Cyclic nucleotides and contractility of isolated soleus muscle

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1   The effects of isoprenaline, terbutaline and forskolin were examined on cyclic nucleotide concentrations and contractile responses in guinea-pig isolated soleus muscles.

2.   Isoprenaline and terbutaline induced rapid, concentration-related reductions in the tension and degree of fusion of subtetanic contractions of the soleus muscle. These changes were associated with increases (about 2 fold) in the levels of adensoine 3′:5′-cyclic monophosphate (cyclic AMP) in the muscle cells. Propranolol competitively inhibited these responses.

3   Forskolin failed to elicit a sympathomimetic response in the soleus muscles despite increasing (by about 20 fold) the intracellular concentration of cyclic AMP. Forskolin also failed to potentiate the effects induced by isoprenaline.

4   The levels of cyclic GMP in the soleus were increased by isoprenaline (about 1.5 fold) and forskolin (about 2.5 fold). Terbutaline was without effect on cyclic GMP levels.

5   These data suggest either that cyclic AMP is not involved as the mediator underlying β-adrenoceptor-induced changes in contractility of slow contracting skeletal muscles or that forskolin does not stimulate the particular adenylate cyclase that leads to appropriate increases in cyclic AMP in those functional compartments associated with modulation of intracellular Ca^{2+} movements. Cyclic GMP is not involved in modifying changes in contractility of the soleus muscle.

Introduction

β-Adrenoceptor agonists modify the contractility of skeletal muscle in different ways depending upon the predominant types of muscle fibre present in the muscle. The evoked twitches of fast-contracting muscles, such as the cat tibialis anterior, are increased in amplitude and duration, whereas the twitches of slow-contracting muscles, such as the cat soleus, are decreased in amplitude and duration (Bowman & Zaimis, 1958; Bowman & Raper, 1965). The effects on the more physiologically relevant incomplete tetanic contractions of the muscles are more pronounced than are those on twitches because the changes in duration of the unit responses affect the fusion of the contractions. Thus, fusion of incomplete tetanic contractions of the cat tibialis muscle is increased so that a substantial increase in overall tension is produced; the opposite effects occur to a pronounced degree in the cat soleus muscle. The effect on slow-contracting muscles is more pronounced and is produced by smaller concentrations of drug than the opposite effect on fast-contracting muscles, and it has been concluded that the depre-

ssant effect on slow-contracting muscles is the more relevant from both physiological and therapeutic viewpoints (Bowman & Nott, 1969; Bowman, 1980).

Neuromuscular transmission is not involved in the effects, which are exerted directly on the muscle fibres via adrenoceptors that have been classified as of the β₂ subclass (Bowman & Nott, 1969; 1970; Apperley & Daly, 1972). Stimulation of β₂-adrenoceptors in both fast- and slow-contracting skeletal muscles produces a rise in the cellular content of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (Posner *et al.*, 1965; Sullivan & Zaimis, 1973; Lefkowitz & Durham, 1974; Al-Jeboory & Marshall, 1978; Fellenius *et al.*, 1980). Bowman & Nott (1974) showed that the effects of isoprenaline and salbutamol on contractions were potentiated by a range of cyclic nucleotide phosphodiesterase inhibitors, and that the rank orders of potency of the phosphodiesterase inhibitors in potentiating the sympathomimetics *in vivo* and in inhibiting the enzyme *in vitro* were broadly similar. They proposed that the effects on contractility, in line with most other β-adrenoceptor mediated effects, were mediated by cyclic AMP. The whole subject has been reviewed in

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detail by Bowman (1980) and Rodger & Bowman (1983).

The experiments described in this paper were designed to explore the role of cyclic nucleotides further by making use of the compound forskolin which selectively stimulates adenylate cyclase in a variety of different cell types leading to large elevations in intracellular cyclic AMP concentration (Seamon & Daly, 1981; Vegesna & Diamond, 1983; Rodger & Shahid, 1984). The effects of forskolin on cyclic nucleotide content (cyclic AMP and cyclic GMP) and on evoked contractions have been compared with the effects of the β-adrenoceptor agonists isoprenaline (non-selective) and terbutaline (β_2-selective) on the isolated soleus muscle of the guinea-pig which is known to respond to sympathomimetic amines in an essentially similar way to other slow-contracting muscles (Tashiro, 1973; Waldeck, 1976).

