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Effects of isoprenaline on contractions of directly stimulated fast and slow skeletal muscles of the guinea-pig

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Summary

1. The actions of isoprenaline on the contraction and the resting potential of the isolated extensor digitorum longus (EDL), a fast contracting muscle, and the soleus, a slow contracting muscle, of the guinea-pig were investigated. Twitch tension was elicited by direct supramaximal stimulation and recorded isometrically.

2. The twitch tension of EDL elicited by pulses of 0·5–10 ms duration was increased in the presence of isoprenaline (1 μg/ml). Isoprenaline increased the twitch tension of the soleus elicited by a pulse of more than 5 ms duration, but decreased it when elicited by a pulse of less than 1 millisecond. These effects were blocked by propranolol (1–3 μg/ml) but not by phentolamine (1–5 μg/ml).

3. In EDL, isoprenaline prolonged the time to peak tension and the half-relaxation time. The twitch of the soleus was shortened by isoprenaline due to an acceleration of relaxation. These findings were independent of stimulus duration.

4. The potentiating effects of isoprenaline on the twitch tension of EDL and the soleus were not observed in K^+-free Krebs solution and were abolished by ouabain (1 μg/ml) and by reduction of the temperature from 33° to 18° C. The effects of isoprenaline on the relaxation process were not affected by these treatments.

5. In EDL, the resting potential increased from 77·3 mV to 78·5 mV after isoprenaline, whereas in the soleus it increased from 69·1 to 74·7 mV. These effects were blocked by propranolol, K^+-deficiency, ouabain, and cooling to 18° C. Hyperpolarization by isoprenaline was increased by substitution of isethionate for the external chloride.

6. There was a good correlation between the potentiation of the mechanical response and the hyperpolarization of the membrane by isoprenaline. The hyperpolarization seems to be due to activation of the Na^+-K^+ pump.

Introduction

Mammalian skeletal muscles have been classified as either fast or slow muscle on the basis of their speed of contraction, and their different biochemical properties (Romanul, 1964; Bárány, 1967; Buller, Mommaerts & Seraydarian, 1969; Eversole & Standish, 1970). Adrenaline and isoprenaline increase the tension and the duration of maximal twitches of fast-contracting mammalian skeletal muscles, but decrease those of the slow-contracting muscles (Goffart & Brown, 1947; Brown,

Bülbring & Burns, 1948; Goffart & Ritchie, 1952; Bowman & Zaimis, 1958; Bowman, Goldberg & Raper, 1962; Jurna & Rummel, 1962; Bowman & Raper, 1967; Bowman & Nott, 1969).

Since most of these studies have been made on preparations *in situ*, the present experiments were aimed at similar studies with isoprenaline in isolated fast- and slow-contracting muscles. Isoprenaline is the most potent catecholamine in producing a direct effect on the muscle contractions and this effect is mediated via the β-adrenoceptor. Dockry, Kernan & Tangney (1966) and Somlyo & Somlyo (1969) have suggested that isoprenaline stimulates the Na^+-K^+ pump. The other aim of the present experiments was, therefore, to investigate the possibility that the β-effect on the contraction of fast- and slow-contracting muscles is mediated through the Na^+-K^+ pump.

Methods

The experiments were carried out on guinea-pigs of either sex, weighing 300–400 g. They were stunned by a blow on the head and bled to death. The extensor digitorum longus (EDL) or soleus muscle was isolated and mounted under slight tension (0·5–1 g) on a holder placed in an organ bath (7 ml) through which Krebs solution flowed at a rate of 1·5 ml/minute. In many experiments both muscles were mounted on the same holder for simultaneous recording of their contractions under the same condition.

In most experiments, direct electrical stimulation was applied longitudinally through Ag-AgCl electrodes placed at each end of the muscle which was curarized with (+)-tubocurarine (5 μg/ml). The muscle was stimulated every 10 s by rectangular pulses of various durations (0·5, 1, 5, or 10 ms) and supramaximal intensities. In some experiments, the pulses were applied transversely through a Ag-AgCl multi-electrode assembly similar to that described by Hill (1949). These two methods of stimulation did not produce any significant difference in the effect of isoprenaline, confirming the observation by Goffart & Ritchie (1952).

