The effects of β -adrenoceptor activation on contraction in isolated fast- and slow-twitch skeletal muscle fibres of the rat

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1 The aim of the experiments was to examine the effects of β -adrenoceptor activation on twitch and tetanic contractions in fast- and slow-twitch mammalian skeletal muscle fibres. Isometric force was recorded from bundles of intact fibres isolated from the normal and denervated slow-twitch soleus and normal fast-twitch sternomastoid muscles of the rat.

2 Terbutaline (10 μ M), a β_2 -adrenoceptor agonist, induced an average 15% potentiation of peak twitch and peak tetanic force in normal soleus fibres and abbreviated twitch and tetanic relaxation. In white- and red-sternomastoid fibres, 10 μ M terbutaline potentiated peak twitch force by about 7% and slowed twitch relaxation.

3 The potentiation of twitches and tetani by terbutaline was quantitatively similar in normal and denervated soleus fibres. However, in contrast to the normal soleus, terbutaline slowed twitch relaxation and had no effect on tetanic relaxation in denervated soleus fibres.

4 Adrenaline (10 μ M) increased peak tetanic force by about 7% in both normal and denervated soleus fibres.

5 Exposure to ($\pm$)-propranolol (0.1 μ M), a general β -adrenoceptor blocker, completely abolished the tetanus potentiation by terbutaline.

6 Dibutyryl-cyclic AMP (2 mM) mimicked the effects of 10 μ M terbutaline on peak tetanic force and tetanic relaxation in normal and denervated soleus fibres. Dibutyryl-cyclic AMP also potentiated peak twitch force in denervated soleus fibres but only after a brief period of twitch depression: the twitch depression might be due to butyrate.

7 The results suggest that the increase in peak twitch and tetanic force and abbreviation of tetanic relaxation induced by terbutaline depend on the activation of β -adrenoceptors and a consequent increase in the myoplasmic cyclic AMP concentration.

Keywords: β -Adrenoceptor; terbutaline; cyclic AMP; skeletal muscle contraction

Introduction

Sympathomimetic-amines modulate contraction and relaxation in mammalian skeletal muscles (reviewed by Tomita, 1975; Bowman, 1980; Williams & Barnes, 1989). The nature of the response depends on the fibre-type composition of muscles. In fast-twitch muscles (a) peak twitch force is increased by 10–20% and twitch relaxation is slowed (Goffart & Ritchie, 1952; Bowman & Zaimis, 1958; Bowman *et al.*, 1962; Tashiro, 1973; Tomita, 1975; Holmberg & Waldeck, 1980) and (b) the amplitude of unfused tetani is increased by up to 60% and the degree of fusion increased (Bowman & Zaimis, 1958; Bowman *et al.*, 1962; Holmberg & Waldeck, 1977; 1980). In contrast, peak twitch force is depressed by 10–25% in slow-twitch muscles and twitch relaxation is accelerated (Bowman & Zaimis, 1958; Bowman *et al.*, 1962; Tashiro, 1973; Tomita, 1975). The amplitude of unfused tetani is decreased by as much as 60% and fusion is reduced (Bowman & Zaimis, 1958; Bowman & Nott, 1970; Holmberg & Waldeck, 1979; 1980). However, under some conditions force is increased in slow-twitch muscles (Tashiro, 1973; Tomita, 1975; Holmberg & Waldeck, 1980). Sympathomimetics in these previous studies had little or no effect on peak tetanic force *in vivo* (Goffart & Ritchie, 1952; Bowman & Zaimis, 1958; Bowman & Nott, 1970; Williams & Barnes, 1989).

Most previous studies have examined the effects of sympathomimetic-amines on twitches and unfused tetani evoked by nerve stimulation in anaesthetized animals or in directly stimulated whole muscles *in vitro*. The aim of the present study was to investigate the direct effects of β -adrenoceptor activation on isometric contractions by using

small bundles of fibres isolated from various rat skeletal muscles. The use of these preparations allowed us to: (i) eliminate indirect sympathomimetic effects on contraction via neuromuscular transmission; (ii) reduce drug diffusion problems to inner fibres of whole muscles; (iii) distinguish between effects in different fibre-types.

Adrenoceptors in the sarcolemma of mammalian skeletal muscle are predominantly of the β_2 -subtype (Hirata *et al.*, 1986; Elfellah & Reid, 1987). Consequently, terbutaline, a selective β_2 -adrenoceptor agonist (Holmberg & Waldeck, 1977; McArdle & D'Alonzo, 1981), was chosen as the principal drug for the present study. Novel findings of this study were that terbutaline increased peak tetanic force in all types of preparation studied and abbreviated the relaxation of tetanic force in normal slow-twitch soleus fibres. Twitch force was increased and the relaxation of the twitch was faster in the presence of terbutaline. Furthermore, dibutyryl cyclic AMP mimics the effects of β -receptor activation on contractile force and relaxation, suggesting that cyclic AMP is the second messenger responsible for the effects of terbutaline on contraction.

Preliminary accounts of some of this work have been reported elsewhere (Cairns & Dulhunty, 1989a,b).