Methods

Male Dunkin-Hartley guinea-pigs weighing between 125–175g were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and soleus muscles prepared for the measurement of contractions using a modification of the method described by Waldeck (1976). Muscles from each leg were rapidly dissected out and placed in cold (4 °C) well oxygenated Krebs-Henseleit solution (KHS) of the following composition (in mmol l^{-1}): NaCl 118, KCl 4.7, MgSO$_4$ 1.2, KH$_2$PO$_4$ 1.2, CaCl$_2$ 2.5, NaHCO$_3$ 25 and glucose 11.7. In this cold KHS each muscle was carefully mounted on a perspex electrode block such that the belly of the muscle was surrounded by (but not touching) two platinum wire ring electrodes. The cut origin end of each muscle was attached to the base of the electrode block whilst the tendon end projected uppermost. The electrode-muscle assembly was next fixed in a 60 ml organ bath containing KHS at room temperature (approx 18 °C) and bubbled vigorously with 95% O$_2$ and 5% CO$_2$ so as to produce a PO$_2$ of 525–540 mmHg, a PCO$_2$ of 38–40 mmHg and a pH of 7.3–7.4. The tendon end of the muscle was attached to a force-displacement transducer (Grass FTO3c) in order to record isometric contractions of the muscle. Initially the soleus muscles were stretched by adjusting the baseline tension to 7g (which in preliminary experiments was established as giving optimal twitch responses) and allowed to equilibrate for 60 min. During this equilibration period the temperature of the organ bath was gradually increased from room temperature up to 37 °C, the temperature at which all experiments were performed. Contractions of the muscles were elicited using rectangular pulses of 1 ms duration and a voltage 10–20% above that required to elicit a maximal

twitch (constant in any one experiment; Grass S 88 stimulator attached to stimulus isolation units, SIU5, capacity coupled). Throughout each experiment the soleus muscles were stimulated with trains of pulses (10–15 Hz for 1.25 s) every 20 s so as to produce incomplete tetanic contractions. In some experiments single maximal twitches were elicited interspersed midway between each subtetanus. The isometric contractions of the muscles were recorded on a Grass (model 7) ink writing, curvilinear polygraph.

Cyclic AMP and cyclic GMP determinations

In each experiment soleus muscles were rapidly removed from the organ bath at the desired time (peak of drug-induced effect as assessed by the plateau on the tension record), blotted dry on absorbent tissue paper and frozen in liquid nitrogen (time elapsed not greater than 10 s). The individual soleus muscles were then weighed. Frozen tissue was pulverised under liquid nitrogen and then transferred to a precooled tube and homogenized in 1 ml of 6% trichloroacetic acid (TCA) with an 8N Ultra-turrax (TP 18/10, 8N shaft) cell disrupter for a period of 90 s (9 × 10 s bursts) at 4 °C. The homogeniser shaft was then washed with a further 0.5 ml 6% TCA to recover any residual cell extract. The remainder of the extraction and assay procedure was identical to that already described (Rodger & Shahid, 1984).

In the text the absolute concentrations of both
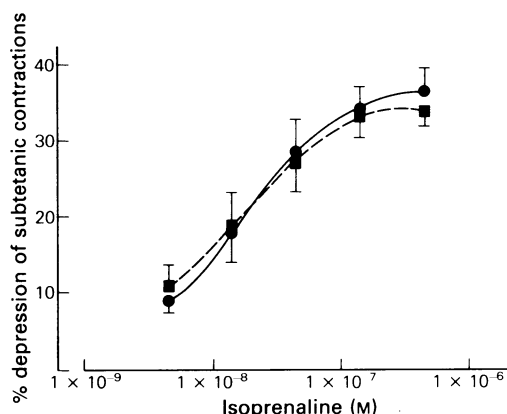
Figure 1 Cumulative concentration-effect curves to isoprenaline on tension responses of guinea-pig isolated soleus muscle in the absence (●) and presence (■) of forskolin 1 µmol l^{-1}. The ordinate scale shows percentage depression of the incomplete tetanic contractions of the muscle. Each point represents the mean (s.e. mean shown by vertical bars) of 10 preparations.

cyclic nucleotides are expressed in $pmol\,mg^{-1}$ wet weight of the tissues. Recovery of known amounts of unlabelled cyclic AMP (40 pmol) and cyclic GMP (20 pmol) added to 6% TCA before homogenization of the muscles was performed. Recovery values were $98 \pm 4\%$ (mean $\pm$ s.e. mean; $n = 10$) for cyclic AMP and $99 \pm 2\%$ ($n = 10$) for cyclic GMP.