Contractions were recorded isometrically by means of a strain gauge and displayed on a cathode-ray oscilloscope and on an ink-writing pen recorder.

The Krebs solution contained (mM): NaCl, 120·7; KCl, 5·9; $MgCl_2$, 1·2; $CaCl_2$, 2·5; NaH_2PO_4, 1·2; $NaHCO_3$, 15·5; glucose, 11·5. In the Cl-deficient solution, NaCl and KCl were substituted by their isethionate salts, leaving 7 mM Cl^- as $CaCl_2$ and $MgCl_2$. A mixture of 3% CO_2 and 97% O_2 was bubbled through the bathing solution maintained at 33° C.

The resting membrane potential of the muscle fibres was measured intracellularly with glass capillary micro-electrodes (10–30 MΩ) filled with 3 M KCl. EDL and the soleus from the same guinea-pig were both mounted in a small chamber of 7 ml capacity for measurements of the resting potential under the same conditions. About 20 fibres were penetrated to obtain the average resting potential from EDL and soleus muscles. In order to study the time-courses of changes in resting potential, measurements were made alternately on the different muscles at 5 min intervals.

(−)-Isoprenaline hydrochloride (Nikken–Kagaku) was injected into the organ bath to give a final concentration of 1 μg/ml (4 μM). This concentration was present only at the moment immediately after injection and was thereafter pro-

gressively diluted by the Krebs solution flowing continuously at about 1·5 ml/minute. Other drugs used were (+)-tubocurarine chloride (Sigma), phentolamine methanesulphonate (Ciba), (±)-propranolol hydrochloride (Sumitomo–Kagaku), and ouabain (Merck). The concentrations of the drugs given in the text, figures and tables refer to their salts.

Results

Effects of isoprenaline in normal Krebs solution

The time-course of the twitch tension of the extensor digitorum longus (EDL) and the soleus differed depending on the stimulus duration (Table 1). When the stimulus duration was increased the time from stimulation to the peak tension was prolonged and the peak tension was increased. However, the time taken for relaxation to half-amplitude (half-relaxation time) was almost independent of the stimulus duration.

TABLE 1. *Effects of pulse duration and of isoprenaline on time to peak twitch tension and to half-relaxation in extensor digitorum longus (EDL) and soleus (SOL)*

Duration of stimulating pulse (ms)	EDL Mean time (±S.E.M.) to Peak tension (ms)	EDL Mean time (±S.E.M.) to Half-relaxation (ms)	SOL Mean time (±S.E.M.) to Peak tension (ms)	SOL Mean time (±S.E.M.) to Half-relaxation (ms)
(a) Controls				
0·5	16·7±0·4 (10)	11·4±0·7 (10)	55·3±2·2 (10)	32·3±1·3 (10)
1	20·2±0·2 (23)	11·1±0·3 (23)	54·7±1·9 (14)	32·4±1·2 (14)
5	25·8±0·4 (20)	11·1±0·3 (20)	56·5±1·6 (26)	33·1±0·9 (26)
10	33·4±0·9 (14)	10·7±0·3 (14)	61·1±1·9 (14)	35·2±1·7 (14)
(b) Treated with isoprenaline (1 μg/ml)				
0·5	18·5±0·3 (5)	12·3±0·5 (5)	50·9±0·7 (5)	25·8±1·1 (5)
1	20·9±0·4 (11)	11·8±0·2 (11)	51·8±0·4 (11)	27·4±0·8 (11)
5	28·2±0·5 (11)	11·4±0·2 (11)	53·4±1·1 (11)	28·4±0·6 (11)
10	36·8±0·9 (9)	10·8±0·3 (9)	59·0±1·0 (9)	31·4±0·4 (9)

Parameters of stimulation: 0·1 Hz, varying pulse duration, supramaximal voltage. Temperature, 33° C. The values in parentheses are the numbers of observations.