Methods

Biological preparations

Adult male Wistar rats (250–550 g) were killed with an overdose of either halothane or CO₂. Soleus or sternomastoid muscles were isolated, pinned by their tendons

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into a Petri dish lined with Sylgard (Dow Corning) and bathed in a modified Krebs solution (Solution A, Table 1) at room temperature (18–22°C). Small bundles of 5–50 fibres were dissected from tendon-to-tendon. Soleus muscles contain predominantly slow-twitch type I fibres (McArdle & D'Alonzo, 1981; Williams *et al.*, 1984) whereas red- or white-sternomastoid muscle segments contain predominantly fast-twitch type IIa or IIb fibres, respectively (Dulhunty & Dlutowski, 1979).

Denervation procedure

Rats were anaesthetized with ether and a 4–5 mm segment of the sciatic nerve was removed just distal to the sciatic notch. Soleus muscles were usually removed about 3–6 weeks later and denervation confirmed by: (i) whole muscle atrophy (Festoff *et al.*, 1977; Dulhunty, 1985); (ii) fibrillations (Smith & Thesleff, 1976; Dulhunty, 1985); (iii) a higher than normal twitch-to-tetanus ratio (Dulhunty, 1985).

Force recording and stimulation

Fibre bundles were mounted horizontally in a small volume (1.4 ml), rapid flow bath, with a solution change-over time of 700 ms. One tendon was held in spring-loaded clamping forceps and the other tendon was attached to a piezo-resistive force transducer (AME, 0–6 g, Horton, Norway). Isometric contractions were evoked by massive transverse electric field stimulation via platinum electrodes. Supramaximal stimulation was achieved by setting the voltage pulses to 0.5 ms duration and 1.1–1.3 times the voltage amplitude which just produced maximum twitch force. The pulse parameters were set using an isolated stimulator constructed in our laboratory. The length of the preparation was adjusted until maximum twitch force was obtained. Temperature was monitored by a thermistor placed in the bathing solution near the preparation and was set at 24.0°C, maintained to within 0.3°C, with a circulating water jacket.

Tetani were evoked at 100 Hz (unless stated otherwise) for a duration that ensured a force plateau was established (usually 1 s in soleus fibres and 250–300 ms in sternomastoid fibres). Stimulation was initiated by computer (Digital PDP 11/03) and pulse parameters set with an isolated stimulator constructed in our laboratory. Force was recorded continuously on a chart recorder (Hewlett Packard 7402A) and selected twitches and tetani sampled by the computer for later analysis.

Solutions and drugs

Most experiments were done in Solution A (Table 1). Solutions were usually bubbled with 100% O₂, although the absence of 100% O₂ did not influence contractile force or the viability of the fibres, suggesting that sufficient atmospheric O₂ equilibrated with the rapidly flowing solutions to maintain contractile function. All fine dissection took place in Solution A since it took about 1 h for the fibres to equilibrate in this solution.

Table 1 Composition of bathing solutions (in mM)

Solution	NaCl	Na ₂ SO ₄	KCl	K ₂ SO ₄	CaCl ₂	CaSO ₄	Sucrose
A	16	32.25	–	1.75	–	7.6	170
B	16	67.00	–	1.75	–	7.6	
Normal Krebs	150	–	3.5	–	2.5	–	–

In addition all solutions contained: 1 mM MgSO₄; 11 mM glucose; 2 mM TES(N-tris-(hydroxymethyl)-methyl-2-aminoethanesulphonic acid) pH buffer. The pH was adjusted to 7.4 ± 0.1 pH units with NaOH. The measured free Ca²⁺ concentration in solutions A and B was 2 mM.

Drugs were added to the bathing solution. Force was monitored until the maximum effect was seen and the drug was then washed out of the bath. Drugs used and sources were: terbutaline sulphate (ASTRA Pharmaceuticals); adrenaline hydrogen tartate (BDH Chemicals Ltd); N⁶,2'-O-dibutyryl adenosine 3':5'-cyclic adenosine monophosphate sodium salt (Sigma); (±)-propranolol hydrochloride (Sigma); n-butyric acid sodium salt (Sigma); ethylenediaminetetraacetic acid sodium salt (EDTA, AJAX); (+)-tubocurarine chloride (Boehringer). Terbutaline, adrenaline, dibutyryl-cyclic AMP and propranolol were all stored in anhydrous form in light-proof containers at 4°C. EDTA (50 µM) was added to all solutions to prevent the oxidation of sympathomimetic-amines. EDTA has no direct effect on force.

Analysis

The contractile properties measured were: peak twitch and peak tetanic force; 80–20% relaxation time; 20–80% rise time (twitch) and 20–60% rise time (tetanus). Maximum drug-induced changes in peak force or rise times were usually expressed relative to control contractions recorded immediately prior to drug application. However, the effect of terbutaline on peak tetanic force in sternomastoid fibres was difficult to quantify because of a progressive run-down of force with repetitive tetanic stimulation. Consequently, the control value for peak tetanic force was estimated from the values for peak tetanic force recorded immediately prior to and following wash-out terbutaline. A constant rate of force run-down was assumed and the control value was determined by interpolation to the time of the maximum terbutaline-induced effect. Changes in relaxation times were compared to the mean of controls obtained immediately prior to drug administration and following wash-out, since relaxation slowed progressively during the experiment. The effect of dibutyryl cyclic AMP (dbcyclic AMP) on relaxation was compared with the initial control since the effect of this drug on force did not readily reverse on wash-out. Values presented in the text are the mean ± s.e.mean of *n* preparations. Student's *t* test (paired data) was used to examine if drug-induced effects were significant and ANOVA (paired + unpaired data) was used to compare quantitatively the effects of different drugs or the effect of a drug in different types of preparation.