Drugs

The following drugs were used: forskolin (Cal Biochem-Behring Corporation), (−)-isoprenaline bitartrate (Wyeth), (±)-propranolol hydrochloride (ICI), terbutaline sulphate (Draco), sodium pentobarbitone (Abbott). Solutions of terbutaline and propranolol were freshly prepared in 0.9% w/v NaCl solution (saline). Isoprenaline solutions were prepared in acidified (pH 3.5) saline to enhance stability. Forskolin was dissolved in 95% ethanol to provide a stock solution which was, thereafter, diluted in saline.

Results

Tension studies

Isoprenaline (5 to $500\,nmol\,l^{-1}$) and terbutaline (0.05 to $3\,\mu mol\,l^{-1}$) induced concentration-dependent reductions in both the tension and degree of fusion of the incomplete tetanic contractions of the guinea-pig isolated soleus muscles. These effects were rapid in onset (within 20 s), peak responses being achieved within 4 min, and were readily reversed by washing out the drugs.

In control experiments the reproducibility of these responses was checked. There were no significant differences between two successive cumulative concentration-effect curves performed 30 min apart; the graphs of the effects were almost superimposable. The responses to both agonists were competitively inhibited by propranolol which at a concentration of $250\,nmol\,l^{-1}$ induced an approximate 20 fold rightward shift in the concentration-effect curves to each agonist. The maximum depression of soleus contractions induced by either β-agonist ranged from 25% to 50% in different experiments but it was always constant throughout any one experiment.

In contrast, forskolin, at concentrations ranging from 0.01 to $6\,\mu mol\,l^{-1}$, was without effect on the tension responses of the soleus muscle even when left in contact with the tissue for 60 min. Concentrations of forskolin in excess of $10\,\mu mol\,l^{-1}$ produced an increase in the degree of fusion of the subtetanic contractions of the soleus muscle resulting in an enhancement of the overall tension developed. This effect, however, was solely attributable to the vehicle

(95% alcohol) in which forskolin was dissolved. The amount of alcohol present in solutions of forskolin up to $6\,\mu mol\,l^{-1}$ was without significant effect on the tension responses of the soleus muscle, neither did it modify in any obvious way the effects induced by either isoprenaline or terbutaline. Concentration-effect curves to isoprenaline performed in the presence of forskolin ($1\,\mu mol\,l^{-1}$) were usually not significantly different from control (Figure 1). In a few experiments, however, in which the maximum depression of the subtetanic contractions of the soleus muscle induced by isoprenaline alone was relatively small (25% to 30%), there was a clear enhancement of the maximum effect of isoprenaline when its concentration-effect curve was repeated in the presence of forskolin. However, as illustrated in Figure 1, when the data from all the experiments were taken together to produce mean curves, these differences were not significant.

Cyclic nucleotide studies

In a separate series of experiments the effects of isoprenaline, terbutaline and forskolin on the cyclic AMP and cyclic GMP content of the soleus muscle were compared. In the case of the β-adrenoceptor agonists the concentrations of each drug chosen for study were those inducing maximum reductions in the tension responses as described above; isoprenaline ($150\,nmol\,l^{-1}$) and terbutaline ($3\,\mu mol\,l^{-1}$). In these experiments each agonist was added to the tissue bath and at the peak of its response as judged by the tension record the muscles were rapidly cut down and frozen in liquid nitrogen to be assayed later for cyclic nucleotide content. The protocol used and typical effects of terbutaline on the tension record are shown in Figure 2.

In the case of forskolin it was not possible to gauge responses based upon the tension record. An incubation period of 30 min was used, therefore, since it has been shown that in isolated cardiac muscle preparations this time period is necessary to allow for full development of the positive inotropic effect of forskolin (Rodger & Shahid, 1984). The results are summarised in Table 1.

