British Journal of Pharmacology (1998) 123, 558–564

Effects of the enantiomers of R,S-salbutamol on incompletely fused tetanic contractions of slow- and fast-twitch skeletal muscles of the guinea-pig

[1,3]Chris Prior, [1]Maureen B. Leonard & [2]John R. McCullough

[1]Department of Physiology and Pharmacology, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, Scotland and [2]Sepracor Inc., 111 Locke Drive, Marlborough, MA 01752, U.S.A.

1 The effects of racemic **R,S**-salbutamol, and its individual enantiomers have been studied on incompletely fused (sub-tetanic) contractile responses of fast- and slow-contracting isolated skeletal muscles of the guinea-pig.

2 **R,S**-salbutamol (2–4 μM) decreased the peak force of sub-tetani in the slow-contracting soleus muscle and increased the peak force of sub-tetani in the fast-contracting peroneus longus muscle. It also increased the force of the first twitch of sub-tetani in both muscles. The decrease in the peak force of sub-tetani in the soleus muscle was due to defusion of the individual twitches caused by a shortening of their time course.

3 The effects of 4 μM of the racemate on both fast- and slow-contracting muscles were mimicked by 2 μM **R**-salbutamol (levalbuterol). However, 2 μM **S**-salbutamol was devoid of activity in both muscles.

4 We concluded that all the effects of **R,S**-salbutamol on guinea-pig skeletal muscles are due to the activity of the **R**-enantiomer. Thus there is a common enantiomeric profile for the skeletal muscle and bronchorelaxant activity of the compound.

Keywords: Salbutamol; levalbuterol; soleus muscle; peroneus longus muscle; isolated muscle tension recording; tetanic defusion; tremor

Introduction

Skeletal muscle function is modulated by β-adrenoceptor agonists (reviewed by Bowman & Nott, 1969; Bowman, 1980; Rodger & Bowman, 1983). The effect depends on the mix of white, intermediate and red fibres present in the muscle. In fast-contracting muscles, there are increases in single twitch tension, single twitch duration and the degree of fusion of partially fused tetanic (sub-tetanic) responses. Conversely, in slow-contracting muscles, twitch tension, twitch duration and the degree of fusion of sub-tetani are all decreased. These effects of β-adrenoceptor agonists are possibly due to an increase (fast-twitch muscle) or decrease (slow twitch-muscle) in the duration of muscle filament interdigitation following an adenosine 3′:5′-cyclic monophosphate (cyclicAMP)-mediated increase in the sequestration of Ca^{2+} by the sarcoplasmic reticulum. The actions of β-adrenoceptor agonists on slow-contracting skeletal muscle have been implicated in their tremorogenic effects (Bowman & Zaimis, 1958). The receptors in all skeletal muscles are of the β_2-type as defined by Lands and co-workers (Lands *et al*., 1967a;b). Thus their pharmacology (Bowman & Nott, 1969) and ligand binding (Grefrath *et al*., 1978) conform to the classic β_2-adrenoceptor pattern.

Like many other β_2-adrenoceptor ligands, salbutamol (racemic albuterol) has a chiral centre and it is marketed as a 50:50 mixture of its (−)-(**R** configuration) and (+)-(**S** configuration) enantiomers. The **R**-enantiomer (levalbuterol) is the active bronchorelaxant, the **S**-enantiomer being 68 fold less active (Brittain *et al*., 1973). Thus, the **S**-enantiomer is often thought of as an inert part of the marketed racemate. However, recent evidence from both preclinical and clinical studies suggest that the **S**-enantiomer may not be inert but may in fact have actions that oppose the therapeutically beneficial bronchorelaxant activity of the **R**-enantiomer (Mazzoni *et al*., 1994; Perrin-Fayolle, 1995; Yamaguchi & McCullough, 1996). Further, the **S**-enantiomers of β-agonists have been implicated as a cause of the increased asthma morbidity seen clinically with the increased use of racemic β_2-adrenoceptor agonists (Morley, 1996).

Skeletal muscle tremor is the extrapulmonary side-effect described most frequently with the use of β_2-adrenoceptor agonists. As previously noted, it is generally accepted that activation of skeletal muscle β_2-adrenoceptors is responsible for the incidence of tremor implicated with these compounds. However, the enantiomeric selectivity for the effects of salbutamol on the mechanical responses of skeletal muscle is unknown. Therefore, we examined the effects of **R,S**-salbutamol and its enantiomers on sub-tetani in guinea-pig isolated muscles and demonstrate that the effects on skeletal muscle are solely due to the pharmacological activity of the **R**-enantiomer.

Methods

Guinea-pig muscle preparations

Twitch responses were recorded from guinea-pig isolated soleus and peroneus longus muscles by use of a standard organ-bath technique, as described by Waldeck (1976). The soleus muscle consists entirely of slow-contracting intermediate-type fibres (Brooke & Kaiser, 1969; Barnard *et al*., 1971), while the peroneus longus muscle has the experimental characteristics typical of a muscle composed predominantly of fast-contracting fibres (Leonard, 1996).

[3] Author for correspondence.

