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A Comparison of the Effects of Temperature and Metabolic Inhibition on the Contraction of Smooth Muscle

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Abstract The effects of temperature and metabolic inhibitors on short tetanus contraction of vas deferens were studied. The contraction height increased by lowering temperature from 35°C, and decreased with a decrease in temperature below 25°C. The maximum speed of contraction and relaxation decreased by lowering the temperature, showing a transition of Q_{10} values at 25°C. The Q_{10} value for the maximum speed of contraction was 1.7 between 35 to 25°C, and 5.3 between 25 to 10°C. The Q_{10} value for the maximum speed of relaxation was 1.9 between 35 and 25°C, and 13.0 between 25 and 15°C. The time to peak tension and to half relaxation increased by lowering temperature. The Q_{10} values also showed a transition at 25°C. The Q_{10} value for the time to peak tension was −2.5 between 35 and 25°C, and −5.0 between 25 to 5°C. The Q_{10} value for the time to half maximum relaxation was −3.1 between 35 and 25°C, and −5.7 between 25 and 5°C. NaN_3 and KCN and DNP inhibited the contraction. However, time to peak tension and half maximum relaxation time were not altered. Although the maximum speed of contraction and relaxation decreased, this was caused only by the reduction in peak tension. O_2-free or glucose-free treatment caused only a slight decrease in peak tension. Neither time to peak tension nor half maximum relaxation time was altered. The differences between the effects of temperature and metabolic inhibition are discussed.

The effects of temperature on the twitch contraction of skeletal muscle have been studied in detail, but in smooth muscle, they have not been well established. This might be due to the difficulty of evoking single twitch contraction, since most smooth muscles show spontaneous activity and elevated active tone (BRADING *et al.*, 1969).

SUNANO and MIYAZAKI (1976) have studied the effects of temperature on the electrical and mechanical activity of the guinea-pig ureter which shows no spontaneous contractions under normal conditions. It was revealed that the duration of the twitch contraction was prolonged at low temperature and that the effects could not

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be explained solely by any of the changes in membrane electrical activity, in excitation contraction coupling, or in the reaction of contractile proteins, but could be explained by a combination of these. In these experiments, however, it was not ascertained whether the effects of temperature were mainly due to the inhibition of the metabolic rate or to some other factors.

In the present investigations, the effects of temperature were studied and compared with the effects of metabolic inhibition in the guinea-pig vas deferens. The vas deferens was used because it shows no spontaneous contraction and because the effects of temperature and of metabolic inhibition on high-K induced contracture of the vas have been studied by us (SUNANO and MIYAZAKI, 1979; SUNANO, 1980).

MCGRATH (1978) recently reported that contraction induced by indirect stimulation becomes slow at low temperature. However, the effect of temperature on neuromuscular transmission might be involved in the change in contraction. In the present investigation, direct stimulation was used in the presence of tetrodotoxin to initiate short tetanic contraction, and the contraction curves were analysed.

METHODS

Vasa deferentia of the guinea-pig were dissected from the abdomen. After dissection, connective tissues and blood vessels were carefully removed. Preparations were made using the prostatic one-third of the vasa, since preparations made from the epididymal side often show more complex responses (Sunano, unpublished observation; MCGRATH, 1978, indirect stimulation).

The preparations were incubated in a modified Tyrode solution of the following composition. NaCl, 137 mM; KCl, 2.7 mM; $CaCl_2$, 2.0 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 11.9 mM; NaH_2PO_4, 0.4 mM; glucose, 5.6 mM; equilibrated with a gas mixture of 95% O_2 and 5% CO_2.