Isoprenaline and terbutaline increased the levels of cyclic AMP in the muscles to a similar extent (about two fold), an event associated with closely similar reductions in the tension responses of the soleus muscle (approximately 30%) in each case (Table 1). In contrast, forskolin increased cyclic AMP to levels that were almost ten fold greater than those achieved by either isoprenaline or terbutaline without modifying the tension responses in any obvious way (Table 1). This effect of forskolin on nucleotide content was unaltered by pretreatment with propranolol ($250\,nmol\,l^{-1}$). Cyclic GMP levels were unaffected
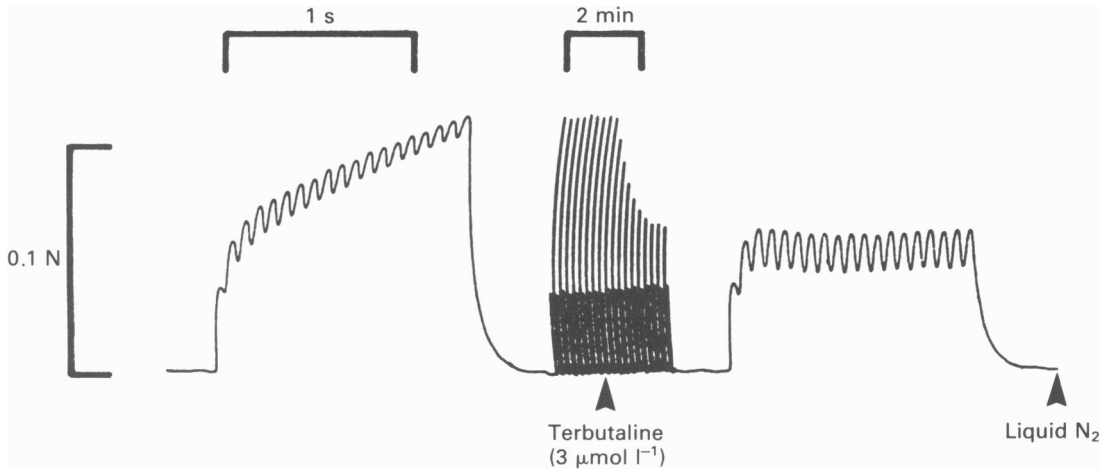
Figure 2 Typical polygraph recording of the effects of terbutaline upon twitches and incomplete tetanic contractions of a guinea-pig isolated soleus muscle. Subtetanic contractions were elicited every 20 s (in this experiment 13 Hz for 1.25 s). Single maximal twitches were interspersed midway between sub-tetani (for a full description see Methods). The chart speed was increased at two points, prior to the addition of terbutaline (3 μmol l^{-1}) and at the peak of its effect, in order to demonstrate more clearly the changes taking place. Terbutaline caused a small increase in the amplitude of the twitch and markedly reduced (by approximately 40% in the experiment illustrated) the tension and degree of fusion of the incomplete tetanic contractions. The muscle was removed from the tissue bath and placed in liquid nitrogen at the point marked Liquid N_2.

by terbutaline but were increased significantly by both isoprenaline (approximately 1.5 fold) and forskolin (approximately 2.5 fold).

Discussion

The results described here clearly show that β-adrenoceptor agonists depress the contractility of the soleus muscle whilst simultaneously elevating cyclic AMP levels within the soleus cells. That both of these effects are mediated via stimulation of β-adrenoceptors is confirmed by the fact that propranolol competitively antagonized the responses to

the β-adrenoceptor agonists. Such observations are in agreement with those reported previously (Sullivan & Zaimis, 1973; Al-Jeboory & Marshall, 1978; Merican & Nott, 1981; Merican *et al.*, 1983). However, forskolin, a substance known to stimulate adenylate cyclase directly in many tissues (including skeletal muscle) and cell types (Seamon & Daly, 1981) thereby bypassing any involvement of β-adrenoceptors, failed to modify in any obvious way the contractile characteristics of the soleus muscle despite increasing the cyclic AMP content within the muscle cells to levels that were some ten fold higher than those produced by terbutaline or isoprenaline. Furthermore, forskolin failed to potentiate the ef-

Table 1 Effects of isoprenaline, terbutaline and forskolin on cyclic nucleotide concentrations and tension responses in guinea-pig isolated soleus muscles

Treatment	Cyclic AMP (pmol mg^{-1})	Cyclic GMP (pmol mg^{-1})	Soleus depression (%)
Control	0.35 ± 0.01	0.029 ± 0.009	–
Isoprenaline (500 nmol l^{-1})	$0.67 \pm 0.09^*$	$0.046 \pm 0.002^*$	30.5 ± 4.6
Terbutaline (3 μmol l l^{-1})	$0.71 \pm 0.06^*$	0.021 ± 0.002	29.3 ± 6.3
Forskolin (6 μmol l^{-1})	$6.73 \pm 0.50^*$	$0.077 \pm 0.007^*$	0

Each value is the mean $\pm$ s.e. mean, $n = 4$–6.
*indicates significant difference from appropriate control: $P < 0.01$ Student's *t* test.

fects of isoprenline on the tension responses, an effect contrary to that which might have been expected if isoprenaline was acting via a cyclic AMP-dependent mechanism. Waldeck & Widmark (1984) have recently reported a similar absence of effect of forskolin on tension responses of guinea-pig isolated soleus and Alade & Nott (1983) likewise recorded no effect on the cat soleus muscle in vivo. However, neither of these groups measured cyclic nucleotide content.