Results

Effects of terbutaline on slow-twitch soleus fibres

Terbutaline (10 µM) did not induce contractures, but induced positive inotropic effects (i.e. an increase in peak twitch and tetanic force) in slow-twitch soleus fibres (Figure 1). Maximum potentiation of the twitch and tetanus took 5–15 min and this potentiation was usually maintained until terbutaline was washed out of the bath. Full recovery from the effects of terbutaline were seen within 10–15 min after wash-out.

The average increase in peak twitch force was 14.6 ± 2.9% (*n* = 15, *P* < 0.001). Holmberg & Waldeck (1980) reported that terbutaline induced an initial depression of twitch amplitude in soleus muscles followed by potentiation. An early depression was seen in 3 out of 15 preparations in the present study but it was small (never more than 4% of peak twitch force): the decrease in force reversed and a potentiation was observed by 5 min.

An average increase in peak tetanic force of 14.6 ± 1.9% (*n* = 30, *P* < 0.001, Table 2) was recorded. This was a surprising result because sympathomimetic-induced tetanus potentiation of this magnitude had not been seen previously (Bowman, 1980; Williams & Barnes, 1989). The considerable variation in tetanus potentiation (s.d. = 10.5%, maximum increase 38.1%) could not be explained by submaximal receptor activation, preparation thickness, age of the animal,

Figure 1 Effects of 10 μ M terbutaline on (a) twitch force and (b) tetanic force in slow-twitch soleus fibres. Solution A, 24°C. Control, is the force record obtained immediately prior to the addition of terbutaline. Terbutaline, is the force record showing the maximum effect with terbutaline.

Table 2 Effects of terbutaline, adrenaline and dibutyryl-cyclic AMP on peak tetanic force in normal and denervated soleus fibres

Drug	Peak tetanic force (% increase)	
	Normal soleus	Denervated soleus
Terbutaline (10 μ M)	14.6 $\pm$ 1.9% (n = 30)	13.5 $\pm$ 1.1% (n = 44)
Adrenaline (10 μ M)	7.8 $\pm$ 2.6%* (n = 4)	6.9 $\pm$ 2.3% (n = 6)
dbCyclic AMP (2 mM)	15.2 $\pm$ 2.0% (n = 5)	16.0 $\pm$ 2.8% (n = 7)
Terbutaline** (10 μ M)	12.7 $\pm$ 4.6%	17.0 $\pm$ 3.8%

Values shown are the mean $\pm$ s.e.mean of n preparations. The maximum increase in peak tetanic force (100 Hz) was expressed as a percentage increase relative to the immediately preceding control tetanus. Solution A, 24°C. Drug induced effects were tested by paired t test. All values were significant for $P < 0.001$ except * where $P < 0.05$; **indicates paired with dbcyclic AMP.

season, or the extent of run-down (as in isolated cardiac muscle, Kurihara & Konishi, 1987). Indeed, the same variation was observed when different preparations from the same muscle were used, indicating a biological origin.

Although sympathomimetics have been reported to depress the amplitude of unfused tetani in slow-twitch muscles (Bowman & Nott, 1970; Holmberg & Waldeck, 1979), 10 μ M terbutaline in our hands induced a 7–37% potentiation of unfused tetani (evoked at 20 Hz for 1 s at 31°C ($n = 2$) or 15 Hz for 1 s at 24°C ($n = 1$)).

Since sympathomimetic-amines facilitate neuromuscular transmission by stimulating α -adrenoceptors (Bowman *et al.*, 1962; Bowman & Nott, 1970; Tomita, 1975), it was unlikely that terbutaline (a β_2 -agonist) potentiated force by acting at the neuromuscular junction. This was confirmed when ter-

butaline, applied in the presence of 1.5 μ M tubocurarine, potentiated tetanic force in the usual way ($n = 2$), suggesting that the drug acted directly on the muscle fibres.

Terbutaline also abbreviated the time-course of twitch and tetanic relaxation (Figure 1): the 80–20% relaxation time was abbreviated from 169 $\pm$ 15 ms to 153 $\pm$ 7 ms ($n = 9$, $P < 0.05$) for the twitch and from 156 $\pm$ 8 ms to 119 $\pm$ 6 ms ($n = 20$, $P < 0.001$) for the tetanus. The effect of terbutaline on tetanic relaxation has not been described previously and was significantly greater than the effect on twitch relaxation ($P < 0.05$). No significant changes were observed in rise times for either the twitch or tetanus.

Effects of terbutaline on fast-twitch sternomastoid fibres

No previous studies have compared the effect of sympathomimetic-amines on contraction in different types of mammalian fast-twitch fibres. Terbutaline (10 μ M) increased peak twitch force in both white- and red-sternomastoid fibres (Figure 2). Twitch potentiation was the same, i.e. 7.3 $\pm$ 2.1% ($n = 8$, $P < 0.025$) and 7.2 $\pm$ 2.8% ($n = 5$, $0.1 < P < 0.5$) in white- and red-sternomastoid fibres, respectively and the average pooled potentiation (7.3 $\pm$ 1.6) was significantly smaller than in slow-twitch soleus fibres ($P < 0.05$). Peak tetanic force was increased by terbutaline in two preparations (5.1% and 7.5%) while in seven other preparations the run-down was slowed. The average terbutaline-induced increase in peak tetanic force was 6.5 $\pm$ 1.3% ($n = 9$, $P < 0.001$, maximum increase 13.5%) following correction for run-down.