Guinea-pigs (Duncan-Hartley, 200–300 g) were killed by severing the spine. Both soleus muscles and both peroneus longus muscles were removed from the animals and mounted, under 10 g of resting tension, in organ baths filled with a standard Krebs-Henseliet solution (37°C) of the following composition (mM): NaCl 118, KCl 5, K_2HPO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11. The solution in the organ baths was constantly bubbled with 95% O_2 and 5% CO_2 to maintain a pH of 7.2–7.4. A constant temperature was critical since even slight (less than 0.5°C) changes in tissue temperature had a profound effect on the rate of relaxation of twitches in the soleus muscle and hence markedly affected the degree of fusion of sub-tetanic responses.

Tension recording

Isometric responses were elicited by field stimulation of the muscle. Stimulation was via a pair of platinum wire ring-shaped (5 mm diameter) electrodes placed, 15 mm apart, around the central portion of the muscle. The electrodes were attached to a Grass S88 stimulator via a Grass SIU5 stimulus isolation unit. A sub-tetanic contractile response was elicited every 100 s by a 1 s train of impulses at either 10–15 Hz (soleus muscles) or 25–30 Hz (peroneus longus muscles). Pulses of 5 ms duration and supramaximal voltage (typically 50–70 volts) were used.

Following set-up and an equilibration period of approximately 1 h, the frequency of stimulation within the train of impulses was adjusted to produce optimal contractile responses for investigating drug effects. For each soleus muscle a frequency was chosen to produce a control sub-tetanic response that was approximately 90–95% fused (range: 12–17 Hz). For peroneus longus muscles the frequency was set to produce approximately 50% fusion at the end of the stimulation period (range: 25–31 Hz). Once the optimal sub-tetanic frequency had been determined, each muscle preparation was exposed to either 2 and 4 μM (soleus muscles) or 2 μM (peroneus longus muscles) of one of the three test compounds.

