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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

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1 Increasing the frequency of twitches and treatment with isoprenaline have been been compared for effects on twitch tension, tension-time integral and cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) levels in the slow-contracting soleus muscle of cats, anaesthetized with chloralose and pentobarbitone. The effect of change in frequency of contractions on cyclic AMP in the fast-contracting extensor digitorum longus muscle was also examined.

2 Although both isoprenaline and increasing the frequency of contractions depressed twitch tension in the soleus, only isoprenaline enhanced cyclic AMP levels. The effects of isoprenaline were independent of the existing frequency of contractions of the muscle. Increasing the frequency of contractions enhanced twitches in the extensor digitorum longus muscle but did not change cyclic AMP levels.

3 It is concluded that cyclic AMP may mediate effects of β-adrenoceptor agonists but not those caused by increasing the frequency of contractions on slow- or fast-contracting skeletal muscles.

Introduction

Sympathomimetic amines enhance the tension and duration of maximal twitches of non-fatigued, fast-contracting mammalian skeletal muscles. In contrast, the tension and duration of slow-contracting muscle twitches are reduced (Bowman, 1980; Rodger & Bowman, 1982). These differing effects of sympathomimetic amines are to some extent similar to those caused by previous contractions (either tetanus or repetitive twitches). Thus, in slow-contracting skeletal muscle such as the cat soleus, adrenaline and previous contractions decrease twitch tension, time to peak tension and time to half relaxation while not affecting the rate of rise of tension. These effects of both types of stimuli are attributed to a curtailment of the active state (Bowman, Goldberg & Raper, 1962), but a distinction appears in their intensity, that caused by sympathomimetic amines being greater. In fast-contracting muscles, the effect common to adrenaline and previous activity is enhanced twitch tension. In this muscle type, the twitch-enhancing effect of previous activity is more marked than the maximal effect of adrenaline (Bowman *et al.*, 1962). This, and other effects of adrenaline (prolongation of contraction time and little or no slowing of rate of rise of tension), are attributed to a prolongation of the active state of the muscle (Goffart & Ritchie, 1952; Close & Hoh, 1968). Previous contractions intensify as well as prolong active state; thus rate of rise of twitch tension as well as maximal twitch tension increases. The relative contribution of the two factors to enhanced twitch tension, hence the degree of similarity to the effect of adrenaline, depend on the characteristics of the previous contractions (Bowman *et al.*, 1962).

Evidence suggests that cyclic 3′,5′ adenosine monophosphate (cyclic AMP) mediates the effects of sympathomimetic amines on contractions of non-fatigued, fast- and slow-contracting skeletal muscles (Sullivan & Zaimis, 1973; Nott & Merican, 1978; Al-Jeboory & Marshal, 1978; Merican & Nott, 1981). The present experiments examine whether cyclic AMP mediates the effects caused by increasing the frequency of twitches. Two approaches have been taken. Firstly, muscle cyclic AMP levels were measured at different frequencies of contractions in cat fast- and slow-contracting muscles. Secondly, the

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interacting effects of increasing the frequency of twitches and of (−)-isoprenaline on cat soleus muscle twitch tension and cyclic AMP levels were assessed.

The effects of previous contractions on twitch characteristics are exerted directly on muscle fibres, and are seen whether the muscle is stimulated directly or indirectly by the motor nerve (Brown & von Euler, 1938). Similarly, the effects of sympathomimetics are direct (Goffart & Ritchie, 1952; Bowman & Zaimis, 1958). Indirect stimulation was used in the present experiments to reduce muscle trauma and to facilitate the removal of muscle for cyclic AMP assay.