Twitch relaxation was prolonged with terbutaline in sternomastoid fibres (Figure 2), in contrast to the faster twitch relaxation in soleus. The average 80–20% relaxation time increased from 44.5 $\pm$ 3.5 ms to 50.0 $\pm$ 3.0 ms ($n = 8$, $P < 0.01$) in white-sternomastoid fibres and from 60.0 $\pm$ 11.0 ms to 64.0 $\pm$ 11.0 ms ($n = 5$, $P < 0.05$) in red-sternomastoid fibres. There was no significant change in the rise time for twitches. We noted that, although the average relaxation time increased, relaxation times in some individual preparations remained unchanged in the presence of terbutaline, despite significant twitch potentiation. This suggested that the pro-

Figure 2 Effects of 10 μ M terbutaline on isometric twitches in (a) fast white-sternomastoid and (b) fast red-sternomastoid fibres. Solution A, 24°C. Note the prolonged relaxation with terbutaline in (a) but no change in the relaxation time-course in (b). Terbutaline and control are defined in the legend of Figure 1.

cess responsible for twitch potentiation was independent of the process responsible for prolonged relaxation.

Effects of terbutaline on denervated soleus fibres

Skeletal muscle is thought to become supersensitive to catecholamines following chronic denervation as catecholamine-induced contractures have been reported (Bowman & Raper, 1965; Evans & Smith, 1976). It was possible that the denervated muscles may also become supersensitive to terbutaline. Thus we investigated the possibilities that contractures might be induced by terbutaline and that potentiation of force would be enhanced in denervated fibres. Terbutaline ($10\text{ }\mu\text{M}$) did not induce contractures. Instead it caused a small depression of resting force in 50% of the preparations and reduced the amplitude of fibrillations (asynchronous twitches produced by the spontaneous firing of action potentials in some fibres, Smith & Thesleff, 1976; Dulhunty, 1985).

Terbutaline did potentiate twitches and tetani in denervated soleus fibres (Figure 3). The average increase of peak twitch force of $13.7 \pm 2.1\%$ ($n = 23$, $P < 0.001$) was not significantly different from that in normal soleus fibres ($P > 0.1$). As in normal soleus fibres, maximum twitch potentiation occurred 10–15 min after the drug was applied and the effect was reversed when terbutaline was washed out of the bath. However, in contrast to normal soleus fibres, relaxation of the twitch in the denervated soleus was prolonged by terbutaline (Figure 3a). On average the 80–20% relaxation time increased from $114 \pm 6\text{ ms}$ to $151 \pm 9\text{ ms}$ ($n = 19$, $P < 0.001$). The potentiation of peak tetanic force of $13.5 \pm 1.1\%$ ($n = 44$, $P < 0.001$, Table 2) was not different from that in the normal soleus ($P > 0.1$). Thus, denervation did not alter the extent of twitch or tetanus potentiation, nor the variability in tetanus potentiation (s.d. = 7.4%, maximum increase 29.7%). Terbutaline did not influence tetanic relaxation in denervated soleus fibres. The 80–20% relaxation times were $92 \pm 3\text{ ms}$ (control) and $95 \pm 3\text{ ms}$ (terbutaline) ($n = 37$, $P > 0.1$).

There was another notable change in the response to terbutaline following denervation. Repeated exposure to terbutaline (at least 20 min between applications) induced

similar increases in peak tetanic force in denervated soleus fibres. In contrast, normal soleus fibres displayed progressively smaller response with successive applications, i.e. tachyphylaxis. The effect of terbutaline on peak tetanic force on the third application was $80 \pm 5\%$ ($n = 12$) of the increase on the first application in denervated fibres, but was only $44 \pm 7\%$ ($n = 5$) in normal fibres. Thus denervation resulted in some protection against tachyphylaxis.

The effect of increasing concentrations of terbutaline on peak twitch force (not shown) was similar to the effect on peak tetanic force (Figure 4) and these effects were quantitatively similar in four preparations examined. Terbutaline ($0.1\text{ }\mu\text{M}$) first slightly depressed peak force (the largest decrease was 8%) and then augmented force. Progressively increasing the concentration of terbutaline caused further potentiation; the maximum increase was with $10\text{ }\mu\text{M}$ terbutaline.

Tetanus potentiation by terbutaline under different experimental conditions

The tetanus potentiation with terbutaline was very much greater than reported previously for other sympathomimetic amines (Bowman, 1980). We considered the possibility that the large tetanus potentiation was a consequence of our experimental conditions. The effects of the composition of the bathing solution and stimulation frequency during the tetanus were examined.