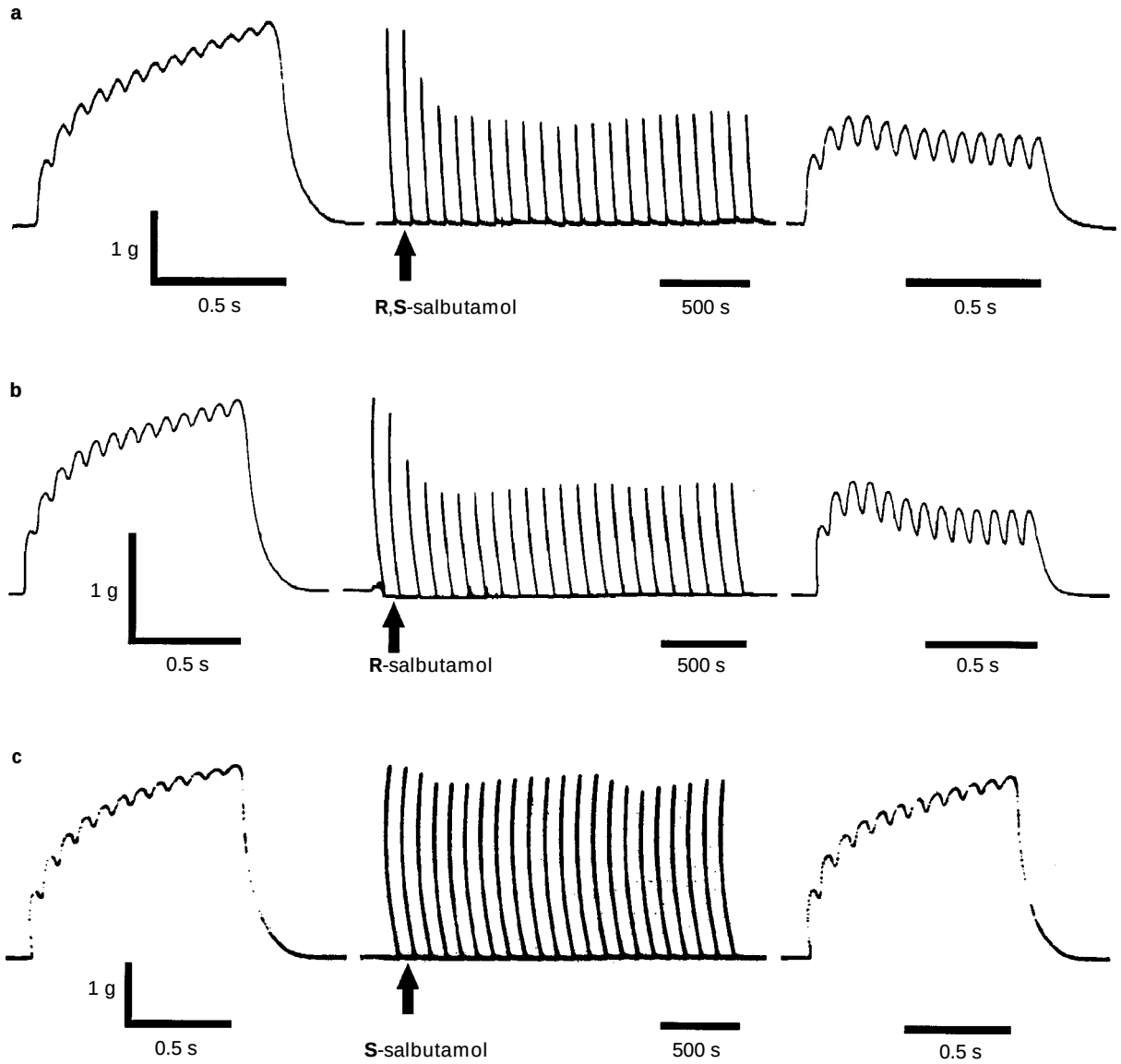

Figure 1 Representative traces showing effects of 2 μM of **R,S**-salbutamol (a), **R**-salbutamol (b) and **S**-salbutamol (c) on sub-tetanic contractions of the guinea-pig soleus muscle. Each trace shows a control contraction (left) and a contraction 30 min after exposure to 2 μM of the test compound (right) recorded at a fast paper speed to reveal the details of the individual sub-tetanic responses and, in addition, contractions at a slow paper speed (centre) to illustrate the effect of the test compound on peak force of the sub-tetani over the initial 30 min incubation period. Stimulation frequencies were: 13 Hz (a), 14 Hz (b) and 12 Hz (c).

Preparations were exposed to the test compounds for 30 min before their effects on the sub-tetanic responses were determined. All contractile responses were monitored by isometric force displacement transducers (Grass FT03C) linked to a multi-channel flatbed ink-writing recorder (Grass 7D polygraph). Sub-tetani were monitored at both fast chart speeds (to allow for analysis of the degree of fusion and individual twitch responses) and slow chart speed (to determine the time-dependence of drug effects).

Drugs and statistics

R,S-salbutamol and its two pure enantiomers were supplied by Sepracor Inc. (Marlborough, MA, U.S.A.). All drug solutions were made, fresh from powder, on the day of use. Each of the three compounds studied was tested in 6 different soleus muscle preparations and 4–6 different peroneus longus muscle preparations. Experiments with the enantiomers were performed blind–i.e. without any prior awareness of the compound. Traces were measured by hand and data were compiled by use of an Excel spreadsheet. Three measurements were taken from each sub-tetanus: (1) the peak force of the first individual twitch (which was always easily identifiable); (2) the maximum force of the whole sub-tetanus and (3) the degree of fusion of the sub-tetanus. The degree of fusion was calculated from the force measured at the last individual trough and peak in the sub-tetanus. All data are presented as mean and s.e.mean. Statistical testing between groups of data was performed by use of either a one sample Student's t test (against 100%) or a two sample (paired) Student's t test. In all cases statistical significance was assumed if $P<0.05$ (two tailed).

Results

Effects of salbutamol on the soleus muscle

In all soleus muscle preparations a consistent pattern of results with the three compounds was observed. Representative examples of data obtained from the soleus muscle with 2 μM of **R,S**-salbutamol and each of its enantiomers are shown in Figure 1. The racemate and the **R**-enantiomer both produced a 30–40% decrease in the peak force of the sub-tetanic responses (Figure 1a,b and Figure 2a). With both these compounds the effects were maximal at 2 μM, since doubling the concentration of either compound produced no further decreases in peak force (Figure 2a). The decrease in peak force of the sub-tetanus was accompanied by a decrease in the degree of fusion of the sub-tetanus (Figure 3). This suggests that the underlying cause of the decreased peak force of the sub-tetanus was an accelerated relaxation of each individual twitch. In addition to their effect on the peak force of the sub-tetanus both the racemate and the **R**-enantiomer at concentrations of 2 and 4 μM produced a small (10–20%), but statistically significant increase in the peak force of the first twitch of the sub-tetanus (Figure 2b). In contrast to the effects of the racemate and the **R**-enantiomer, **S**-salbutamol, at either 2 or 4 μM, had no effect on the peak force (Figures 1c and 2a) or the degree of fusion (Figure 3) of sub-tetanic responses in the soleus muscle, or on the peak force of the first twitch in the sub-tetanus (Figure 2b).

Effects of salbutamol on the peroneus longus muscle

In all peroneus longus muscle preparations the three compounds showed a consistent pattern of results. Represen-

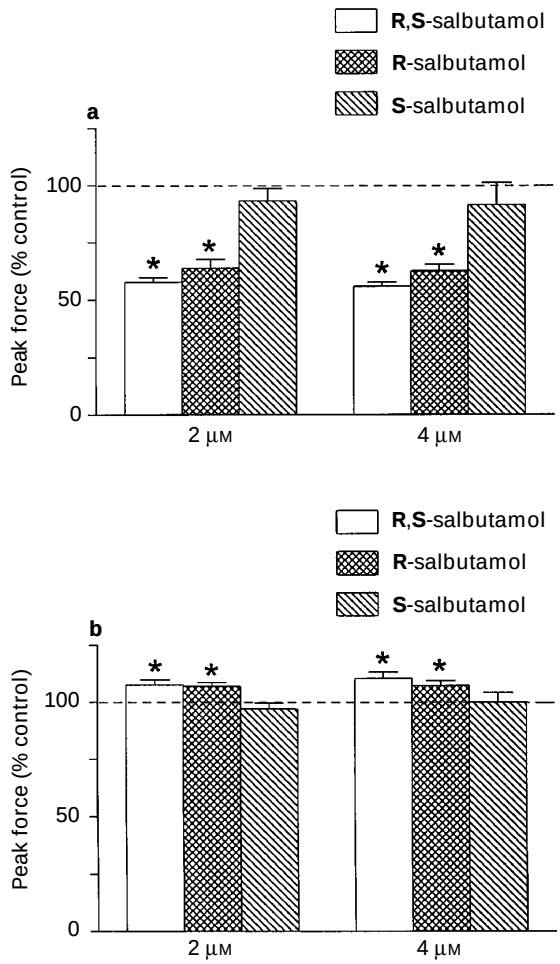

Figure 2 Effects of **R,S**-salbutamol and each of its two individual enantiomers (2 and 4 μM) on the peak force of the whole sub-tetanus (a) and the first twitch response (b) in sub-tetanic contractions of the guinea-pig isolated soleus muscle. In each muscle preparation, contractile force in the presence of the test compound was expressed as a % of control and the plot shows mean and s.e.mean of data from 6 different muscle preparations for each compound. Mean values statistically different from 100% are indicated with an asterisk ($P<0.05$, two-tailed, one-sample Student's t test).