In EDL, isoprenaline (1 μg/ml) slightly delayed the time to peak and prolonged the duration of the contraction (Table 1). The mean maximum tension produced with a 1 ms pulse was 14·2±1·1 g (n=5), and that with a 10 ms pulse 44·8±1·2 g. Isoprenaline potentiated the twitch tension by about 7%, independently of the stimulating pulse duration.

In the soleus, the mean maximum twitch tension evoked by a 1 ms pulse was 8·3±1·5 g (n=7) and that by a 10 ms pulse 12·0±1·6 g. The effects of isoprenaline depended on the stimulating pulse duration. The peak tension evoked by a supramaximal 1 ms pulse was decreased by 15%, but that evoked by a 10 ms pulse was increased by 20%. The time to peak tension and the half-relaxation time were both decreased, although these effects were less with longer pulses (Table 1).

The effect of isoprenaline was not blocked by phentolamine (5 μg/ml) but was abolished by propranolol (1–3 μg/ml). Twitch tensions were decreased by propranolol itself, as observed in the cat *in situ* (Wislicki & Bosenblum, 1967).

Effects of isoprenaline in low potassium solution

The twitch tension of EDL was scarcely affected by lowering the external K^+ to one-tenth of the normal concentration. In K^+-free solution there was a gradual decrease in twitch tension and potentiation by isoprenaline became weak and short-lasting (Figure 1).

In the soleus, the reduction of external K^+ to 0·06 mM or to zero increased the twitch tension evoked by 5 or 10 ms pulses and prolonged it initially whereas the twitch tension evoked by a 1 ms pulse was decreased. Thus, the removal of K^+

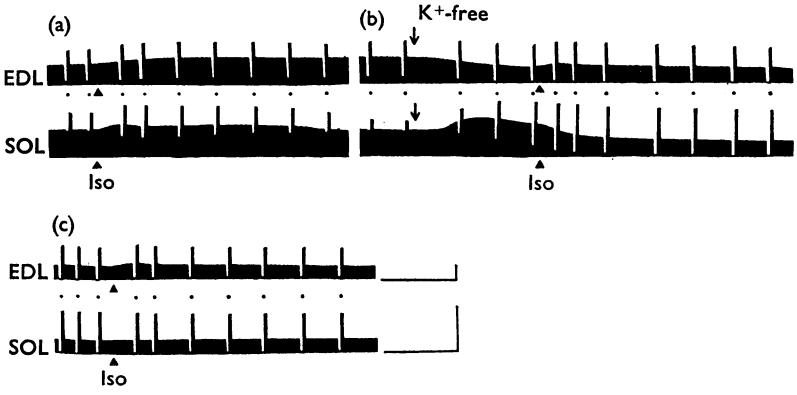

FIG. 1. Effects of isoprenaline on contractions of extensor digitorum longus (EDL) and soleus (SOL) in K^+-free solution. Twitches were evoked by supramaximal pulses of 5 ms duration at 0·1 Hz; at the dots, the pulse duration was changed to 1 ms for 40 s and then to 10 ms for 40 seconds. (a) Control. (b) In K^+-free solution twitches of EDL decreased gradually while in the soleus they increased transiently and then decreased gradually. Isoprenaline (1 μg/ml; Iso at ▲) accelerated depression of twitches of the soleus in K^+-free solution. (c) After about 100 min in K^+-free solution the effects of isoprenaline became very weak in EDL and were nearly abolished in the soleus. Vertical bars: 10 g; horizontal bars: 20 minutes.

produced transiently an effect which was similar to that caused by isoprenaline in normal Krebs solution. This was in contrast to the effect of removal of external K^+ in EDL in which isoprenaline in normal Krebs solution had the opposite effect.