The action of terbutaline in different bathing solutions The amplitude of action potentials during tetanic stimulation may have been reduced in the modified Krebs solution which contained 80.5 mM Na^+ (Solution A), compared with the normal 150 mM Na^+ (Table 1). A smaller action potential amplitude would have reduced peak tetanic force to allow room for greater potentiation with terbutaline. However, this was not the case because $10\text{ }\mu\text{M}$ terbutaline produced a similar increase in peak tetanic force in the normal Krebs solution ($n = 5$). In three denervated soleus preparations, where paired data were obtained, terbutaline potentiated peak tetanic force by $19.4 \pm 10.1\%$ in the normal Krebs solution and then by $15.2 \pm 4.7\%$ in the 80.5 mM Na^+ solution. In addition, peak tetanic force was increased when the bathing solution was changed from Solution A to one containing 196 mM Na^+ (Solution B, Table 1), but this did not prevent tetanus potentiation by terbutaline (Figure 5). Peak tetanic force was increased by $18.4 \pm 5.0\%$ ($n = 3$) when exposed to the 196 mM Na^+ solution and then by a further $8.9 \pm 0.9\%$ with terbutaline.

Figure 3 Typical effects of $10\text{ }\mu\text{M}$ terbutaline on (a) twitch force and (b) tetanic force in denervated soleus fibres. Solution A, 24°C . Terbutaline and control are defined in the legend of Figure 1.

Figure 4 Concentration- and time-dependent effects of terbutaline on peak tetanic force in denervated soleus fibres. Tetani were evoked at 50 Hz for 2 s every 5 min . Solution A, 24°C . The concentration of terbutaline was progressively increased during the experiment with the period of exposure to terbutaline, at each concentration, indicated by the bars above the force records.

Figure 5 Chart recordings showing the effect of $10\text{ }\mu\text{M}$ terbutaline on peak tetanic force in Solution A ($[\text{Na}^+] = 80.5\text{ mM}$, $[\text{Cl}^-] = 16\text{ mM}$) and then in Solution B ($[\text{Na}^+] = 196\text{ mM}$, $[\text{Cl}^-] = 16\text{ mM}$) in a denervated soleus preparation. Tetani were evoked at 30 Hz for 2 s, 24°C . Terbutaline increased peak tetanic force by 22% in Solution A. The third tetanus was recorded in Solution A following washout of terbutaline. Exposure to Solution B increased peak tetanic force by 17% and then terbutaline potentiated peak tetanic force by 10% in Solution B.

The action of terbutaline at different frequencies of tetanic stimulation Although fully fused tetani were evoked at 100 Hz, a greater peak tetanic force was achieved at lower frequencies (around 50 Hz) in normal and denervated soleus fibres (in Solution A, 24°C). Since terbutaline may have acted by preventing this depression of force at 100 Hz, the effect of terbutaline was examined on tetani evoked at 100 and 50 Hz. The peak tetanic force at 100 Hz was $87.4 \pm 2.0\%$ ($n = 9$) of the 50 Hz tetanus in normal soleus fibres. Terbutaline increased the amplitude of tetani at 100 Hz by $14.6 \pm 1.9\%$ ($n = 30$) and by $8.6 \pm 1.6\%$ ($n = 9$) at 50 Hz. In paired experiments on seven denervated soleus preparations, the peak tetanic force at 100 Hz was $79.8 \pm 1.8\%$ of that at 50 Hz. Terbutaline potentiated the 100 and 50 Hz tetani by $15.0 \pm 1.8\%$ and $12.9 \pm 1.6\%$, respectively. Therefore tetanus potentiation by terbutaline did not depend on the frequency of tetanic stimulation.

Effects of adrenaline on contraction

It was of interest to see if adrenaline, a physiological catecholamine, induced similar effects on contraction to those of terbutaline. Adrenaline ($10\text{ }\mu\text{M}$) potentiated tetani by about 7% in normal and denervated soleus fibres (Table 2), and potentiated twitches by $17.3 \pm 3.4\%$ ($n = 6$, $P < 0.01$) (pooled normal and denervated soleus fibres). There was no consistent effect on relaxation times. Adrenaline did not alter resting force in normal soleus fibres, but induced small contractions (associated with an increased amplitude of fibrillations) in denervated soleus preparations. This was in contrast to the decrease in resting force and the depressed fibrillations with terbutaline.

Effect of propranolol on tetanus potentiation by terbutaline

Since high concentrations of terbutaline were used, the drug may have produced effects that were independent of β -adrenoceptor stimulation, perhaps by activating α -adrenoceptors which may also be found in rat skeletal muscle (Hirata *et al.*, 1986). Propranolol, a general β -adrenoceptor blocker, was used to test this possibility. Addition of $0.1\text{ }\mu\text{M}$ propranolol alone had no direct effect on force. The tetanus potentiation with terbutaline was completely abolished following a 15 min exposure to $0.1\text{ }\mu\text{M}$ propranolol (Figure 6) in three denervated and one normal soleus. Thus force potentiation by terbutaline requires β -receptor activation.

Effects of dibutyryl cyclic AMP on contraction

Effects of β -agonists may occur via cyclic AMP (Oota & Nagai, 1977; Fellenius *et al.*, 1980b) or directly via G-

Figure 6 Effect of $10\text{ }\mu\text{M}$ terbutaline on peak tetanic force (100 Hz) in the absence and presence of $0.1\text{ }\mu\text{M}$ propranolol in a denervated soleus preparation. Solution A, 24°C . Terbutaline maximally increased peak tetanic force by 11% in 10 min under control conditions. Propranolol added for 15 min had no direct effect on force production and then terbutaline failed to potentiate force during exposure to propranolol when added for 20 min.