Electrical stimulation of alternating current (AC) was applied as a short stimulus with a duration of 0.1 to 0.5 sec through two silver-silver chloride plates placed 1 cm apart parallel to the preparation. Alternating current was chosen for the stimulation, since it was more effective than direct current (DC) and could induce contraction of constant height. The cycles and the strength of the alternating current and the duration of its application were altered to obtain constant and maximum contraction in the ranges of 50 to 100 Hz, 10 to 50 V, and 0.1 to 0.5 sec, respectively. Contractions were initiated at intervals of at least 10 min after the equilibration of the preparation for 20 min at the desired temperature. Tetrodotoxin (5×10^{-7} g/ml) was used to avoid indirect effects of stimulation.

The temperature of the incubation medium was controlled by the flow of water at the desired temperature into the outer chamber of an organ bath made of glass. The change in temperature was monitored continuously by a thermistor (Shibaura-Electric).

A glucose-free solution was made by the omission of glucose from the Tyrode solution. Although correction of the change in osmotic pressure was made by increasing NaCl in the solution, no obvious difference was observed. An oxygen-free condition was created by equilibration of the solution with 95% N_2 and 5% CO_2.

The metabolic inhibitors used in the present experiments were; 2,4-dinitrophenol (DNP), potassium cyanide (KCN), and sodium azide (NaN_3).

RESULTS

Effects of temperature on the contraction

Figure 1 shows the effect of lowering the temperature on contractions induced by electrical stimulation applied at intervals of 3 min. When the temperature was lowered from 35°C, the contraction height first increased, and then decreased as the temperature decreased. The maximum speeds of contraction and of relaxation were decreased with the decrease in temperature without any acceleration. At these intervals, the basic tension increased gradually. By rewarming the preparation, the contraction height and the maximum speed of contraction and of relaxation returned to the initial level. During the recovery course, a transient potentiation of contraction was observed. This potentiation was not associated with the decrease in the speed of contraction and of relaxation, being different from that observed at the beginning of the decrease in temperature. Spontaneous contractions were also frequently observed at the beginning of rewarming.

The contraction curves at various temperatures are shown in Fig. 2. In this experiment, equilibration of the preparation at the desired temperature and intervals of the stimulation were respectively set as described in METHODS. The intervals of the stimulation at 5°C, for example, were every 20 min. As shown in this

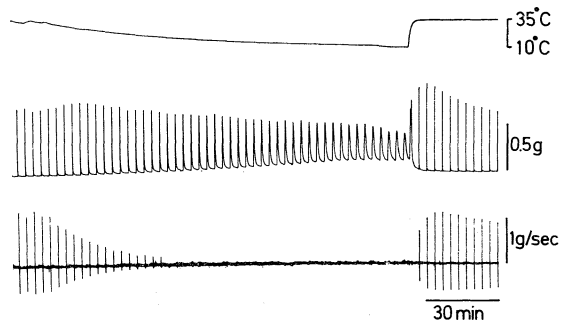

Fig. 1. The effects of temperature on the contractions evoked at constant intervals. The temperature of the organ bath was lowered slowly from 35 to 10°C over a period of 150 min, and then elevated again. Electrical stimulation was applied at constant intervals of 3 min. Top, temperature; middle, tension; bottom, speed of contraction (upstroke) and of relaxation (downstroke). Note the elevation of basic tension.

figure, the duration of contraction was increased by lowering the temperature with the decrease in the speed of contraction and of relaxation. The time to peak tension increased from 1.6±0.4 sec (M±SE, n=5) to 118±21.4 sec (M±SE, n=5) and for half relaxation time, from 1.7±0.3 sec (M±SE, n=5) to 221±22.1 sec (M±SE, n=5). Thus, single twitch contractions with durations longer than 10 min were often observed at 5°C. The contraction height first increased and then