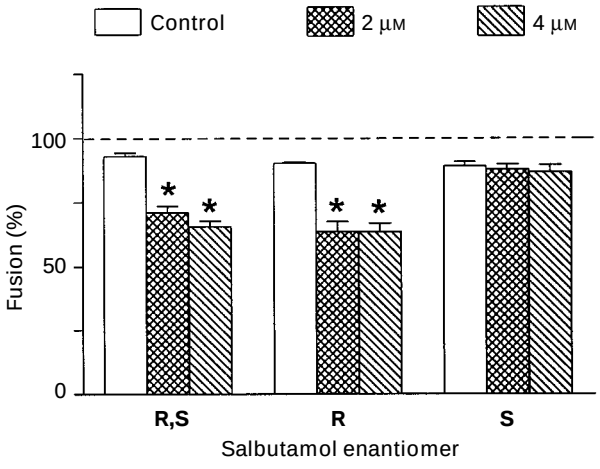

Figure 3 The effect of **R,S**-salbutamol and its enantiomers on the degree of fusion of sub-tetanic contractions of the guinea-pig isolated soleus muscles. The degree of fusion was calculated as described in the Methods. Data are mean and s.e.mean of values from 6 individual recordings in each case. Asterisks denote values significantly different from control ($P<0.05$, two-tailed paired Student's t test).

tative examples of data obtained from the peroneus longus muscle with 2 μM **R,S**-salbutamol and each of its enantiomers are shown in Figure 4. In contrast to their effects on sub-tetanic responses in the soleus muscle, in the peroneus longus muscle 2 μM of both **R,S**- and **R**-salbutamol produced statistically significant increases in the peak force of sub-tetanic contractions and in the peak force of the first twitch of the sub-tetanus (Figures 4 and 5). **S**-salbutamol (at 2 μM) had no effect on the peak force of either the whole sub-tetanus or the first twitch in the sub-tetanus (Figures 4 and 5). Increasing the concentration of **R,S**-salbutamol to 4 μM in the peroneus longus muscle preparation produced essentially the same pattern of results as seen at 2 μM of this compound. This suggests that the effects observed at 2 μM were maximal. Neither the racemate nor its enantiomers caused any changes in the degree of fusion of sub-tetanic contractions in the peroneus longus muscle.

Discussion

Effects of salbutamol on skeletal muscle contractions

The increase in peak sub-tetanic force in the fast-contracting peroneus longus muscle and the decreases in peak sub-tetanic force and degree of fusion in the soleus muscle seen with **R,S**- and **R**-salbutamol are consistent with previously obtained effects of this compound on fast- and slow-contracting isolated skeletal muscles of the guinea-pig (Al-Jeboory & Marshall, 1978). In contrast, the salbutamol-induced increase in single twitch force in the soleus muscle that we found might, at first, seem inconsistent with respect to the published theories of the β-adrenoceptor-mediated effects of sympathomimetic amines on slow-contracting skeletal muscle. Thus, in the *in vivo* soleus muscles of both cats (Bowman & Saimis, 1958) and guinea-pigs (Bohmer & Raper, 1976; Bohmer & O'Donnell, 1977), β-adrenoceptor agonists produce decreases in the peak force of singly evoked twitch responses. That the increase we observed is due to the applied drug, rather than any time-dependent change in the experimental conditions, is supported by the observation that no similar increase in single twitch force was seen with the inactive **S**-enantiomer.

A resolution to the above paradox comes from the work of Holmberg & Waldeck (1980) who showed that the *in vivo* and *in vitro* responses of the guinea-pig soleus muscle to terbutaline are not the same. In *in vitro* studies they showed that although the compound initially decreased single twitch force, this effect gradually declined with time until, eventually, there was an increase in single twitch force. The change in single twitch force was not accompanied by any changes in the degree of fusion of sub-tetanic responses - i.e. sub-tetanic contractions remained depressed. The reversal of the effect on single twitch