Figure 7 Representative effects of $10\text{ }\mu\text{M}$ terbutaline and 2 mM dibutyryl cyclic AMP on peak tetanic force (100 Hz) in a normal soleus preparation. Solution A, 24°C . Tetani were recorded every 5 min. Terbutaline maximally increased peak tetanic force by 10% in 10 min. The second control was obtained about 30 min after wash-out of terbutaline. Dibutyryl cyclic AMP maximally increased peak tetanic force by 19% in 20 min.

proteins (Yatani *et al.*, 1988). We tested the hypothesis that an increased concentration of myoplasmic cyclic AMP ([cyclic AMP]) was responsible for the effects of terbutaline on contraction using, dibutyryl cyclic AMP (dbcyclic AMP), a derivative of cyclic AMP which is more membrane permeable and less susceptible to intracellular hydrolysis than is cyclic AMP (Skelton *et al.*, 1970; Drummond *et al.*, 1974).

dbcyclic AMP (2 mM) mimicked the tetanus potentiation seen with $10\text{ }\mu\text{M}$ terbutaline in normal soleus fibres (Figure 7). Notably a longer time was required to develop maximum effects with dbcyclic AMP and these effects reversed only slowly on wash-out of the drug. Consequently, in paired experiments, terbutaline was added first and post-wash controls for dbcyclic AMP were not obtained. The tetanus potentiation by terbutaline and dbcyclic AMP was quantitatively similar in normal and denervated soleus fibres (Table 2). As with terbutaline, dbcyclic AMP had no effect on tetanic relaxation in denervated soleus fibres, but abbreviated relaxation in normal soleus fibres (Figure 8). The 80–20% relaxation time was reduced from the control $165 \pm 15\text{ ms}$ to $126 \pm 7\text{ ms}$ with dbcyclic AMP ($n = 5$, $P < 0.001$).

Unexpectedly, dbcyclic AMP reduced peak twitch force by $7.4 \pm 2.0\%$ ($n = 6$, $P < 0.05$) at 5 min; an effect seen with $0.1\text{ }\mu\text{M}$ terbutaline, but seldom seen with $10\text{ }\mu\text{M}$ terbutaline (see above). Twitch potentiation slowly developed and the initial twitch depression was reversed (Figure 9). In nine paired experiments, maximum twitch potentiation with dbcyclic AMP ($10.3 \pm 4.1\%$) was similar to that with ter-

Figure 8 Typical effects of (a) $10\ \mu\text{M}$ terbutaline and (b) $2\ \text{mM}$ dibutyl cyclic AMP on tetanic relaxation in normal soleus fibres. The relaxation portion of the force records (from the end of the stimulation period) were normalized and superimposed. Tetani were evoked at $100\ \text{Hz}$ for $1\ \text{s}$, Solution A, 24°C . The control in (b) was obtained following wash-out of terbutaline in (a).

Figure 9 Chart recordings showing the effect of $2\ \text{mM}$ dibutyl cyclic AMP (dbcyclic AMP) on twitches and fibrillations in a denervated soleus preparation. Solution A, 24°C . The twitches were depressed by 9% in $9\ \text{min}$ and showed a maximal potentiation of 22% at $39\ \text{min}$.

butaline ($10.9 \pm 2.2\%$) dbcyclic AMP slowed twitch relaxation in the same way as terbutaline.

Some effects of dbcyclic AMP might have been due to butyrate which is formed following deacylation of dbcyclic AMP (Skelton *et al.*, 1970; Kurihara & Konishi, 1987). Exposure to butyrate ($2\ \text{mM}$) depressed peak twitch force by $7.5 \pm 0.8\%$ ($n = 3$) and either depressed or had no effect on peak tetanic force. Therefore force potentiation by dbcyclic AMP cannot be attributed to butyrate. However the initial reduction of force with dbcyclic AMP might be caused by butyrate.

Discussion

The results of this study demonstrate that (a) β -adrenoceptor agonists potentiate force in all types of mammalian skeletal

muscle fibres – a clear potentiation of tetanic force is shown for the first time; (b) β -adrenoceptor agonists alter the rate of relaxation of twitch and tetanic contractions in a manner that varies with the type of muscle fibre; (c) dbcyclic AMP mimics the effects of β -receptor activation, providing strong evidence for a role of cyclic AMP in the end effects β -adrenoceptor activation on contraction.

Effects of β -adrenoceptor activation on contraction in different fibre-types and after denervation

The greater twitch potentiation by terbutaline in soleus than in sternomastoid is consistent with a greater density of β -adrenoceptors in slow-twitch fibres (Williams *et al.*, 1984) and greater enhancement of adenylate cyclase activity (Festoff *et al.*, 1977; Fellenius *et al.*, 1980a; Williams *et al.*, 1984). The similar twitch potentiation in different types of fast-twitch sternomastoid fibre indicates similar β -adrenoceptor densities and activation of adenylate cyclase. Terbutaline induced a dramatic acceleration of tetanic relaxation which had not been reported previously. The smaller acceleration of twitch relaxation with terbutaline in soleus fibres and slowing of relaxation in sternomastoid fibres was in agreement with earlier studies (Bowman & Zaimis, 1958; Bowman *et al.*, 1962; Tashiro, 1973; Tomita, 1975).

