevertheless, the slight enhancement of caffeine contractures by 20 μM (±)-propranolol (Figure 4) and the marked prolongation of tetanic tension relaxation at propranolol concentrations ⩾10 μM (Table 1) are probably indicative of inhibitory effects on SR Ca^{2+} uptake at higher concentrations.

Actions of propranolol on membrane excitability

(±)-Propranolol (20 μM) had no effect on the resting membrane potential in soleus fibres, ruling out membrane depolarization as a possible mechanism of action. A similar lack of effect was seen by Larsen & Teräväinen (1978) in rat diaphragm muscles perfused with either (+)- or (−)-propranolol (68 μM).

Perfusion of mammalian skeletal muscle fibres with solutions containing raised extracellular K^+ leads to activation of excitation-contraction coupling in a manner which is independent of the sarcolemmal and transverse-tubular action potential (Dulhunty, 1992). The inhibition of sub-maximal K^+ contractures by (±)-propranolol (Figure 5) was significant but modest when compared to the effects of equivalent concentrations of the drug on twitch and tetanic tension. Previous studies in frog skeletal muscle fibres have also shown decreased K^+ contracture amplitude in the presence of high concentrations (50 μM) of (±)-propranolol (Oota & Nagai, 1977). As we have shown that (±)-propranolol has no direct effects on the SR Ca^{2+} release channels, these results suggest that (±)-propranolol has a small, direct inhibitory effect on a prior step in excitation-contraction coupling. One possibility is that this lipophilic drug inhibits the function of the voltage sensors (dihydropyridine receptors) located in the transverse tubular system, either by a membrane stabilizing action or by direct binding to a hydrophobic region of the receptor.

A further indication of a membrane stabilizing action of (±)-propranolol was shown by its ability to decrease markedly both the overshoot and the rate of rise of the intracellularly recorded action potential in the presence of (+)-tubocurarine (Figure 6). This effect suggests a blocking action on voltage-dependent sodium channels located in the sarcolemma and/or transverse tubular system, rather than a 'curare-like' action of propranolol that has been previously suggested in mammalian skeletal muscle (Larsen & Teräväinen, 1978; Chiarandini, 1980). The effects of 20 μM (±)-propranolol on action potentials in the present study (80% decrease in rate of rise, 50% decrease in amplitude) are quantitatively similar to those previously observed in rat diaphragm muscles at concentrations around 100 μM (Larsen, 1978), indicating a higher sensitivity of the rat soleus muscle to the effects of (±)-propranolol.

Possible clinical relevance

A direct, negative inotropic effect of propranolol on mammalian skeletal muscle fibres via a membrane-stabilizing mechanism could potentially contribute to its therapeutic efficacy as a tremorolytic agent (Ogawa *et al*., 1987). It might also partly explain other propranolol-induced side effects such as premature fatigue during endurance exercise (van Baak *et al*., 1995). Previous authors have argued against any clinical significance of the membrane stabilizing actions of propranolol on the basis that the concentrations required for such effects are 50 to 100 times the plasma concentrations that are effective in cardiovascular disorders and in essential tremor (Larsen & Teräväinen, 1982). However, this issue is complicated by the hydrophobicity of propranolol, which has often led to a poor correlation between plasma propranolol levels and the clinical variable being studied (eg. the degree of tremor reduction, Findley & Capildeo, 1984). Indeed, our results demonstrated that perfusion with relatively low concentrations of extracel-

lular propranolol (eg. 0.1 μM) produced an extremely slow depression of muscle twitch tension that had not reached steady-state at 30 min (Figure 1).

Both of the observations above could be explained if the concentration of propranolol in the plasma membrane (or hydrophobic pocket on a receptor) was a better indicator of efficacy of the drug and was slowly increasing over time. Such an idea implies that the IC_{50} obtained for the effects of propranolol could effectively decrease with prolonged incubation periods, a phenomenon which has been previously observed (Hellenbrecht *et al*., 1973). Thus, the relatively low IC_{50} for the 'membrane-stabilizing' action of propranolol on tetanic tension (3.5 μM), combined with the ability of the drug to gradually accumulate in biological membranes may contribute to a peripheral component of the tremorolytic and fatigue-inducing actions of propranolol on skeletal muscle.

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References

BENET, L.Z., ØIE, S. & SCHWARTZ, J.B. (1996). *The Pharmacological Basis of Therapeutics*. ed. Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. & Gilman, A.G., p. 1776, New York: McGraw-Hill.