Isoprenaline reduced the twitch tension evoked by 5 ms pulses, when given after the twitch had been augmented in K^+-free solution (Fig. 1b). However, the potentiating effect of isoprenaline on the twitch tension of the soleus was abolished after prolonged immersion (150 min) in K^+-free solution.

Effects of ouabain and temperature on the action of isoprenaline

Ouabain. The tension in EDL was at first slightly potentiated by ouabain (1 μg/ml), but this was followed by depression. No potentiating effect of isoprenaline was observed in either EDL or the soleus when applied after the twitch had been depressed by ouabain. However, the prolongation of the time to peak tension and of the half-relaxation time caused by isoprenaline in EDL was not affected by ouabain. In the soleus the time to peak tension was still slightly shortened by isoprenaline and the half-relaxation time was accelerated.

Low temperature. It has been reported that in both the tibialis anterior and soleus of the cat and of the rabbit, the twitch tension is reduced when the tempera-

ture is lowered from 36° to 30° C, although the effects of adrenaline on the developed tension and on the time-course of twitches are not affected (Bowman *et al.*, 1962).

The twitch tension of the guinea-pig EDL evoked by 0·5 and 1 ms pulses was increased and prolonged when the temperature was lowered from 33° to 18° C. In contrast, the twitch elicited by a 5 ms pulse was decreased to the size of the twitch tension produced by a 1 ms pulse at 18° C (Fig. 2). At 18° C, the poten-

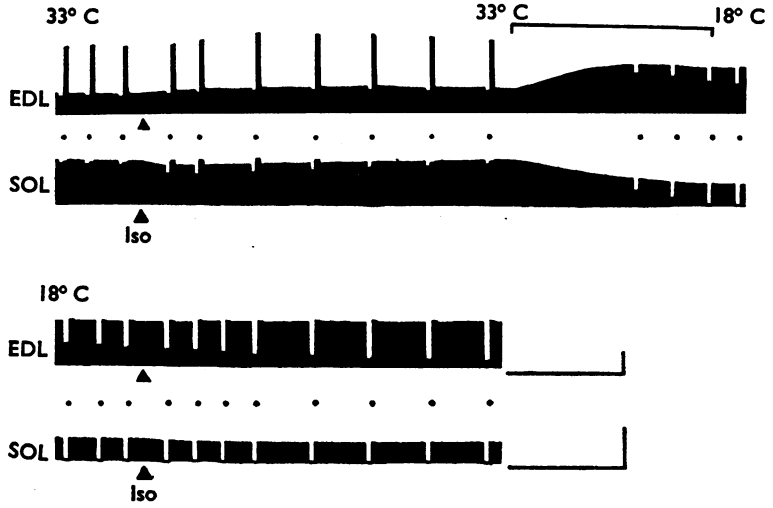

FIG. 2. Effects of temperature and isoprenaline on contractions of extensor digitorum longus (EDL) and the soleus. Twitches were evoked by supramaximal pulses of 1 ms at 0·1 Hz; at the dots, the pulse duration was changed to 0·5 ms for 40 s then 5 ms for 40 seconds. By lowering the temperature from 33° to 18° C (⌐¬), twitches of EDL evoked by 0·5 and 1 ms pulses were increased in size whereas those by 5 ms pulse were decreased. No potentiation of twitches by cooling was observed in the soleus. Isoprenaline (1 μg/ml) was injected at ▲ and was thereafter progressively washed out by the continuous flow of Krebs solution. No clear effect of isoprenaline on twitches was observed in either muscle at 18° C. Vertical bars: 5 g; horizontal bars: 20 minutes.

tiating effect of isoprenaline on the twitch tension was nearly abolished, but both the time to peak and half-relaxation time were still prolonged by isoprenaline (Fig. 3). The twitch tension evoked by a 0·5 ms pulse progressively decreased, independently of the application of isoprenaline.

In the guinea-pig soleus, reduction of the temperature from 33° to 18° C reduced the twitch tensions evoked by pulses of 0·5, 1 and 5 ms (Fig. 2). When stimulation was submaximal, there was an initial small potentiation of the twitch during cooling. As the temperature was lowered, the depressant effect of isoprenaline on the twitch tension appeared later and became weaker but isoprenaline still accelerated relaxation (Figure 3).