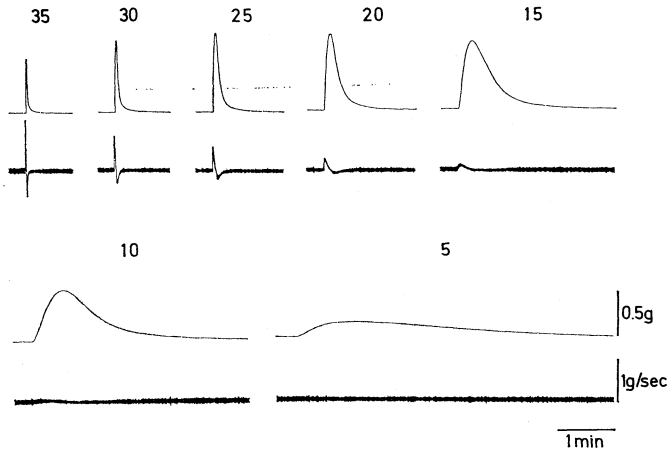

Fig. 2. Contractions at various temperatures. Top, contraction; bottom, the speed of contraction and of relaxation. The contractions were induced at intervals of at least 20 min. Numbers at the top of each recording are the temperatures at which the contractions were observed.

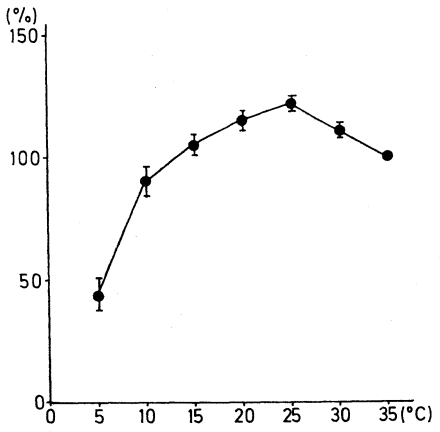

Fig. 3. The relation between temperature and the contraction height. Each point represents the mean value±SE of 5 experiments, expressed as the percentage of the contraction height at 35°C.

decreased. The maximum speed of contraction and of relaxation was decreased by lowering the temperature and became unobservable at 10 or 5°C with this magnification. Since the absolute values of these parameters varied greatly among the preparations, each value was expressed as a percentage or ratio of the values obtained at 35°C and shown in Figs. 3, 4, and 5.

As shown in Fig. 3, the maximum contraction height was observed at 25°C and was 122±3.2% of that observed at 35°C. It then decreased with the decrease in temperature, and was 43±5.4% of the control at 5°C. The maximum speed of

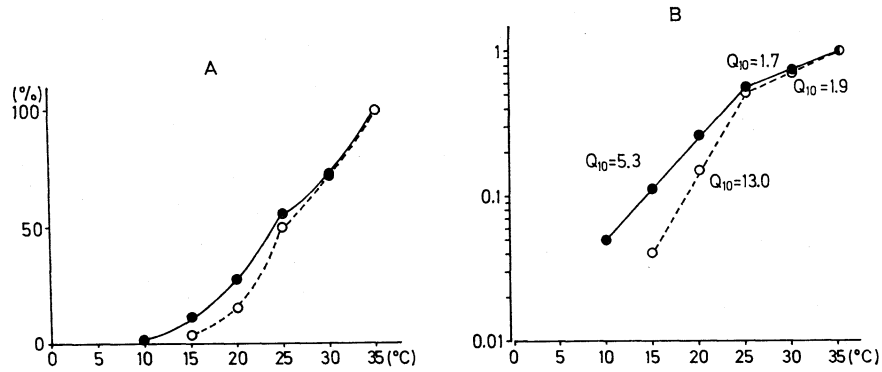

Fig. 4. The relation between temperature and maximum speed of contraction and of relaxation. A: each point represents the mean value of 5 cases expressed as the percentage of the speed observed at 35°C with SE. B: semilogarithmic plot of these relationships showing Q_{10} values. Closed circles: maximum speed of contraction. Open circles: maximum speed of relaxation.

