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Cooling-induced contraction in ileal longitudinal smooth muscle of guinea-pig

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The ileal longitudinal smooth muscle developed a transient contraction on cooling from 37 °C to 1 °C in normal Ca^{2+} (2.5 mM) medium. The transient contraction was not inhibited by pretreatment with the Ca^{2+} antagonist, D-600 (1×10^{-6} M). The contractions were sustained by cooling to 1 °C in the presence of added Ca^{2+} greater than 10 mM. After the pretreatment with D-600, when the muscle incubated in normal medium with added 20 mM Ca^{2+} had been cooled to 1 °C, a phasic response was only seen. However, D-600 did not inhibit the sustained contraction at 1 °C after incubation in the presence of added 20 mM Ca^{2+}. It is suggested that the transient and sustained contraction at 1 °C is maintained by Ca^{2+} release from a cellular site, probably the cell membrane and it requires more calcium for the sustained tension.

It has previously been demonstrated (Conway & Sakai 1960; Sakai et al 1971) that skeletal muscle pretreated with caffeine contracts when cooled. This probably occurs because the cooler temperature augments the Ca^{2+} releasing action of caffeine (Weber & Herz 1968) in the sarcoplasmic reticulum. However, skeletal muscle did not contract only as a result of lowered surrounding temperature (Conway & Sakai 1960; Lüttgau & Oetliker 1968). However, it has been reported (Magaribuchi et al 1973) that a contraction occurs with membrane depolarization after a lowering of the temperature from 32 to 10 °C in the absence of caffeine in smooth muscle of taenia coli and stomach of guinea-pig. Our experiments were designed to study the sources of Ca^{2+} involved in the contraction which occurs at low temperature in the ileal smooth muscle.

METHODS

Strips of longitudinal smooth muscle were isolated from ileum of male Hartley strain guinea-pigs, 400 g, by the method of Rang (1964), and immersed in Tyrode solution bubbled with 95% O_2 and 5% CO_2 at 37 °C. The Tyrode contained (mM): NaCl 136·8, KCl 2·7, $CaCl_2$ 2·5, $MgCl_2$ 1·0, $NaHCO_3$ 11·9 and glucose 5·5. The water jacket could be connected with one of two water circuits controlled at 37 °C and 1, 3, 5, 15 or 20 °C. The bathing fluid was changed simultaneously with the circulating fluid in the jacket. The change of temperature was complete within 1 s as measured with a thermometer (Yellow Springs Instruments Co, 43TA). The pH of the solution was 7·3 at 37 °C and the change of the pH of the solution between 37 °C and 1 °C was within 0·15. The high K^+ (40 mM) was prepared by the addition of an appropriate amount of 2 M KCl solution. A gas mixture of 95% N_2 and 5% CO_2 was used to produce the anoxic condition.

The muscle strips were suspended under a resting tension of 0·6 g and allowed to equilibrate for 60 min with several changes of solution. After equilibration, the tissue was conditioned by adding 40 mM K^+ to the bath. Isometric contractions of the muscle were recorded by a strain gauge transducer (Nihon Kohden, MR-150).

RESULTS

Fig. 1A shows that the ileal longitudinal muscle developed a transient contraction when cooled from 37 to 1 °C in a normal Ca^{2+} (2·5 mM) medium. The maximum tension was 1·71 ± 0·06 g (n = 50) (mean ± s.e.) and was 53·1 ± 0·6% of the maximum response to high-K^+ (40 mM) at 37 °C. The time required to reach the maximum tension was 6·3 ± 0·3 s. The tension gradually reduced and the half time of the relaxation was 10·7 ± 0·6 s.

When muscles were cooled to 1 °C after incubation in Ca^{2+}-free medium for 30 min at 37 °C, no responses were seen.

To determine the relationship between the cooling-induced tension and the external Ca^{2+} concentration, the muscles were incubated in medium containing in addition various concentrations of Ca^{2+} at 37 °C for 30 min, and were then cooled to 1 °C. In added 1 or 5 mM Ca^{2+}, only transient