Effects of isoprenaline on the resting membrane potential

The mean resting potential at 33° C of EDL was 77·4 ± 0·1 (S.E.M.) mV (750 fibres from 20 muscles) and that of the soleus, 69·4 ± 0·1 mV at 33° C. These values are in good agreement with those reported for rat muscles, i.e. 79 mV for EDL and 68 mV for the soleus (Yonemura, 1967). Effects of isoprenaline were studied on 450 fibres from 12 EDL and 12 soleus muscles. In EDL, the resting

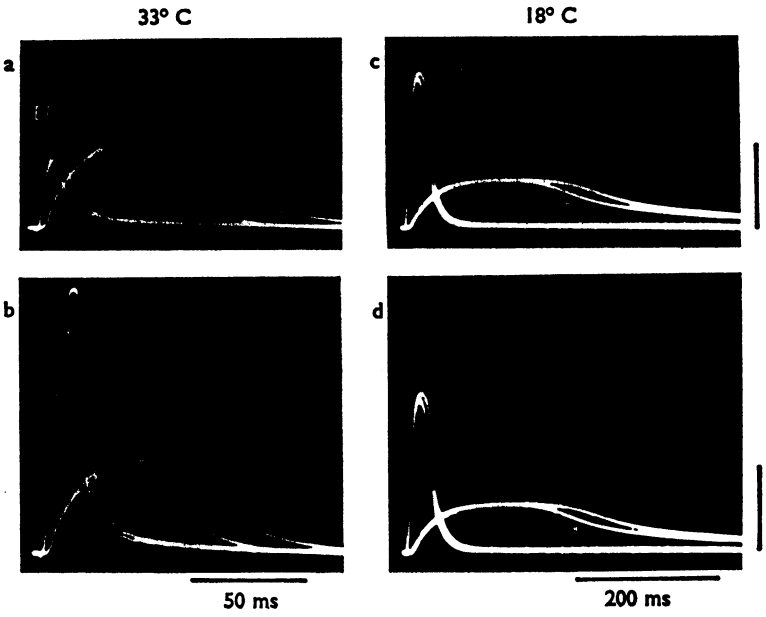

FIG. 3. Effects of isoprenaline at 33° and 18° C in extensor digitorum longus (EDL) and soleus. Superimposed records of twitches elicited by 1 ms (a, c) and 5 ms pulses (b, d) before and 20 min after application of isoprenaline (1 μg/ml; arrows). At 33° C, isoprenaline increased and prolonged the twitches in EDL. In the soleus, twitches elicited by 1 ms pulse were decreased and shortened (a), whereas those elicited by 5 ms pulse were increased, in spite of shortening (b). At 18° C, contractions were prolonged. Twitches of EDL were prolonged but only slightly potentiated by isoprenaline. The twitch size of the soleus was not affected, but relaxation was still accelerated by isoprenaline. Vertical lines: 10 g for EDL, 5 g for the soleus.

TABLE 2. *Effects of external K^+-concentration, isoprenaline, ouabain or cooling on the resting potentials of extensor digitorum longus (EDL) and soleus (SOL)*

	Mean resting potential (±S.E.M. mV)			
	EDL		SOL	
Treatment	Control	Isoprenaline	Control	Isoprenaline
5·9 mM K^+	77·3±0·1 (450)	78·5±0·1 (450)‡	69·1±0·1 (450)	74·7±0·1 (450)‡
0·6 mM K^+	98·1±0·4 (149)	99·1±0·3 (153)*	89·3±0·1 (152)	91·7±0·1 (150)‡
0 mM K^+	109·9±0·3 (137)	108·8±0·3 (142)†	96·6±0·2 (135)	97·2±0·2 (140)*
Ouabain (1 μg/ml) at 5·9 mM K^+	74·2±0·2 (60)	73·9±0·2 (60) NS	67·9±0·2 (60)	68·5±0·2 (60)*
18° C at 5·9 mM K^+	72·3±0·1 (180)	72·5±0·1 (180) NS	66·2±0·1 (180)	66·7±0·1 (180)†