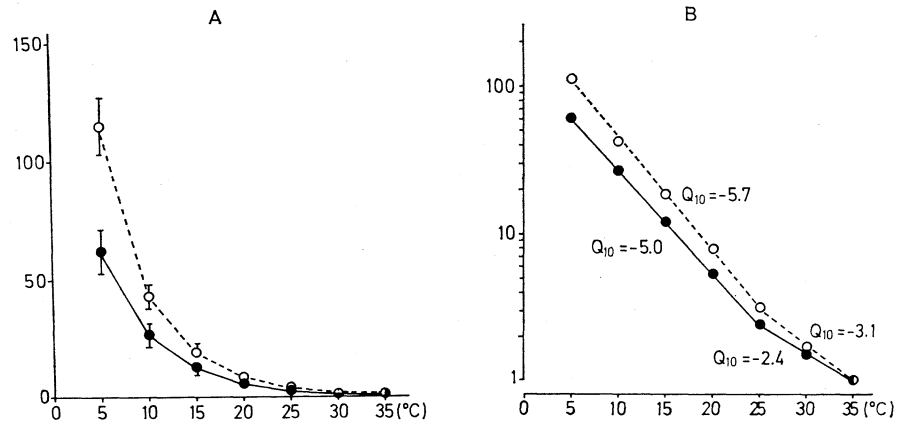

Fig. 5. The relation between temperature and times to peak tension and half relaxation. A: each point represents the mean value of 5 cases with SE. The values obtained at 35°C. B: semilogarithmic plot of the relationship with Q_{10} values. Closed circles, time to peak tension; open circles, half relaxation time.

contraction was decreased by lowering the temperature (Fig. 4). The Q_{10} value was 1.7 between 35 and 25°C, and 5.3 at the temperatures below 25°C. The maximum speed of relaxation decreased more steeply and became unobservable at 10°C with this magnification. The Q_{10} values between 35 and 25°C, and at temperatures below 25°C were 1.9 and 13.0, respectively. The relation between the temperature and the maximum speed showed a curve composed of two exponential curves of different factors, as shown in Fig. 4A and B. The transition of the two curves was observed at 25°C. The times to peak tension and half relaxation were increased by lowering the temperature. At 5°C, the time to peak tension was prolonged 62 times, and for half relaxation time, to 115 times those observed at 35°C (Fig. 5). As shown in Fig. 5B, a linear relationship was observed between the temperature and the logarithm of these times, which deflected at 25°C. The Q_{10} value for the time to peak tension between 35 and 25°C was 2.4 and that observed at the temperatures below 25°C was 5.0. The Q_{10} value for half relaxation time between 35 and 25°C was 3.1 and that observed at the temperatures lower than 25°C was 5.7.

Effects of metabolic inhibitors

Figure 6 shows the effects of NaN_3 on the contractions induced by electrical stimulation. Contraction height, and the maximum speed of contraction and of relaxation were reduced by 2 mM of NaN_3. The threshold concentration for the inhibition was 0.2 mM. Twenty minutes after the application of 2.0 mM of NaN_3, the contraction height was decreased to $13\pm2.1\,\%$ (M±SE, $n=5$) of that of the con-