The increase of peak twitch and tetanic force by terbutaline and adrenaline was unaltered by denervation in soleus fibres, although relaxation of the twitch force was slowed with terbutaline, rather than accelerated. Tachyphylaxis with repeated drug application was reduced, possibly because β -adrenoceptors in denervated fibres are less susceptible to desensitization, or because adenylate cyclase activity is enhanced (Hashimoto *et al.*, 1989). Changes in resting force were also observed in denervated fibres. Catecholamines induce contractures in denervated muscle (Bowman & Raper, 1965; Evans & Smith, 1976) and adrenaline induced contractures with increased fibrillations in this study. In contrast, terbutaline and dbcyclic AMP reduced resting force and depressed fibrillations (Figure 9). These changes in resting force could be due to changes in membrane potential and spontaneous depolarizations, which cause fibrillations and initiate action potentials (Bowman & Raper, 1965). The increased frequency of spontaneous depolarizations with adrenaline (Bowman & Raper, 1965; Smith & Thesleff, 1976) could cause a contracture by synchronizing fibrillations in different fibres. Terbutaline and dbcyclic AMP hyperpolarize fibres (McArdle & D'Alonzo, 1981; Cairns & Dulhunty, unpublished observations) and spontaneous depolarizations no longer trigger action potentials (Cairns & Dulhunty, unpublished observations).

The role of β -adrenoceptors and myoplasmic cyclic AMP

Sympathomimetics activate β -adrenoceptors in mammalian skeletal muscle (Bowman & Nott, 1970; Holmberg & Waldeck, 1977; 1980; Bowman, 1980; McArdle & D'Alonzo, 1981). Propranolol abolished the tetanus potentiation by terbutaline. This is consistent with previous studies with various β -blockers (including selective β_2 -blockers) and demonstrated the involvement of β -adrenoceptors in the effects that we describe.

The gradual development of inotropic effects with terbutaline suggested the involvement of a second messenger. Terbutaline and other sympathomimetics increase [cyclic AMP] in skeletal muscle (Festoff *et al.*, 1977; Fellenius *et al.*, 1980a; Williams *et al.*, 1984). dbcyclic AMP has been shown to potentiate twitches in frog twitch fibres and in rat diaphragm (Oota & Nagai, 1977; Varagic & Kentara, 1978). dbcyclic AMP in the present study mimicked most of the effects of terbutaline, again suggesting that cyclic AMP is the second messenger. The slow action of dbcyclic AMP was attributed to time required for dbcyclic AMP to enter the

cells (Drummond *et al.*, 1974). Studies using phosphodiesterase inhibitors (Bowman & Nott, 1974) or forskolin (Prostran & Varagić, 1986) support the involvement of cyclic AMP in sympathomimetic-induced potentiation of force in fast-twitch fibres.

Mechanism for the terbutaline-induced effects on relaxation

The cyclic AMP-dependent phosphorylation of phospholamban, a 22 kD sarcoplasmic reticulum (SR) protein which regulates the SR Ca^{2+} -pump, is thought to abbreviate relaxation of cardiac twitch force (Kirchberger & Tada, 1976; Lindemann *et al.*, 1983; Kurihara & Konishi, 1987; Tada *et al.*, 1988). Phospholamban is present in slow-twitch, but not fast-twitch, skeletal muscle (Jorgensen & Jones, 1986; Tada *et al.*, 1988) and is probably the 22 kD protein phosphorylated by cyclic AMP-protein kinase to accelerate Ca^{2+} uptake into SR from slow-twitch, but not from fast-twitch, fibres (Kirchberger & Tada, 1976; Salvati *et al.*, 1982).

The cyclic AMP/phospholamban system might also be responsible for the terbutaline-induced acceleration of relaxation in normal soleus fibres. The absence of phospholamban would explain the inability of terbutaline to abbreviate relaxation in sternomastoid fibres and also possibly in denervated soleus. Faster twitch relaxation in cardiac muscle with sympathomimetics may also be due to altered myofilament function following phosphorylation of troponin-I or C-protein (England *et al.*, 1984; Hartzell, 1984). However, this is not the case in skeletal muscle since neither protein is phosphorylated during β -adrenoceptor activation (England *et al.*, 1984). Furthermore, sympathomimetics decrease the resting myoplasmic calcium concentration in slow-twitch fibres (Ballanyi & Grafe, 1988) in a manner that is consistent with increased calcium uptake by the SR, but not consistent with a change in myofilament function (i.e. with faster calcium dissociation from troponin). The effect of terbutaline on relaxation is unlikely to reflect a modulation of cross-bridge function by changes in metabolite concentrations. Intracellular pH in soleus fibres is not altered by adrenaline (Ballanyi & Grafe, 1988) and changes in the concentration of other metabolites due to enhanced glycolysis (Fellenius *et al.*, 1980a,b) may account for the slower relaxation of fast-twitch fibres with terbutaline, but cannot account for the faster relaxation in soleus fibres (Fellenius *et al.*, 1980b; Ballanyi & Grafe, 1988).