Values in parentheses are numbers of observations. Effects of isoprenaline (1 μg/ml): *, $P<0.05$; †, $P<0.01$; ‡, $P<0.001$; NS, not significant. Temperature 33° C, except where stated otherwise.

potential was not much affected by isoprenaline (1 μg/ml), whereas in the soleus it was clearly increased (Table 2). In the rat diaphragm, adrenaline has no significant effect on the resting membrane potential (Krnjević & Miledi, 1958). Thus the guinea-pig EDL seems to behave similarly to the rat diaphragm.

The hyperpolarization due to isoprenaline was blocked by propranolol (1 μg/ml). In the presence of propranolol, the mean resting potential in 90 fibres from 3 EDL muscles was 76·8±0·2 mV before and 76·9±0·3 mV after isoprenaline (1 μg/ml), and in 100 fibres from 3 soleus muscles, it was 68·5±0·2 mV before and 69·2±0·2 mV after isoprenaline.

When the external K^+-concentration was reduced to 0·6 or 0 mM, the resting potential increased but there was no hyperpolarizing effect of isoprenaline in the soleus (Table 2).

Effects of ouabain and low temperature on resting potential

Effects of ouabain were examined in 8 EDL and soleus muscles (Table 2). The mean membrane potential was decreased by 4·3 mV in EDL and by 2·9 mV in the soleus after 30–60 min exposure to ouabain (1 μg/ml). Similar depolarizations by ouabain have been reported for rat skeletal muscles (Locke & Solomon, 1967) and rat diaphragm (Elmqvist & Feldman, 1965). After treatment with ouabain for 30–60 min, the effect of isoprenaline was reduced in the soleus and abolished in EDL.

When the temperature was lowered to 18° C, the resting potential was slightly decreased and the hyperpolarizing effect of isoprenaline was reduced in the soleus and abolished in EDL (Table 2). The effect was measured in 4 muscles after equilibration at a constant temperature for at least 30 minutes.

Hyperpolarization by isoprenaline in chloride-deficient solution

Since ouabain and low temperature reduced the hyperpolarization produced by isoprenaline, it is possible that the effect is due to an activation of the Na^+-K^+ pump. In the rabbit vagus nerve, removal of the short-circuiting effect of external chloride by its replacement with a large foreign anion potentiated the hyperpolarization due to the electrogenic pump (Rang & Ritchie, 1968). When the external chloride concentration was reduced to 7 mM by substituting it with isethionate, there was a transient large depolarization followed by partial repolarization in both EDL and soleus muscles. After 60–90 min, the membrane (80 fibres from 3 muscles) was still depolarized from $76·9 \pm 0·1$ mV to $72·5 \pm 0·3$ mV in EDL and from $68·8 \pm 0·2$ mV to $67·3 \pm 0·1$ mV in the soleus.

In chloride-deficient solution, hyperpolarization by isoprenaline was much more pronounced than in normal Krebs solution (Fig. 4). In EDL, isoprenaline (1 μg/ml)