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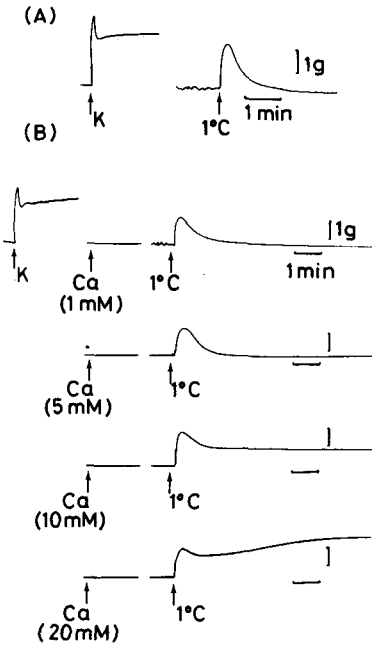

FIG. 1. Effects of cooling on mechanical activity in normal Ca^{2+} and high concentration Ca^{2+} medium. (A) The ileal longitudinal muscle was cooled from 37 to 1 °C in normal Ca^{2+} (2·5 mM) medium. (B) The muscles incubated with medium containing various concentrations of Ca^{2+} at 37 °C for 30 min were then cooled to 1 °C. Each concentration of Ca^{2+} was added to normal medium containing 2·5 mM Ca^{2+}.

contractions were seen. The tonic response was not seen until the concentration of added Ca^{2+} was greater than 10 mM (Fig. 1B).

The effects of rapid change of temperature on contraction were investigated in the high concentration Ca^{2+}-treated muscle. Twenty mM Ca^{2+} was added to the normal medium at 37 °C, and 30 min later, the temperature in the medium was changed. By cooling to 1 °C, the tonic response followed a transient response. By cooling to 3 °C, the tonic response developed, but the response was small. However, by cooling to 5 °C, the tonic response did not occur. By cooling to 20 °C, only a small response was seen (Fig. 2).

The maximum tension which developed after cooling to 1 °C following the addition of 20 mM Ca^{2+} was 1·90 ± 0·06 g (n = 50), and the contraction was sustained for 180 min so long as the low temperature was maintained. When the muscles were rewarmed, the contractions disappeared, and when they were recooled from 37 to 1 °C, the tension was observed again (data not shown). Thus, the cooling-induced contraction was reversible. When 20 mM Ca^{2+} was added after a transient contraction induced by cooling at 1 °C, a gradual increase in tension was seen thereafter (Fig. 3A).

The effects of the calcium antagonist, D-600 (gallopamil) on the cooling-induced contraction were investigated. The transient contraction occurring in normal Ca^{2+} medium with cooling to 1 °C was not inhibited by pretreatment with 1×10^{-6} M D-600, which completely suppressed the K^+-induced contractions (Fig. 3B, upper figure). After pretreatment with D-600, when the muscle incubated in 20 mM Ca^{2+} at 37 °C was cooled to 1 °C, a phasic response was seen, but no tonic response occurred (Fig. 3B, middle figure). In another experiment, when muscle was preincubated in 20 mM Ca^{2+} added medium, D-600 failed to inhibit the sustained contraction at 1 °C (Fig. 3B, bottom figure).

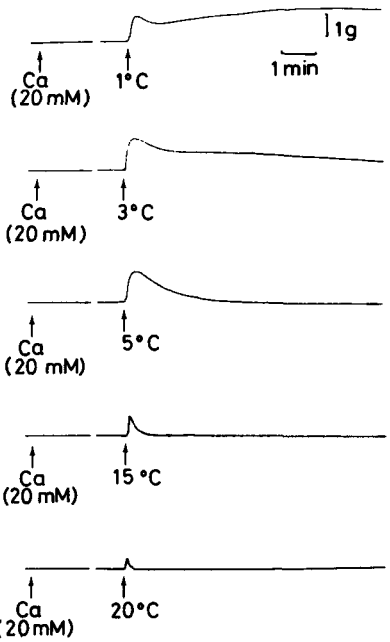

FIG. 2. Effect of rapid change of temperature on contraction. 20 mM Ca^{2+} was added in normal medium at 37 °C. After 30 min, the temperature was changed from 37 °C to 1, 3, 5, 15 or 20 °C, respectively.

The effects of substituting Sr^{2+} or Ba^{2+} for Ca^{2+} on the cooling-induced contraction were examined. Muscles were placed in a Ca^{2+}-free medium for 30 min at 37 °C and then cooled to 1 °C. After 10 min, the concentration of Ca^{2+}, Sr^{2+} or Ba^{2+} was increased in a stepwise manner at 1 °C. The tension was increased as the concentration of external Ca^{2+}, Sr^{2+} or Ba^{2+} was raised, respectively and the order of tension was $Ca^{2+} > Sr^{2+} > Ba^{2+}$.