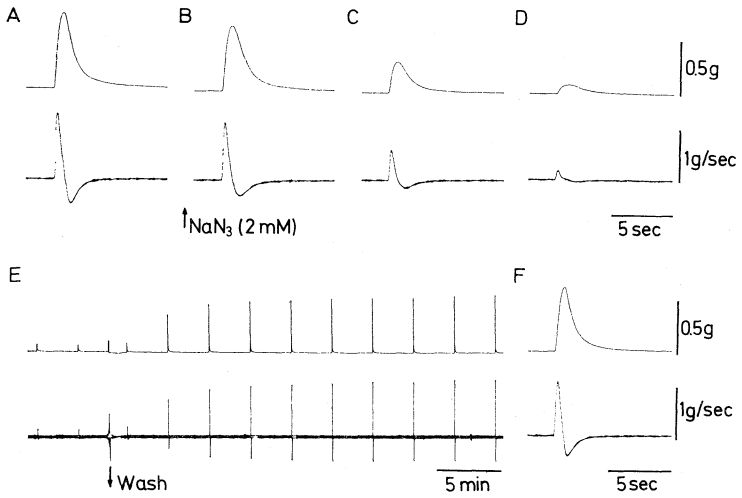

Fig. 6. Effects of NaN_3 on the contraction. A: control. B, C, and D: 5 min, 10 min, and 20 min after the application of NaN_3, respectively. E: 5 min after D, NaN_3 was washed out. Stimulation was applied every 3 min. F: 30 min after washing out NaN_3. Note the difference in the time scale in E.

Table 1. Comparison of the effects of low temperature and some metabolic inhibition.

Treatment	Peak tension	Time to peak	$V_{max\,c}$	$V_{max\,r}$	$Time_{1/2\,r}$
20°C	1.42	5.52	0.26	0.14	8.35
NaN_3 2.0 mM	0.13	1.12	0.15	0.12	1.23
DNP 0.2 mM	0.08	1.13	0.11	0.11	1.75
KCN 1.0 mM	0.14	0.73	0.18	0.13	0.99
Glucose (−)	0.96	1.02	1.02	1.03	0.94
Anoxia	0.76	1.05	0.79	0.82	0.91

Each number represents the mean value of 5 to 6 experiments expressed as the ratio to the control. The values in the presence of NaN_3, DNP, and of KCN were measured 20 min after the application. The values in glucose-free and O_2-free treatment were measured 120 min after the treatment. $V_{max\,c}$, maximum speed of contraction; $V_{max\,r}$, maximum speed of relaxation; $time_{1/2\,r}$, half relaxation time.

trol (Table 1). At the same time, the maximum speeds of contraction and of relaxation were decreased to 15±2.3% (M±SE, $n=5$) and to 12±1.8% (M±SE, $n=5$) of those of the control, respectively. The time to peak tension and half relaxation, on the other hand, were not strongly affected by NaN_3: NaN_3 prolonged the former by only 12% and the latter by 23%. These effects of NaN_3 were reversible and the values were returned to normal by washing out the drug.

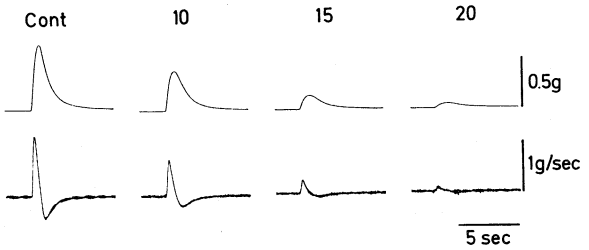

Fig. 7. Effects of DNP on the contraction. Cont: control. 10, 15, and 20: 10, 15, and 20 min after the application of 0.2 mM of DNP.

Figure 7 shows the effects of 0.2 mM 2,4-dinitrophenol (DNP). Similarly to the effects of NaN_3, the contraction height and the maximum speeds of contraction and of relaxation were reduced without showing any significant effect on the times to peak tension and half relaxation. The effects were also reversible and all of these values recovered 20 to 30 min after washing out the drug.

Results similar to those produced by NaN_3 and DNP were obtained by the application 1 mM of KCN. Threshold concentration for the inhibition was 0.2 mM and complete abolition was observed at a concentration higher than 2.0 mM. Complete recovery was also observed by washing out the drug for 10 to 20 min.

The results are summarized in Tables 1 and 2. When higher concentrations

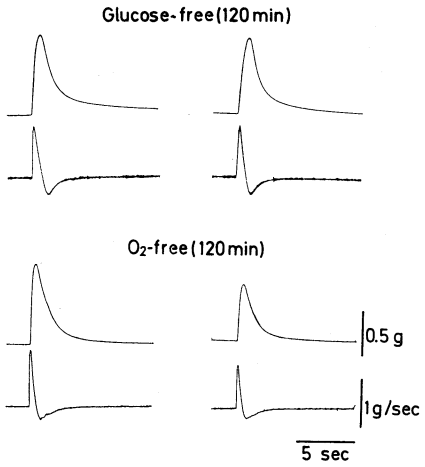

Fig. 8. Effects of glucose removal and anoxia on the contraction. Top, left: control. Top, right: 120 min after equilibration with 95% N_2 and 5% CO_2. Bottom, left: control. Bottom, right: 120 min after the removal of glucose.