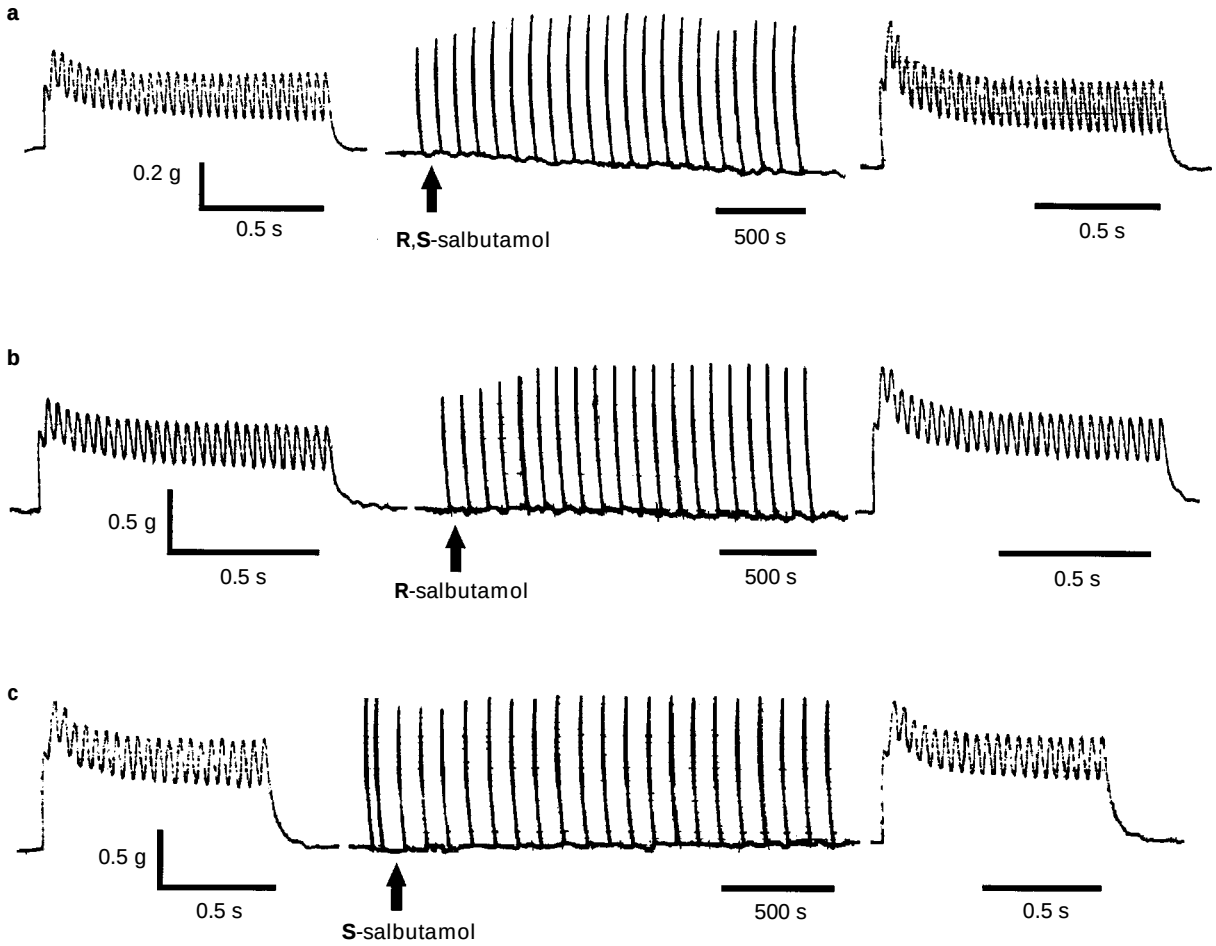

Figure 4 Representative traces showing effects of 2 μM of **R,S**-salbutamol (a), **R**-salbutamol (b) and **S**-salbutamol (c) on sub-tetanic contractions of the guinea-pig peroneus longus. Each trace shows a control contraction (left) and a contraction 30 min after exposure to 2 μM of the test compound (right) recorded at a fast paper speed to reveal the details of the individual sub-tetanic responses and, in addition, contractions at a slow paper speed (centre) to illustrate the effect of the test compound on peak force of the sub-tetani over the initial 30 min incubation period. Stimulation frequencies were: 28 Hz (a), 25 Hz (b) and 28 Hz (c).