Methods

Cat soleus and extensor digitorum longus muscle

Cats of either sex were anaesthetized with a mixture of α-chloralose (80 mg kg^{-1}) and sodium pentobarbitone (4.8 mg kg^{-1}) injected intraperitoneally. The trachea was intubated but the cat was allowed to breathe spontaneously in all experiments. A carotid artery was cannulated and blood pressure measured with a Statham pressure transducer coupled to a Grass 79D polygraph. Drugs were injected intravenously through a brachial vein. Soleus or extensor digitorum longus muscles were dissected free from surrounding tissues and their tendons attached to isometric strain transducers (FT10C) also coupled to the polygraph. Tension-time integral of muscle twitches was determined with a Grass integrator 7P10B. The sciatic nerves were ligated and cut, and shielded bipolar stimulating electrodes were placed distal to the ligation point. The motor nerves were stimulated with a Grass S48 stimulator with pulses of 100 μs duration and amplitude (3–5 V) required to evoke maximal twitches. Resting tension (0.5 to 1.5 N for soleus, 1 to 2 N for extension digitorum longus) was set up and maintained at that giving the greatest twitch tension. Twitch frequency was set at 0.05 Hz and was maintained there for the control muscle. For the test muscle, stimulation was increased successively from 0.05 through 0.1, 0.2, 0.5 to 1, or further to 2 Hz. The peak twitch tension reached a steady level 3 to 5 min after each increase in frequency. The next higher frequency then was applied. When the peak twitch tension was steady at 1 or 2 Hz, the muscle was excised and within 5 s was placed in a microwave oven (Philips Commercial Microwave Oven, Type AAH 050, with a power output of 2000 W and frequency 2450 Hz) where it was immediately irradiated for 10 s. It was then assayed for cyclic AMP. The contralateral soleus or extensor digitorum longus muscle acted as a control. It was excised and immediately microwave irradiated 30–40 min before or 30–40 min after removal of the test muscle.

Interaction between increasing the frequency of twitches and (−)-isoprenaline

The frequency of stimulation of soleus muscles was increased progressively from 0.05 to 2 Hz. The effects of isoprenaline on twitch tension and tension-time integral were compared at the lowest and highest frequency. Cyclic AMP levels were examined in muscles excised while contracting at 2 Hz in the absence of (−)-isoprenaline, or at the nadir of the responses to (−)-isoprenaline.

Cyclic AMP estimation

After microwave irradiation, the tissue was weighed and homogenized in Tris-EDTA buffer solution of 0.05 mol l^{-1} Tris and 4 mmol l^{-1} EDTA at pH 7.5. This was followed by heating for 3 min in a boiling water bath to coagulate the protein, centrifugation and assay of cyclic AMP in the supernatant. The assay is based on the competition between unlabelled cyclic AMP and a fixed quantity of tritium-labelled compound for binding to a protein which has a high specificity and affinity for cyclic AMP. The amount of labelled protein-cyclic AMP complex formed is inversely related to the amount of unlabelled cyclic AMP present in the assay sample. Measurement of the protein-bound radioactivity enables the amount of unlabelled cyclic AMP in the sample to be calculated. Separation of the protein-bound cyclic AMP from the unbound nucleotide was achieved by adsorption of the free nucleotide onto coated charcoal, followed by centrifugation. An aliquot of the supernatant was then removed for liquid scintillation counting. The concentration of unlabelled AMP in the sample was then determined from a linear standard curve. The cyclic AMP assay kit was obtained from the Radiochemical Centre, Amersham, England. Cyclic AMP levels are expressed in pmol g^{-1} of microwave irradiated tissue.

Drugs

(−)-Isoprenaline bitartrate (Wyeth) was dissolved in 0.9% w/v NaCl solution at pH 4 with ascorbic acid (100 μmol l^{-1}) as antioxidant. Propranolol HCl (ICI) was dissolved in 0.9% w/v NaCl. The doses quoted in the text refer to the base.

Expression of results

Unless otherwise stated, data are expressed as mean ± s.e.mean of 3 to 4 sample pairs. A paired *t* test was used to test for statistical significance. *P* values < 0.05 were considered to be significant.

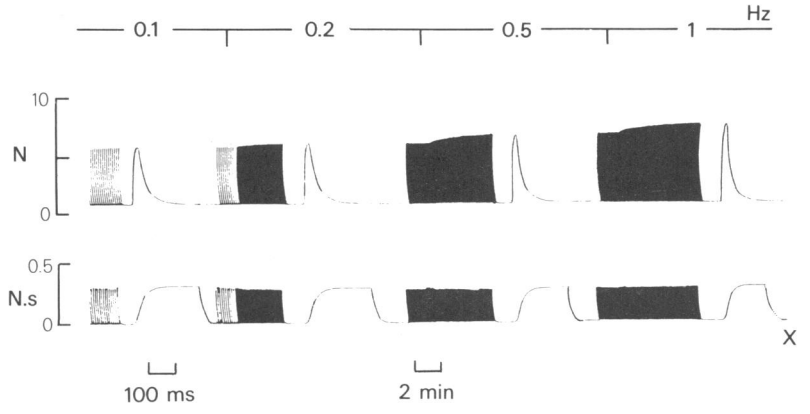

Figure 1 Effect of increasing the frequency of stimulation from 0.05 through 0.1, 0.2, 0.5 to 1 Hz on maximal twitches of the cat extensor digitorum longus muscle. The 0.05 Hz section is not shown. Upper record shows tension; lower record shows tension-time integral. A fast moving trace is shown at each frequency. At × the muscle was excised and assayed for cyclic AMP.