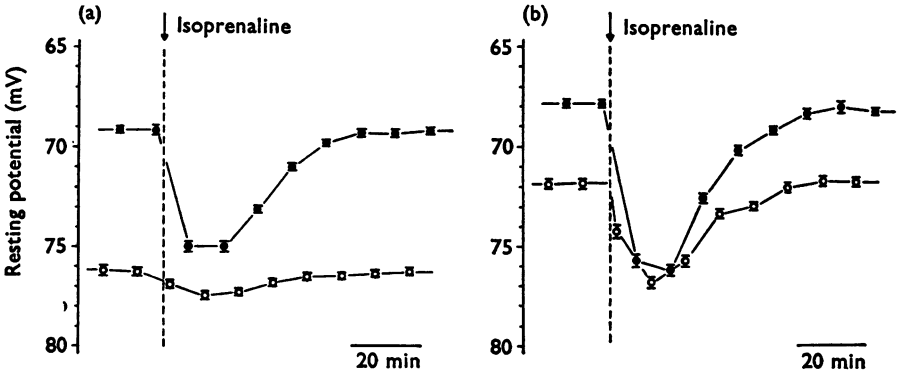

FIG. 4. Effects of isoprenaline (1 μg/ml) on resting potential in the extensor digitorum longus (EDL, open circles) and in the soleus muscle (filled circles) in normal Krebs solution (a) and chloride-deficient solution in which all but 8 mM chloride was substituted with isethionate (b). Measurements in the chloride-deficient solution were started after 50 min equilibration. The points indicate the means of 56 fibres of 3 EDL and 72 fibres of 3 soleus muscles; the vertical bars indicate standard errors of the means.

hyperpolarized the membrane from 72·5 ± 0·3 mV to 77·3 ± 0·3 mV (56 fibres in 3 muscles) and in the soleus, from 67·3 ± 0·1 mV to 76·5 ± 0·2 mV (72 fibres in 3 muscles).

Temperature effect on the resting potential after pretreatment with isoprenaline

Figure 5 shows the temperature effects with and without pretreatment of isoprenaline (1 μg/ml) in 1 out of 4 experiments. In the soleus a small transient hyperpolarization was observed when the temperature was raised from 18° to 33° C, whereas in EDL there was only a gradual recovery of the membrane potential (Fig. 5a). Isoprenaline (1 μg/ml) had little effect at 18° C, but in the soleus, potentiated the hyperpolarization produced by raising the temperature (Fig. 5b). Warming after isoprenaline had no significant effect in the EDL muscle shown in this figure but a small hyperpolarization was observed in two other preparations. In chloride-deficient solution, a large hyperpolarization was produced in both EDL and soleus when the temperature was raised after pretreatment with isoprenaline (Figure 5c).

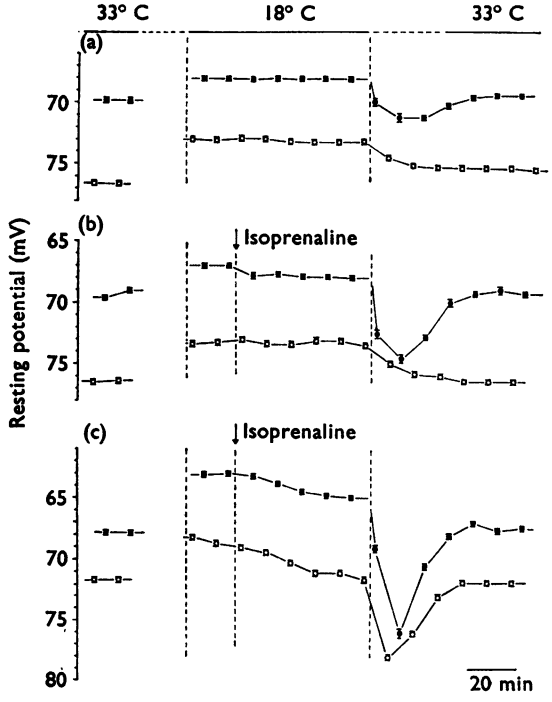

FIG. 5. Effect of raising the temperature from 18° to 33° C without (a) and with isoprenaline pretreatment (b). Measurements at 18° C were started 15 min after cooling to 18° C. In (c), all but 7 mM chloride was replaced by isethionate. The points are the means of 40 observations on the extensor digitorum longus (open circles) and of 40 observations on the soleus (filled circles); the vertical bars are the standard errors of the means.

Discussion

In the guinea-pig extensor digitorum longus (EDL), a fast contracting muscle, the twitch tension evoked by supramaximal stimulation with pulses of 0·5–10 ms duration is potentiated by isoprenaline. On the other hand, in the guinea-pig soleus,

a slow contracting muscle, the twitch tension elicited by supramaximal stimulation with pulses of less than 1 ms duration is always decreased by isoprenaline, as observed by many other workers (see Bowman & Nott, 1969).