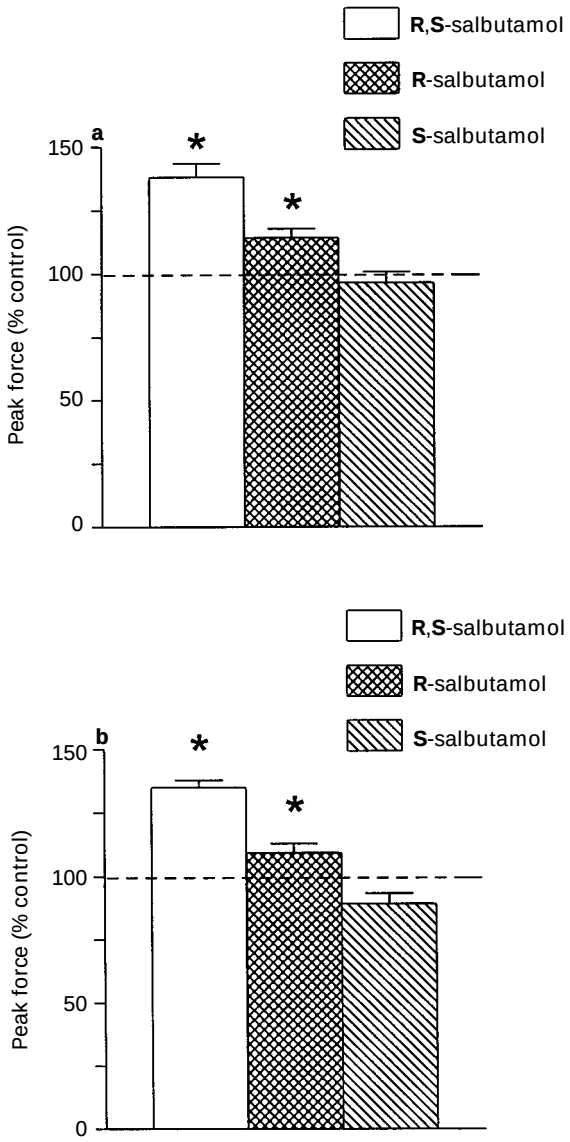

Figure 5 The effects of 2 μM of **R,S**-salbutamol and each of its two individual enantiomers on the peak force of the whole sub-tetanus (a) and the first twitch response (b) in sub-tetanic contractions of the guinea-pig isolated peroneus longus muscle. In each muscle preparation, contractile force in the presence of the test compound was expressed as a % of control and the plot shows mean and s.e.mean of data from between 4 and 6 different muscle preparations for each compound. Mean values statistically different from 100% are indicated with an asterisk ($P<0.05$, two-tailed, one-sample Student's t test).

force is not seen in the *in vivo* guinea-pig soleus muscle (Bohmer & Raper, 1976; Bohmer & O'Donnell, 1977). The only other *in vitro* guinea-pig soleus study in which a β-adrenoceptor agonist-induced decrease in single twitch force has been observed is that of Tashiro (1973). In this study, twitch force was measured immediately after a pulsed application of isoprenaline. Waldeck (1976) showed that terbutaline had no effect on single twitch force in the *in vitro* guinea-pig soleus muscle while, like our present study, Bowman *et al.* (1985) demonstrated that racemic salbutamol increased single twitch force. In both these latter studies the effects of the compounds were observed after a 4–5 min exposure to the β-adrenoceptor agonist. In all our studies, tissues were exposed to racemic salbutamol or its enantiomers for 30 min before assessing any drug effects. Thus our present data for single twitch force in the guinea-pig soleus are consistent with the previously published *in vitro* data. No explanation has been given as to the cause of the β-adrenoceptor agonist-mediated increase in single twitch force in the *in vitro* guinea-pig soleus. However, irrespective of the underlying cause, it is clear from our present studies, that with respect to all the changes in mechanical activity of the *in vitro* guinea-pig soleus muscle, the **R,S**- and **R**-enantiomers are pharmacologically indistinguishable and the **S**-enantiomer is inactive.

A second anomaly in our current findings with respect to previous results is that neither **R,S**- nor **R**-salbutamol enhanced the degree of fusion of sub-tetanic contractions of the peroneus longus muscle. This suggests that there was no significant β_2-adrenoceptor agonist-induced lengthening of the time course of individual twitches. However, given that the exact histological composition of the guinea-pig peroneus longus is unknown, it is possible that the mix of slow-and fast-contracting fibre types in this muscle is such that, in spite of the detectable increases in both single twitch and peak sub-tetanic forces, no change in the degree of fusion is produced by the β-adrenoceptor agonists. However, whatever the underlying reasons for the differences from previously published data, our present data on the peroneus longus muscle show the same enantiomeric selectivity for salbutamol as seen in the soleus muscle.

Resolution of bronchorelaxant and skeletal muscle effects of salbutamol

β_2-Adrenoceptor agonists are an important class of bronchorelaxants used extensively in the treatment of asthma. However, their ability to produce tremor through activation of β_2-adrenoceptors on slow-contracting skeletal muscle is obviously problematical with respect to their therapeutic use. Therefore, any separation of the tremorogenic and bronchorelaxant effects could be of potential benefit for the development of new effective therapies for asthma.

It is known that the bronchorelaxant effects of **R,S**-salbutamol are largely due to the pharmacological activity of the **R**-enantiomer (Brittain *et al.*, 1973). However, to date, nothing was known of the stereo-selectivity of the compound for its effects on the mechanical responses of skeletal muscle. Given that both the bronchorelaxant and tremorogenic effects of β-adrenoceptor agonists have been, in the past, described as classical β_2-adrenoceptor-mediated effects, it might seem unlikely, from the outset, that the stereo-selective profile of salbutamol on skeletal muscle would be any different from that on bronchial smooth muscle. However, in recent years it has become apparent that in addition to the classical β_1- and β_2-adrenoceptor there also exists atypical β-adrenoceptors, such as the β_3- (Arch & Kaumann, 1993) and β_4-adrenoceptors (Kaumann, 1997). The presence of β_3-adrenoceptors in many tissues, including slow-contracting skeletal muscle (Challis *et al.*, 1988; Molenaar *et al.*, 1992), has lead to the suggestion that certain pharmacological responses of β-adrenoceptor agonists may involve mixed receptor populations. Further, it has been shown that some β-adrenoceptor ligands have different degrees of stereo-selectivity for β_2- and β_3-adrenoceptors (Bojanic *et al.*, 1985; Harms, 1976; Harms *et al.*, 1977). Thus there is no guarantee that the enantiomers of salbutamol would possess the same profile of activities in bronchial smooth muscle and skeletal muscle. In addition, there is evidence that the **S**-enantiomers of β_2-adrenoceptor agonists may have pharmacological activities that oppose the therapeutically beneficial bronchodilatation induced by the **R**-enantiomers (Morley *et*

al., 1990; Mazzoni *et al*., 1994; Johansson *et al*., 1996). These results suggest that **S**-enantiomers may have their own pharmacology and that they may not be 'inert' when viewed simply from their β_2-adrenoceptor activity. Indeed, there have been suggestions that **S**-salbutamol may promote the bronchial hyperresponsiveness that has been noted with the increased use of this β_2-adrenoceptor agonist (Perrin-Fayolle, 1995; Morley, 1996; Perrin-Fayolle *et al*., 1996). Thus, our experiments were conducted with the knowledge that the pharmacology of the S-enantiomers of β_2-adrenoceptor agonists has not been completely characterized.