Results

Cyclic AMP levels at different frequencies of twitches

Progressively increasing the frequency of stimulation of the cat soleus from 0.05 to 2 Hz resulted in a frequency-dependent depression of twitch tension and tension-time integral. There was no significant difference in cyclic AMP levels of the muscles when contracting at the lowest and highest frequencies (Table 1).

In the cat extensor digitorum longus muscle, progressively increasing the frequency of stimulation from 0.05 Hz enhanced twitch tension, but tension-time integral increased only slightly (Figure 1). There was no significant difference between cyclic AMP levels measured at the lowest and highest frequencies of contractions (Table 1).

Interaction between increasing the frequency of twitches and (−)-isoprenaline in the soleus muscle

(−)-Isoprenaline (100 ng kg^{-1}, i.v.) was given while twitches of the soleus muscles were evoked at the lowest frequency (0.05 Hz) and the highest frequency (2 Hz). In both cases (−)-isoprenaline depressed twitch tension and tension-time integral. The extent of the effect was independent of the contraction

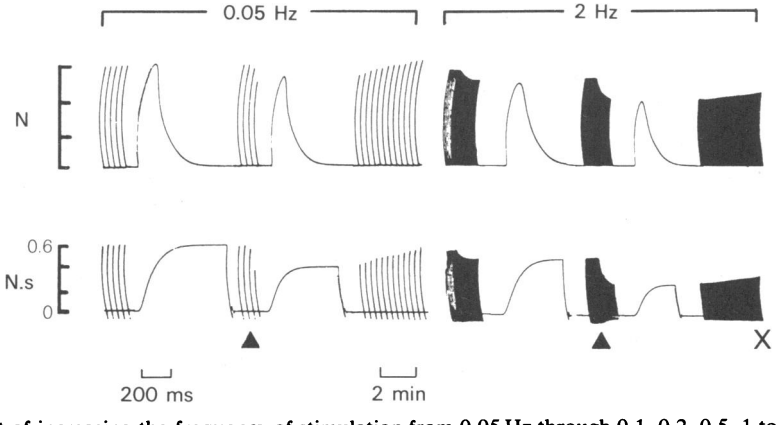

Figure 2 Effect of increasing the frequency of stimulation from 0.05 Hz through 0.1, 0.2, 0.5, 1 to 2 Hz and the injection of (−)-isoprenaline (100 ng kg^{-1}, i.v.; ▲) on maximal twitches of the cat soleus muscle. The trace for twitches at 0.1, 0.2, and 0.5 Hz is not shown. Upper record shows tension; lower record shows tension-time integral. A fast moving trace is shown before and after isoprenaline at the low and high frequency of contractions. At × the muscle was excised and assayed for cyclic AMP.

Table 1 Effect of stimulation frequency on cat extensor digitorum longus (e.d.l.) and soleus muscle twitches and cyclic AMP levels, with additional effect of isoprenaline on soleus

	Nerve stimulation†	*Twitch peak tension* (% of control)	*Twitch tension – time integral* (% of control)	*Cyclic AMP concentration* (pmol g^{-1} of muscle)
e.d.l.	0.05 Hz	100	100	206 ± 47
				NS
	1 Hz	147 ± 4.3 *	103.0 ± 1.7 NS	177 ± 37 NS
soleus	0.05 Hz	100	100	419 ± 21
				NS
	2 Hz	88.6 ± 5.6 *	58.4 ± 3.2 *	421 ± 54 NS
soleus	0.05 Hz	100	100	ND
		*	*	
	2 Hz	76.4 ± 4.1	59.4 ± 2.2	407 ± 11
		*	*	*
	2 Hz with (−)-isoprenaline 100 ng kg^{-1}, i.v.	60.3 ± 3.2	35.0 ± 2.4	723 ± 14

† Pulse parameters: 100 μs, 3–5 V, see Methods. All results expressed as mean ± s.e.mean, $n = 3–4$.
* $P < 0.01$; NS $P > 0.05$; ND not determined.

frequency (Figure 2). Cyclic AMP levels, measured at the 2 Hz contraction frequency, rose in response to (−)-isoprenaline (Table 1).