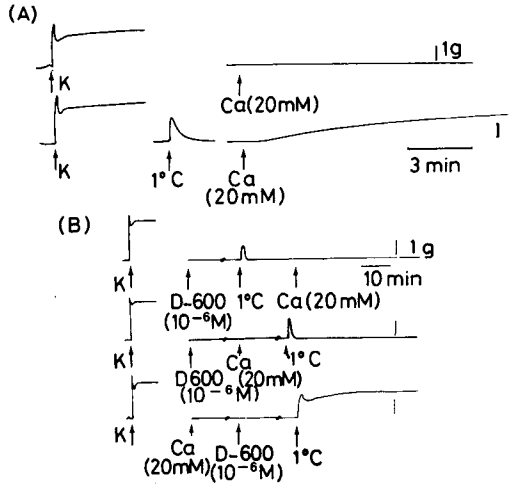

FIG. 3. Effect of D-600 on cooling-induced contraction. (A) Upper figure: When 20 mM Ca^{2+} was added at 37 °C, no tension occurred. Lower figure: After cooling from 37 °C to 1 °C in normal medium, 20 mM Ca^{2+} was added at 1 °C. It was followed by a gradual increase in tension. (B) Upper figure: After pretreatment with 1×10^{-6} M D-600 for 30 min, the muscle was cooled to 1 °C and then a transient response was seen. Middle figure: After the pretreatment with D-600, 20 mM Ca^{2+} was added. 30 min later, the muscle was cooled to 1 °C and then the transient response occurred, but the sustained tension did not occur. Lower figure: After 20 mM Ca^{2+} was added for 30 min, D-600 was added, and 30 min later, the muscle was cooled to 1 °C, and then the tonic response occurred.

In attempt to determine if the tension development after increasing the concentration of Ba^{2+} was maintained by the Ba^{2+} alone or through Ca^{2+}, the effects of various concentration of Ba^{2+} at 1 °C were studied in the presence of 2·5 to 7·5 mM Ca^{2+}, which by itself was usually below threshold for contraction. As shown in Fig. 4B, Ba^{2+} evoked a sustained tension depending on the concentration of the external Ca^{2+}.

In the presence of 1×10^{-4} M 2,4-dinitrophenol (DNP) and 1×10^{-3} M monoiodoacetic acid (IAA), neither a transient nor sustained response was noted by cooling to 1 °C (Fig. 5A). In another experiment, the muscles were incubated in the normal Ca^{2+} solution bubbling N_2 gas, and when cooled from 37 to 1 °C, a small transient response was seen. When 20 mM Ca^{2+} was added to the medium being bubbled with N_2 gas at 1 °C, after approximately 4 min, a gradual rise in tension was seen and the maximum tension was ultimately 60% of that seen in normoxia (Fig. 5A).

The rapid shortening of 1 mm in length of a muscle strip of about 1·5 cm was done under cooling in 20 mM Ca^{2+}. Then, a lessening of the tension occurred and was followed by redevelopment of the tension Fig. 5B).

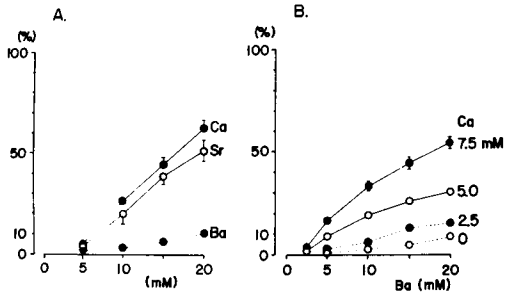

FIG. 4 (A) Effect of Ca^{2+}, Sr^{2+} and Ba^{2+} on cooling-induced contraction. Muscles were incubated in Ca^{2+}-free medium for 30 min at 37 °C and cooled to 1 °C. After 10 min, cumulative concentration-response curves were obtained to $CaCl_2$, $SrCl_2$, $BaCl_2$ by increasing the concentration at 10 min intervals. The responses are plotted as % of maximum tension induced by 40 mM K^+. (B) Effect of Ba^{2+} in the presence of Ca^{2+} on cooling-induced contraction. Muscles were incubated in Ca^{2+}-free medium for 30 min and then incubated in medium containing 2·5, 5 or 7·5 mM Ca^{2+} for 30 min at 37 °C. The muscles were cooled to 1 °C. After 10 min, the concentration of Ba^{2+} was increased in a stepwise manner.