The resting membrane potential of the soleus was hyperpolarized by isoprenaline by 5·6 mV at 33° C, while in EDL, the hyperpolarization was only 1·2 mV. Thus, a decrease in twitch tension evoked by short pulses in the soleus could partly result from an increase in the threshold due to hyperpolarization of the muscle membrane.

However, it was possible to demonstrate also in the soleus the potentiation of the twitch by isoprenaline when the muscle was stimulated by pulses of a duration of more than 5 milliseconds. It may be that there is the same mechanism for potentiation of the twitch by isoprenaline in both EDL and soleus, but that, in the soleus, the hyperpolarization of the membrane masks the effect when the stimulating pulses are not long enough.

The effect of isoprenaline on the peak tension of both EDL and soleus was suppressed by ouabain, K^+-deficiency and by reduction of the temperature although the changes induced by isoprenaline in the time to peak tension and the half-relaxation time were scarcely affected. These results agree with the view that contraction and relaxation are separate processes, as has been observed in the rat diaphragm by Goffart & Ritchie (1952). The hyperpolarization of the membrane by isoprenaline was much reduced or absent in K^+-free Krebs solution, at a low temperature and in the presence of ouabain. It is known that ouabain, K^+-deficiency and reduction in temperature all suppress the Na^+-K^+ pump (Kernan, 1970). Therefore, it is possible that the hyperpolarization by isoprenaline is mediated through an activation of the Na^+-K^+ pump. Activation of the Na^+-K^+ pump by catecholamines is also suggested by the studies on the resting potential in denervated Na^+-rich soleus of the rat (Dockry *et al.*, 1966) and in avian muscle (Somlyo & Somlyo, 1969), although in both these studies depolarization is produced by isoprenaline.

A change in the resting potential does not seem to be the only factor which affects the contraction. In EDL, no clear change in resting potential by isoprenaline was observed although the twitch was potentiated. However, the Na^+-K^+ pump seems to be activated even in EDL, in which isoprenaline causes a significant hyperpolarization only in chloride-deficient solution but not in normal Krebs solution. A similar potentiating effect of removal of external chloride on the Na^+-K^+ pump has been demonstrated in the rabbit vagus nerve by Rang & Ritchie (1968). In other species, there is also no close relationship between the effects of catecholamines on the membrane potential and on the mechanical responses (Bowman & Nott, 1969 ; Somlyo & Somlyo, 1969).

Preliminary results indicate that the action potential was not much influenced by isoprenaline in either EDL or soleus. There is very good correlation between the hyperpolarization of the resting membrane which may be due to activation of the Na^+-K^+ pump and the potentiation of the contraction under the various conditions. It may be possible that the activation of the Na^+-K^+ pump by isoprenaline may affect in some way a Ca^{++}-releasing mechanism for the contraction in both fast and slow muscles. When the activation of the Na^+-K^+ pump seemed to be blocked, the contraction time was prolonged in EDL and shortened in the soleus. The difference in the action of catecholamines on the falling phase of contraction of fast and slow muscles may be explained by Bowman & Nott's hypothesis (1969), that in fast contracting muscles, an increase of cyclic 3′, 5′-AMP by catecholamines

may facilitate the release of Ca^{++} from the sarcoplasmic reticulum or may suppress the re-uptake of Ca^{++}, thereby increasing the intracellular free Ca^{++} concentration and hence prolonging the contraction. On the other hand, in slow contracting muscles, cyclic AMP may potentiate the process of re-uptake of Ca^{++} by the sarcoplasmic reticulum, thus increasing the rate of decay of the active state. In addition, the present results suggest that the process affecting the falling phase of contraction is different from the process which modifies the magnitude of tension development, and that the latter process is closely related to the activation of the Na^{+}-K^{+} pump by catecholamines.

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