Our present results clearly demonstrated that all of the effects of **R,S**-salbutamol on the mechanical responses of both fast- and slow-contracting isolated skeletal muscle of the guinea-pig are due to the pharmacological activity of the **R**-enantiomer. We saw no effects of the **S**-enantiomer on the mechanical responses of skeletal muscles at the same concentrations at which the effects of the **R**-enantiomer appeared maximal. Unfortunately, the absence of quantitative ED_{50} values for the effects of the two enantiomers on bronchial smooth muscle and on fast- and slow-contracting skeletal muscle means that we cannot, at present, confirm whether the pharmacological effects of **R**-salbutamol are produced by the activation of similar populations of β-adrenoceptors in the different tissues. All we can safely conclude is that the **S**-enantiomer has no observable intrinsic activity at β-adrenoceptors and that the **R**-enantiomer is responsible for both bronchorelaxation and skeletal muscle tremor. Clinical studies (undertaken by Sepracor Inc) indicate that all the skeletal muscle tremorogenic effects of racemic salbutamol are caused by the **R**-enantiomer (Lipworth *et al*., 1997). Furthermore, the bronchorelaxant effects of levalbuterol (**R**-salbutamol) may occur at doses lower than those currently used in racemic albuterol; with fewer side-effects such as tremor being seen in the patients. Thus, further quantitative *in vitro*, *in vivo* and clinical studies, using a variety of β-adrenoceptor agonists with differing β-adrenoceptor profiles, need to be performed, to determine whether the populations of β-adrenoceptors in the bronchi and skeletal muscles are the same and the extent to which the tremorogenic and bronchorelaxant effects of β-adrenoceptor agonists can be clinically resolved.

Research supported by a grant from Sepracor Inc. (Marlborough, MA). The authors are indebted to Dr Lijun Tian for her technical assistance with the experimental preparation.

References

AL-JEBOORY, A.A. & MARSHALL, R.J. (1978). Correlation between the effects of salbutamol on contractions and cyclic AMP content of isolated fast-and slow-contracting muscles of the guinea-pig. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **305,** 201 – 206.

ARCH, J.R.S. & KAUMANN, A.J. (1993). β_3 and atypical β adrenoceptors. *Med. Res. Rev.,* **13,** 663 – 729.

BARCROFT, H. & SWAN, H.J.C. (1953). *Sympathetic Control of Human Blood Vessels,* 88 – 89. London: Arnold.

BARNARD, R.J., EDGERTON, V.R., FURUKAWA, T. & PETER, J.B. (1971). Histochemical, biochemical and contractile properties of red, white and intermediate fibres. *Am. J. Physiol.,* **220,** 410 – 414.

BOHMER, K. & O'DONNELL, S.R. (1977). Beta-adrenoceptor-mediated effects of clenbuterol (NAB 365) on trachea, heart and skeletal muscle in anaesthetized guinea-pigs. *Clin. Exp. Pharmacol. Physiol.,* **4,** 383 – 390.

BOHMER, K. & RAPER, C. (1976). Sympathomimetic-induced effects in the soleus muscle of the guinea-pig. *Arch. Int. Pharmacodyn.,* **221,** 60 – 65.

BOJANIC, D., JANSEN, J.D., NAHORSKI, S.R. & ZAAGSMA, J. (1985). Atypical characteristics of the β-adrenoceptor mediating cyclic AMP generation and lipolysis in the rat adipocyte. *Br. J. Pharmacol.,* **84,** 131 – 137.

BOWMAN, W.C. (1980). Effects of adrenergic activators and inhibitors on the skeletal muscle. In *Handbook of Experimental Pharmacology*. ed. Szekeres, L. 47 – 128, Berlin: Springer-Verlag.

BOWMAN, W.C., LAM, F.Y., RODGER, I.W. & SHAHID, M. (1985). Cyclic nucleotides and contractility of isolated soleus muscle. *Br. J. Pharmacol.,* **84,** 259 – 264.

BOWMAN, W.C. & NOTT, M.W. (1969). Actions of sympathomimetic amines and their antagonists on skeletal muscle. *Pharmacol. Rev.,* **21,** 27 – 72.

BOWMAN, W.C. & ZAIMIS, E. (1958). The effects of adrenaline, noradrenaline and isoprenaline on skeletal muscle contractions in the cat. *J. Physiol.,* **144,** 92 – 107.

BRITTAIN, R.T., FARMER, J.B. & MARSHALL, R.J. (1973). Some observations on the β-adrenoceptor agonist properties of the isomers of salbutamol. *Br. J. Pharmacol.,* **48,** 144 – 147.

BROOKE, M.H. & KAISER, K.K. (1969). Some comments on the histochemical characterization of muscle adenosine triphosphatases. *J. Histochem. Cytochem.,* **17,** 431 – 432.