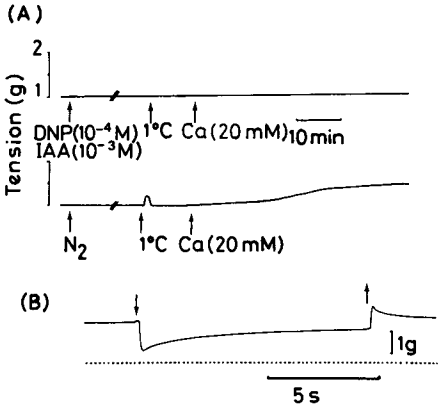

FIG. 5. (A) Effect of metabolic inhibitors on cooling-induced contraction. (B) Effect of quick release in ileal longitudinal muscle during cooling-induced sustained contraction in 20 mM Ca^{2+}. The rapid decrease by 1 mm in muscle length of about 1·5 cm was affected in the contracted state at 1 °C at the first arrow. A broken line indicates the base line.

DISCUSSION

Holzer & Lembeck (1979) showed that the ileal longitudinal muscle would contract with a rapid change in the bath temperature from 37 to 27 °C and the contraction was completely inhibited by the neuronal conduction blocker, tetrodotoxin. However, we found tetrodotoxin (1×10^{-7} M) had no effect on the cooling-induced transient and

sustained contraction at 1 °C (data not shown). It is considered that the contraction occurring at low temperatures such as 1 °C, are not related to the neurogenic element.

Pretreatment with D-600 did not inhibit the transient contraction seen with cooling. Since it is well established that Ca^{2+} antagonists inhibit the movement of Ca^{2+} into smooth muscle, it is proposed that the transient contraction in normal Ca^{2+} medium at low temperature appears to be elicited by Ca^{2+} release from a cellular site taken up Ca^{2+} through a D-600 insensitive pathway.

When muscle was cooled to 1 °C after incubation in 20 mM Ca^{2+} at 37 °C, the sustained contraction was followed by a transient contraction (Fig. 1B). However, when 20 mM Ca^{2+} was added to the normal medium at 1 °C, it produced a gradual increase in tension; phasic contraction did not appear (Fig. 3A). Furthermore, the sustained contraction did not occur on pretreatment with D-600 in spite of the addition of 20 mM Ca^{2+} at low temperature (Fig. 3B). From this, it is suggested that the sustained contraction is also maintained by Ca^{2+} release from a cellular site and it requires more calcium for the sustained tension. As shown in Fig. 3B when calcium was sufficiently accumulated at the cellular site after exposure to high concentration of Ca^{2+}, D-600 failed to inhibit the sustained contraction.

There was a substituting effect of Sr^{2+} or Ba^{2+} for Ca^{2+} on the cooling-induced sustained contraction. The effects of Sr^{2+} or Ba^{2+} were approximately 81 or 17% of the maximum tension on the cooling-induced contraction by Ca^{2+}, respectively. Bando et al (1970) reported that Sr^{2+} and Ba^{2+} have little effect of the contractile proteins in glycerinated taenia coli; 1/28th and 1/79th of that of Ca^{2+}, respectively. Furthermore, Ba^{2+} evoked a sustained tension which was dependent on the concentration of Ca^{2+} at 1 °C. Thus, it seems that the contractions caused by Sr^{2+} or Ba^{2+} at low temperature develop via Ca^{2+}.

It has been shown that rigor occurred during metabolic depletion due to removal oxygen and glucose with a decrease below critical level of ATP in guinea-pig taenia coli (Bose & Bose 1975; Knull & Bose 1975; Nasu et al 1983). There is some evidence that the cooling-induced sustained contraction in ileal muscle is not a rigor state. (i) The cooling-induced contraction depends on external Ca^{2+}; (ii) the cooling-induced contraction is inhibited by metabolic inhibitors (DNP + IAA, N_2 gas); (iii) quick release of cooling-induced contraction redeveloped the tension. Daniel et al (1971) showed the tissue ATP content remained under low temperature (4 °C) in uterine smooth muscle. The rate of superprecipitation in ileal smooth muscle myosin was decreased by lowering the temperature (Matsumoto et al 1974), but superprecipitation has been reported in actomyosin even at 0 °C (Endo 1964).

In conclusion, it is suggested that the transient and sustained contraction at 1 °C is maintained by Ca^{2+} release from a cellular site, probably the cell membrane, and the sustained contraction at 1 °C results from Ca^{2+} release from the site that has taken up Ca^{2+} through a D-600 sensitive pathway.

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