Two possible explanations for a role of cyclic AMP in the increased rate of relaxation of the soleus muscle produced by β-adrenoceptor agonists have been suggested. One is that (as in cardiac muscle) the nucleotide, acting via its dependent protein kinase, phosphorylates certain sarcoplasmic reticulum proteins resulting in enhanced Ca-ATPase activity and consequently increased sequestration of Ca^{2+} (for references, see Rodger & Bowman, 1983). The other is that the tension changes are secondary to enhancement, by cyclic AMP, of membrane Na^+-K^+ ATPase activity (Tashiro, 1973; Ebashi, 1976; Clausen, 1981; Holmberg & Waldeck, 1980a, b).

Presumably the fall in intracellular Na^+ achieved by enhanced pump activity is considered to increase the removal of Ca^{2+} from the cytosol. The simplest explanation of the present observation that forskolin produced a 20 fold increase in cyclic AMP content, yet failed to modify the contractions, is that both of the above proposed explanations are incorrect in that cyclic AMP is not involved. The increases in nucleotide levels produced by β-adrenoceptor agonists would then reflect events occurring in parallel, but not causally related to the tension changes. Support for the idea that cyclic AMP is not involved comes from experiments by Festoff et al. (1977) who found that adenylate cyclase activity is almost totally lost after denervation of rat soleus muscles, yet Bowman

& Raper (1965) found that directly evoked contractions of chronically denervated cat soleus muscles are, if anything, even more sensitive than innervated muscles to the characteristic modifying action of β-adrenoceptor agonists. Although a species difference is a possibility (rat vs cat), it seems unlikely.

Despite these conflicting observations, it is probably premature, in view of the large amount of circumstantial evidence in its favour (Rodger & Bowman, 1983), to abandon the idea of a second messenger role for cyclic AMP in mediating the contractile changes produced by β-adrenoceptor agonists. The main negative evidence depends upon the ability of forskolin to stimulate the activity of a particular adenylate cyclase that may be involved, directly or indirectly, in Ca^{2+} movements. However, it is not unlikely that adenylate cyclase is compartmentalised in skeletal muscle cells, in much the same way as has been proposed for cardiac muscle cells (Brunton et al, 1981; Hayes & Brunton, 1982). Furthermore, adenylate cyclases that differ greatly in their sensitivity to forskolin, or are insensitive to it, have been described (Seamon et al., 1981; Forte et al., 1983; Zahler, 1983). It is therefore possible that, despite the huge rise in cellular cyclic AMP content produced by forskolin, the particular adenylate cyclase that may be involved in the control of Ca^{2+} sequestration is unaffected by it. Until it becomes possible to separate the various adenylate cyclases in their postulated compartments and study the interaction of forskolin and other drugs with them, it will remain inappropriate to decide for or against a definite role of cyclic AMP as a modulator of intracellular Ca^{2+} movements in skeletal muscles.

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-Schmiedebergs Arch. Pharmac., 305, 201–206.

ALADE, I. & NOTT, M.W. (1983). Comparison of isoprenaline and forskolin, a direct activator of adenylate cyclase, in the anaesthetised cat. Proc. Austral. Soc. exp. Biol. Med., (in press).

APPERLEY, G.H. & DALY, M.J. (1972). Simultaneous measurement of skeletal muscle, pulmonary mechanical and vascular responses to bronchodilator in the cat. J. Pharm. Pharmac., 24, 152–153.

BOWMAN, W.C. (1980). Effects of adrenergic activators and inhibitors on the skeletal muscles in Handbook of Ex-

perimental Pharmacology, vol 54/2. ed. Szekeres, L. pp. 47–128. Berlin: Springer Verlag.

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

BOWMAN, W.C. & NOTT, M.W. (1970). Actions of some sympathomimetic bronchodilator and β-adrenoceptor blocking drugs on contractions of the cat soleus muscle. Br. J. Pharmac., 38, 37–49.

BOWMAN, W.C. & NOTT, M.W. (1974). Effects of catecholamines, cyclic nucleotides and phosphodiesterase inhibitors on contractions of skeletal muscles in anaesthetised cats. Clin exp. Pharmac. Physiol., 1, 309–323.