Table 2. Correction of the maximum speeds of contraction and of relaxation.

Treatment	$V_{max\ c}/T_{max}$	$V_{max\ r}/T_{max}$
20°C	0.18	0.10
NaN_3 2.0 mM	1.24	0.97
DNP 0.2 mM	1.09	1.29
KCN 1.0 mM	1.29	1.25
Glucose (−)	1.06	1.05
Anoxia	1.03	1.07

T_{max}: maximum tension (peak tension) obtained under each treatment.

of the drugs were applied, complete abolition of twitch contraction was observed.

The effects of glucose depletion and of anoxia

Glucose depletion from the incubation medium caused a decrease in the tonic component of high-K induced contracture of the vas deferens, whereas the phasic component remained unchanged. It took about 120 min in a glucose-free solution to obtain complete abolition of the tonic component. Contractions by electrical stimulations were observed after incubating the preparations in the glucose-free solution for at least 120 min. As shown in Fig. 8, no obvious changes in contraction height, time to peak tension, half relaxation time, or maximum speed of contraction and of relaxation were observed.

Similar results were obtained with anoxia. In this case, however, a slight decrease in the contraction height was observed 120 min after changing the equilibration gas from 95% O_2 to 95% N_2 and 5% CO_2. These results are also summarized in Tables 1 and 2.

DISCUSSION

The contractions evoked by electrical stimulations might be due to direct stimulation of muscles, since the experiments were performed in the presence of tetrodotoxin, which has been known to block contractions due to nerve stimulation (SJÖSTRAND and SWEDIN, 1974; HEDQVIST and VON EULER, 1976; SUNANO *et al.*, 1978).

The contraction by AC stimulation can be regarded as a twitch-like contraction, since the duration of the stimulation was much shorter than the time to peak tension observed by DC single pulse, and the time courses of contractions from both types of stimulation were similar (Sunano, unpublished observation).

Although excitation-contraction coupling in smooth muscle has not been well established, it may be said that such contraction may be induced by Ca released from some intracellular binding sites (SOMLYO and SOMLYO, 1968) and/or by influxed Ca during the action potential (SUNANO and MIYAZAKI, 1968; LÜLLMANN and MOHNS, 1969). These Ca movements have been thought to involve a passive process in various types of muscles. Relaxation, on the other hand, has been thought to be induced by uptake of Ca into intracellular binding sites and/or by the extrusion of Ca through the plasma membrane, both of which are energy dependent processes (CARSTEN, 1969; CASTEELS and VAN BREEMEN, 1975; JANIS *et al.*, 1977; HURWITZ *et al.*, 1977).

In the present experiments, the speed of contraction was decreased and the time to peak tension increased by lowering temperature. Since it has been reported that the ATPase activity of smooth muscle myosin B is depressed at low temperature (MATSUMOTO *et al.*, 1974), this may be the main cause of slowed contraction as reported in heart muscle (HAMRELL and LOW, 1978). However, the large Q_{10} values obtained at the temperatures lower than 25°C indicate the involvement of some changes in excitation-contraction coupling as reported in relation to ureter smooth muscle (SUNANO and MIYAZAKI, 1976). The observation that the release of Ca from microsomes of smooth muscle was depressed at low temperature (HURWITZ *et al.*, 1975, 1977) may support this assumption.