CLEEVES, L. & FINDLEY, L.J. (1984). Beta-adrenoceptor mechanisms in essential tremor: a comparative single concentration study of the effect of a non-selective and a beta-2 selective adrenoceptor antagonist. *J. Neurol. Neurosurg. Psychiat*., **47,** 976–982.

CHIARANDINI, D.J. (1980). Curare-like effect of propranolol on rat extraocular muscles. *Br. J. Pharmacol*., **69,** 13–19.

COLTART, D. & MELDRUM, S.J. (1971). The effect of racemic propranolol, dextro-propranolol and racemic practolol on the human and canine cardiac transmembrane action potentials. *Arch. Int. Pharmacodyn*., **192,** 188–197.

DULHUNTY, A.F. (1992). The voltage-activation of contraction in skeletal muscle. *Prog. Biophys. Molec. Biol*., **57,** 181–223.

DULHUNTY, A.F. & GAGE, P.W. (1985). Excitation-contraction coupling and charge movement in denervated rat extensor digitorum longus and soleus muscles. *J. Physiol*., **358,** 75–89.

FINDLEY, L.J. & CAPILDEO, R. (1984). *Movement Disorders: Tremor*. New York: Oxford University Press.

HA, T. & FRYER, M.W. (1995). β-Adrenoceptor antagonists decrease isometric force production in rat slow-twitch skeletal muscles. *Proc. Australian Physiol. Pharmacol. Soc*., **26,** 125P.

HA, T. & FRYER, M.W. (1996). Inhibitory effects of (±)-propranolol on excitation and contraction of rat slow-twitch skeletal muscles. *Proc. Australian Physiol. Pharmacol. Soc*., **27,** 163P.

HELLENBRECHT, D., LEMMER, B., WIETHOLD, G. & GROBEEKER, H. (1973). Measurement of hydrophobicity, surface activity, local anaesthesia, and myocardial conduction velocity as quantitative parameters of the non-specific membrane affinity of nine β-adrenergic blocking agents. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **277,** 211–226.

HOWE, R. & SHANKS, G. (1966). Optical isomers of propranolol. *Nature*, **210,** 1336–1338.

LARSEN, A. (1978). Effects of pindolol, sotalol and the optical isomers of propranolol on muscle action potentials and depolarization-secretion coupling in the rat. *Acta Physiol. Scand*., **102,** 357–363.

LARSEN, A. & TERÄVÄINEN, H. (1978). Effects of the optical isomers of propranolol on neuromuscular transmission in the rat. *Exp. Neurol*., **59,** 435–443.

LARSEN, T.A. & TERÄVÄINEN, H. (1982). Propranolol and essential tremor: role of the membrane effect. *Acta Neurol. Scand*., **66,** 289–294.

MORALES-AGUILERÁ, A. & VAUGHAN WILLIAMS, E.M. (1965). The effects on cardiac muscle of β-receptor antagonists in relation to their activity as local anaesthetics. *Br. J. Pharmacol*., **24,** 332–338.

NOACK, E., KURZMACK, M., VERJOBSKI-ALMEIDA, S. & INESI, G. (1978). The effect of propranolol and its analogues on Ca^{2+} transport by sarcoplasmic reticulum vesicles. *J. Pharmacol. Exp. Ther*., **206,** 281–288.

OGAWA, N., TAKAYAMA, H. & YAMAMOTO, M. (1987). Comparative studies on the effects of β-adrenergic blockers in essential tremor. *J. Neurol*., **235,** 31–33.

OOTA, I. & NAGAI, T. (1977). Effects of catecholamines on excitation-contraction coupling in frog single twitch fiber. *Jap. J. Physiol*., **27,** 195–213.

SU, J.Y. & MALENCIK, D.A. (1985). Effects of (+)-propranolol on intracellular mechanisms of contraction in striated muscle of the rabbit. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **331,** 194–201.

VAN BAAK, M.A., DE HAAN, A., SARIS, W.H., VAN KORDELAAR, E., KUIPERS, H. & VAN DER VUSSE, G.J. (1995). Beta-adrenoceptor blockade and skeletal muscle energy metabolism during endurance exercise. *J. Appl. Physiol*., **78,** 307–313.

WESTERBLAD, H. & ALLEN, D.G. (1994). The role of sarcoplasmic reticulum in relaxation of mouse muscle; effects of 2,5-di(tertbutyl)-1,4-benzohydroquinone. *J. Physiol*., **474,** 291–301.

ZCHUT, S., FENG, W. & SHOSHANBARMATZ, V. (1996). Ryanodine receptor calcium release channel conformations as reflected in the different effects of propranolol on its ryanodine binding and channel activity. *Biochem. J*., **315,** 377–383.

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