BOWMAN, W.C. & RAPER, C. (1965). The effects of sym-

pathomimetic amines on chronically denervated skeletal muscles. *Br. J. Pharmac. Chemother.*, **24**, 98–109.

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.

BRUNTON, L.L., HAYES, J.S. & MAYER, S.E. (1981). Functional compartmentation of cyclic AMP and protein kinase in heart. *Adv. Cyclic Nucl. Res.*, **14**, 391–397.

CLAUSEN, T. (1981). The hormonal regulation of active electrogenic Na^+– K^+-transport in skeletal muscle In *Physiology of Non-excitable Cells, Advances in Physiological Sciences* Vol. 3, ed. Salanki, J. pp. 209–220. Budapest: Pergamon Press.

EBASHI, S. (1976). Excitation – contraction coupling. *A. Rev Physiol.*, **38**, 293–313.

FELLENIUS, E., HEDBERG, R., HOLMBERG, E. & WALDECK, B. (1980). Functional and metabolic effects of terbutaline and propranolol in fast- and slow- contracting skeletal muscle *in vitro. Acta physiol. scand.*, **109**, 89–95.

FESTOFF, B.W., OLIVER, K.L. & REDDY, N.B. (1977). *In vitro* studies of skeletal muscle membranes. Adenylate cyclase of fast and slow twitch muscle and the effects of denervation. *J. memb. Biol.*, **32**, 331–343.

FORTE, L.R., BYLAND, D.B. & ZAHLER, W.L. (1983). Forskolin does not activate sperm adenylate cyclase. *Mol. Pharmac.*, **24**, 42–47.

HAYES, J.S & BRUNTON, L.L. (1982). Hypothesis: Functional compartments in cyclic nucleotide action. *J. Cyclic Nucl. Res.*, **8**, 1–16.

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

HOLMBERG, E. & WALDECK, B. (1980b). The effect of insulin on skeletal muscle contractions and its relation to the effect produced by β-adrenoceptor stimulation. *Acta physiol. scand.*, **109**, 225–229.

LEFKOWITZ, R.J. & DURHAM, N.C. (1974). Beta-adrenergic "isoreceptors" and adenylate cyclase. *J. clin. Invest.*, **53**, 44A.

MERICAN, Z. & NOTT, M.W. (1981). Effects of isoprenaline, levodopa and a phosphodiestecase inhibitor (ICI 63,197) on cyclic AMP levels and contractions of soleus muscles in anaesthetised cats. *Clin exp. Pharmac. Physiol.*, **8**, 57–66.

MERICAN, Z., NOTT, M.W. & SUNBHANICH, M. (1983). Effects of increasing the frequency of twitches and of isoprenaline on maximal twitches and cyclic AMP levels in slow- and fast- contracting cat skeletal muscles. *Br. J. Pharmac.*, **80**, 303–308.

POSNER, J.B., STERN, R. & KREBS, E.G. (1965). Effects of electrical stimulation and epinephrine on muscle phosphorylase, phosphorylase *b* kinase and adenosine 3', 5'-phosphate. *J. biol. Chem.*, **240**, 982–985.

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

RODGER, I.W. & SHAHID, M. (1984). Forskolin, cyclic nucleotides and positive inotropism in isolated papillary muscles of the rabbit. *Br. J. Pharmac.*, **81**, 151–159.

SEAMON, K.B. & DALY, J.W. (1981). Forskolin: a unique diterpene activator of cyclic AMP – generating systems. *J. Cyclic Nucl. Res.*, **7**, 201–224.

SEAMON, K.B., PADGETT, W. & DALY, J.W. (1981). Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. *Proc. natn. Acad. Sci. U.S.A.*, **78**, 3363–3367.

SULLIVAN, A. & ZAIMIS, E. (1973). The effect of isoprenaline on cyclic AMP concentrations in skeletal mscle. *J. Physiol.*, **231**, 102–103P.

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

VEGESNA, R.V.K. & DIAMOND, J. (1983). Comparison of the effects of forskolin and isoproterenol on cyclic AMP levels and tension in bovine coronary artery. *Can J. Physiol, Pharmac.*, **61**, 1202–1205

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

WALDECK, B. & WIDMARK, E. (1984). Actions of and interactions between forskolin and β-adrenoceptor agonists. *Acta physiol. scand.* (In press).

ZAHLER, W.L. (1983). Evidence for multiple interconvertible forms of adenylate cyclase detected by forskolin activation. *J. Cyclic Nucl. Protein Phosphorylation Res.*, **9**, 221–230.