The speed of relaxation was more strongly affected by temperature. The depression of Ca uptake by microsomes at low temperature (HURWITZ *et al.*, 1975, 1977) and/or the decrease in Ca extrusion through cell membrane (NAGASAWA, 1965; CASTEELS and VAN BREEMEN, 1975; DETH, 1978) may be the cause of the prolongation of this relaxation.

The increase in peak tension can be explained by the increase in intracellular free Ca caused by a decrease in the sequestration by some organellae and/or in the Ca extrusion, as has been mentioned concerning the action of caffeine in skeletal muscle (see SANDOW, 1965). Increase in the basic tension observed with repetitive stimulations at low temperature may also be explained in a similar manner. The report that cellular Ca increases at low temperature (BAUER *et al.*, 1965; CASTEELS and VAN BREEMEN, 1975) supports this assumption.

It was also shown that Q_{10} values for the maximum speed of contraction and of relaxation, and, as a result, times to peak tension and half relaxation, go through a transition at 25°C. Regarding this transition, it is of interest that the ATPase activity of sarcoplasmic reticulum also showed a transition at 20°C (INESI *et al.*, 1973). The ATPase activity of the actomyosin of skeletal muscle has also been reported to show a transition, although the temperature at which the transition occurs is different (BARANY, 1967).

Thus, all of these factors could be the cause of the slowed contraction observed at low temperature but might not be the main cause of the change in contraction. This was supported by the experiments performed under metabolic inhibition.

The speeds of contraction and of relaxation were also reduced by metabolic inhibitions. These inhibitions, however, might be the result of reduction of developed tension, since these values *versus* peak tension were not significantly different from the control. In addition, the time to peak tension and half relaxation were not significantly altered by lowering the temperature, as shown in Table 1. Thus, it may be concluded that these metabolic inhibitions had no effect on the rate of contraction and of relaxation, unless the peak tension was unaltered.

Although a reduction of ATP by metabolic inhibitors has been reported in smooth muscle (VAN BREEMEN *et al.*, 1975) residual ATP may be sufficient for the initiation of reduced contraction and for relaxation when contractions are initiated at very long intervals. The reduction of contractions might not be due to the reduction of cellular Ca, since it has been reported that cellular Ca increases somewhat with metabolic inhibition (VAN BREEMEN *et al.*, 1966; CASTEELS *et al.*, 1973; CASTEELS and VAN BREEMEN, 1975). In addition, Ca uptake (CASTEELS and VAN BREEMEN, 1975) and Ca efflux (GOODFORD, 1964; CASTEELS and VAN BREEMEN, 1975; VAN BREEMEN *et al.*, 1975; DETH and CASTEELS, 1977) have also been reported to increase.

The reduction in the amplitude of the action potential could be a cause of the reduction in contraction height, since it has been reported that metabolic inhibition causes depolarization of membrane and a decrease in action potential height (AXELSSON and BÜLBRING, 1961). The reduction of action potential height may result in diminution of the elevation of the intracellular free Ca induced by the action potential (SUNANO and MIYAZAKI, 1968).

The contraction height and the rate of contraction and of relaxation were not greatly affected by the removal of glucose or anoxia. The results were consistent in that the phasic component of high-K induced contracture was not significantly influenced by these treatments, while the tonic component was completely abolished (Sunano, unpublished observation). This may indicate that the energy supply was still adequate for twitch or phasic contraction. The incompleteness of ATP depletion in glucose-free or O_2-free treatment reported in taenia coli (CASTEELS *et al.*, 1972) and in vascular smooth muscle (HELLSTRAND *et al.*, 1977) may explain these results.

Thus, differences in the effects of low temperature and of metabolic inhibition are discussed. Low temperature may decrease the rate of ATP-breakdown without affecting its degree and cause a decrease in the rate of contraction and of relaxation. Metabolic inhibitions, on the other hand, may affect the degree of ATP-breakdown by reducing ATP-content. Thus, a reduction of contraction height was brought about without altering the time to peak tension and relaxation.

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