CHALLISS, R.A.J., LEIGHTON, B., WILSON, S., THURLBY, P.L. & ARCH, J.R.S. (1988). An investigation of the β-adrenoceptor that mediates metabolic responses to the novel agonist BRL 28410 in rat soleus muscle. *Biochem. Pharmacol.,* **37,** 947 – 950.

GREFRATH, S.P., SMITH, P.B. & APEL, S.H. (1978). Characterization of the β-adrenergic receptor and adenylate cyclase in skeletal muscle plasma membrane. *Arch. Biochem. Biophys.,* **188,** 328 – 337.

HARMS, H.H. (1976). Stereochemical aspects of β-adrenoceptor antagonist-receptor interaction in adipocytes. Differentiation of β-adrenoceptors in human and rat adipocytes. *Life Sci.,* **19,** 1447 – 1452.

HARMS, H.H., ZAAGSMA, J. & DE VENTE, J. (1977). Differentiation of beta-adrenoceptors in rat atrium, diaphragm and adipose tissue of the rat using stereoisomers of propranolol, alprenolol, infenalol and practolol. *Life Sci.,* **21,** 123 – 128.

HOLMBERG, E. & WALDECK, B. (1980). On the possible role of potassium ions in the action of terbutaline on skeletal muscle contractions. *Acta Pharmacol. Toxicol.,* **46,** 141 – 149.

JOHANSSON, F., RYDBERG, I., ABERG, A. & ANDERSSON, R.G.G. (1996). Effects of albuterol enantiomers on *in vitro* bronchial reactivity. *Clin. Rev. Allergy Immunol.,* **14,** 57 – 64.

KAUMANN, A.J. (1997). Four β-adrenoceptor subtypes in the mammalian heart. *Trends Pharmacol. Sci.,* **18,** 70 – 86.

LANDS, A.M., ARNOLD, A., MCAULIFF, J.P., LUDUENA, F.P. & BROWN, T.G. (1967a). Differentiation of receptor systems activated by sympathomimetic amines. *Nature,* **214,** 597 – 598.

LANDS, A.M., LUDUENA, F.P. & BUZZO, H.J. (1967b). Differentiation of receptors responsive to isoproterenol. *Life Sci.,* **6,** 2241 – 2249.

LIPWORTH, B.J., CLARK, D.J., KOCH, P. & ARBEENY, C. (1997). Pharmacokinetic and extrapulmonary β_2 adrenoceptor activity of nebulised racemic salbutamol and its R- and S-isomers in healthy volunteers. *Thorax,* **52,** 849 – 852.

LEONARD, M.B. (1996). Contractility and Cyclic Nucleotide Levels of Fast- and Slow-Contracting Skeletal Muscle. M.Phil. Thesis, University of Strathclyde.

MAZZONI, L., NAEF, R., CHAPMAN, I.D. & MORLEY, J. (1994). Hyperresponsiveness of the airways following exposure of guinea-pigs to racemic mixtures and distomers of β_2-selective sympathomimetics. *Pulmon. Pharmacol.,* **7,** 367 – 376.

MOLENAAR, P., ROBERTS, S.J., KIM, Y.S., PAK, H.S., SAINZ, R.D. & SUMMERS, R.J. (1992). Localization and characterization of two propranolol resistant (−)-[^{125}I]cyanopindolol binding sites in rat skeletal muscle. *Eur. J. Pharmacol.,* **209,** 257 – 262.

MORLEY, J. (1996). Anomalous effects of albuterol and other sympathomimetics in the guinea-pig. *Clin. Rev. Allergy Immunol.,* **14,** 65 – 89.

MORLEY, J., CHAPMAN, I.D., FOSTER, A., HOSHIKO, K. & MAZZONI, L. (1990). Effects of (+) and racemic salbutamol on airway responses in the guinea-pig. *Br. J. Pharmacol.,* **99,** 66P.

PERRIN-FAYOLLE, M. (1995). Salbutamol in the treatment of asthma. *Lancet,* **346,** 1101.

PERRIN-FAYOLLE, M., BLUM, P.S., MORLEY, J., GROSCLAUDE, M. & CHAMBE, M.-T. (1996). Differential responses of asthmatic airways to enantiomers of albuterol: implications for clinical treatment of asthma. *Clin. Rev. Allergy Immunol.,* **14,** 139–147.

RODGER, I.W. & BOWMAN, W.C. (1983). Adrenoceptors in skeletal muscle. In *Adrenoceptors and Catecholamine Action,* Part B. ed. Kunos, G. 123–155. New York: John Wiley.

TASHIRO, N. (1973). Effects of isoprenaline on contractions of directly stimulated fast and slow skeletal muscle of the guinea-pig. *Br. J. Pharmacol.,* **48,** 121–131.

WALDECK, B. (1976). An *in vitro* method for the study of β-receptor mediated effects on slow contracting skeletal muscle. *J. Pharm. Pharmacol.,* **28,** 434–436.

YAMAGUCHI, H. & MCCULLOUGH, J.R. (1996). S-Albuterol exacerbates calcium responses to carbachol in airway smooth muscle cells. *Clin. Rev. Allergy Immunol.,* **14,** 47–55.

(Received May 19, 1997
Revised September 5, 1997
Accepted October 20, 1997)