Discussion

Results confirm similarities in effects of increasing the frequency of contractions and β-adrenoceptor agonists in depressing twitches of the slow-contracting soleus muscle. However, the mechanisms of action are different in that cyclic AMP levels increase in response to isoprenaline but do not change in response to an increase in contraction frequency. Furthermore, isoprenaline is as active in curtailing tension and increasing cyclic AMP levels in twitches already depressed by an increased frequency of contraction (2 Hz) as it is at a lower frequency of stimulation (0.05 Hz).

In the fast-contracting extensor digitorum longus muscle, cyclic AMP levels did not rise as twitch enhancement occurred with increasing frequency of stimulation. Again this points to a difference in mechanism of action from that occurring with the twitch-enhancing action of catecholamines which simultaneously increase cyclic AMP levels (Sullivan & Zaimis, 1973). Note that in the present experiments twitch enhancement occurred without increase in twitch tension-time integral. This is consistent with enhanced rate of relaxation, so that the area under the tension-time curve does not rise. Bowman *et al.* (1962) made similar observations on the cat tibialis muscle which is fast-contracting. Desmedt & Hainaut (1968) and Rosenfalck (1974) attributed this to an enhancement of the intensity of the active state (by previous contractions) rather than a prolongation of the active state (as seen with β-adrenoceptor agonists).

Ultimately the tension and duration of a skeletal muscle twitch depend on the concentration of Ca^{2+} and its time in contact with myofibrils (Endo, 1977), these being the determinants of the active state. A depressant action, as occurs in slow-contracting muscles, is probably due to a lowering of the Ca^{2+} concentration by enhanced sequestration by the sarcoplasmic reticulum so that the active state is curtailed. As suggested by Bowman & Nott (1974), the opposite effect of twitch enhancement in fast-contracting muscle may be due to its greater density of sarcoplasmic reticulum (Davey & Wong, 1980) and greater Ca^{2+}-sequestering activity (Briggs, Poland & Solarno, 1977). Thus enhancement of an already optimal Ca^{2+}-sequestering activity would not curtail the twitch, but rather the greater pulse of Ca^{2+} available for release in the next twitch would lead to its potentiation.

Cyclic AMP is probably the second messenger for effects of β-adrenoceptor agonists on contractions. Thus Merican & Nott (1981) showed a close correlation between isoprenaline-induced increase in cyclic AMP levels and depression of soleus contractions. There are two likely trains of events intervening between the rise in cyclic AMP and the effect on contraction. One is activation by cyclic AMP of phos-

phorylase b kinase, which then phosphorylates a Ca^{2+}-dependent ATPase which is linked to the Ca^{2+}-sequestering pump (Schwartz, Entman, Kaniike, Lane, Van Winkle & Bornet, 1976). The second is activation of cyclic AMP-dependent protein kinase which then phosphorylates phospholamban (22,000 dalton protein). This in turn enhances the sarcoplasmic reticular Ca^{2+}-dependent ATPase which is linked to the Ca^{2+}-sequestering pump (Kirchberger & Chu, 1976; Tada, Ohmori, Yamada & Abe, 1979). Recent evidence suggests a third mechanism whereby β-adrenoceptor agonists might affect muscle contractions. This involves stimulating a membrane Na^+/K^+ pump (Holmberg & Waldeck, 1980; Buur, Clausen, Holmberg, Johansson & Waldeck, 1982), presumably through the mediation of cyclic AMP. It may be that previous contractions could also activate this mechanism, or bring about similar changes in ionic concentrations which could lead to effects on contractions through a cumulative ion shift caused by repetitive membrane depolarization.

Although previous contractions and β-adrenoceptor agonists are similar in so far as they depress slow contracting skeletal muscle twitches and enhance fast-contracting muscle twitches, they have differences in quantitative and qualitative effects within each muscle type (see Introduction). These differences probably reflect differences in the mechanisms brought into play. Of the above mechanisms, all three could mediate the effects of β-adrenoceptor agonists. For previous contractions the first mechanism could be ruled out because cyclic AMP levels do not rise. But entry into the second system could be through Ca^{2+} (released through previous contractions) activating the Ca^{2+}-dependent ATPase directly. The third system is also a possibility.

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