Mechanism for the positive inotropic effect of terbutaline

Force potentiation by terbutaline, adrenaline and dbcyclic AMP was not due to an effect at the neuromuscular junction. The drugs apparently modulated a cellular process involved in contraction initiated by action potentials since they did not trigger contractures by themselves. Phosphorylation of phospholamban may have contributed to force potentiation in slow-twitch fibres, possibly by increasing the Ca^{2+} loading of the SR, but phospholamban is not found in fast-twitch fibres (Jorgensen & Jones, 1986; Tada *et al.*, 1988) and hence cannot be involved in those fibres. Force potentiation occurred irrespective of the changes in the rate of relaxation; therefore at least two separate processes were influenced by terbutaline. Sympathomimetics could, in principal, potentiate force by enhancing myofilament function, although cyclic AMP-protein kinase does not influence the myofilaments in skinned fast-twitch fibres (Fabiato & Fabiato, 1978). There may be greater action potential depolarization (Smith & Thesleff, 1976; McArdle & D'Alonzo, 1981) or cyclic AMP might modulate a process in excitation-contraction coupling (Oota & Nagai, 1977; Fabiato & Fabiato, 1978; Yatani *et al.*, 1988; Williams & Barnes, 1989). Indeed, Brum *et al.* (1990) demonstrated that adrenaline increases Ca^{2+} release in voltage-clamped frog fibres.

Why were twitches and unfused tetani in slow-twitch fibres potentiated by terbutaline in this study but depressed in previous studies?

The increased peak force during twitches and unfused tetani in soleus fibres was in striking contrast to most previous reports where sympathomimetic-amines reduce the amplitude of submaximal contractions in slow-twitch muscles (Bowman & Zaimis, 1958; Bowman *et al.*, 1962; Tashiro, 1973; Tomita, 1975; Holmberg & Waldeck, 1979). We think that the differences between our results and those of others arise from the concentration of terbutaline used and the smaller size of the preparations. It is unlikely that the discrepancies were due to the external solution used. Holmberg & Waldeck (1980), using a standard bicarbonate buffered solution, demonstrated that 2.3 μM terbutaline in guinea-pig soleus initially depressed force by 15% before producing a final potentiation of 25%. This was similar to the effect of 0.1 μM terbutaline and to dbcyclic AMP in the present study. One possibility is that the force depression seen with 0.1 μM terbutaline and with dbcyclic AMP was due to the same process that induced twitch depression in other studies.

We suggest that there may be a biphasic response to sympathomimetics if they activate separate processes that firstly depress and later potentiate force, and the depression is activated at lower [cyclic AMP]_i than the potentiation (see Figure 4). This hypothesis is based on the observation of the small (maximum 8%) and transient depression of force, followed by force potentiation, at lower concentrations of terbutaline (Figure 4 above) and with dbcyclic AMP. The hypothesis is also consistent with the twitch depression prior to potentiation noted by Holmberg & Waldeck (1980) with 2.3 μM terbutaline in whole muscles. Diffusion limitations with the whole muscle preparation would mean that concentrations of terbutaline (and [cyclic AMP]_i) away from the surface of the muscle would be significantly less than 2.3 μM for some time after this concentration of the drug had been applied to the external solution.

The depression of force has been attributed to enhanced sodium-pump activity (Tashiro, 1973; Tomita, 1975; Holmberg & Waldeck, 1980) or to increased Ca^{2+} removal by the SR during contraction (Bowman, 1980), but could also be due to reduced myofilament calcium sensitivity. We seldom saw twitch depression with terbutaline, and when we did it rapidly reversed and potentiation was seen. Therefore the force potentiating mechanism dominated in our system.

Sympathomimetic-induced potentiation of peak tetanic force

Terbutaline and adrenaline potentiated peak tetanic force in soleus fibres. This was in contrast to *in vivo* studies where sympathomimetics either reduce, or have no effect on, peak tetanic force (Goffart & Ritchie, 1952; Bowman & Zaimis, 1958; Bowman & Nott, 1970): tetanus depression has been associated with vasoconstriction (Bowman & Zaimis, 1958; Bowman & Nott, 1970). A small tetanus potentiation in soleus, induced by isoprenaline in anaesthetized cats, was attributed to vasodilatation or to a contribution from fast-twitch fibres (Bowman & Nott, 1970).

The lack of tetanus potentiation in previous studies may have been due to the use of *in vivo* preparations with anaesthetics which directly potentiate force (Holmberg & Waldeck, 1979) and may thus prevent the force potentiating effects of the sympathomimetics from being seen. In addition, circulating hormones may increase cyclic AMP levels to mask the effects of added sympathomimetics. Indeed, the [cyclic AMP]_i is considerably higher in resting muscle *in vivo* than *in vitro* (Fellenius *et al.*, 1980b).

Moreover, it has been thought that hormones cannot increase peak tetanic force by increasing calcium release from the SR since it is assumed that troponin-C is saturated with

Ca^{2+} during the tetanus (Blinks *et al.*, 1978). However this is not true in mammalian fibres since caffeine (Cairns & Dulhunty, 1989b; Fryer & Neering, 1989) and external solutions containing high potassium concentrations (Dulhunty & Gage, 1985) allow force production that is greater than the normal peak tetanic force. Therefore sympathomimetics could, in principal, increase force in a normal tetanus by

increasing the myoplasmic Ca^{2+} concentration and allowing further Ca^{2+} activation of the myofilaments.

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