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IODOACETIC ACID-INDUCED RIGOR IN ILEAL LONGITUDINAL SMOOTH MUSCLE OF GUINEA-PIG

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Abstract—1. Ileal tensions to iodoacetic acid (IAA) develop when tissue ATP concentration falls below approximately 60–55% of the control.

2. As the IAA concentration is increased (0.1–10 mM), the ATP concentrations decrease rapidly, and both the time of the onset and the duration of the contraction shorten.

3. In the presence of lactate, IAA failed to decrease the tissue ATP concentration and did not develop tension.

4. The contraction to IAA developed in the presence of Ca^{2+} antagonist, D-600 or in Ca^{2+}-free solution, however, onset time was prolonged.

5. These results suggest that the contraction to IAA is referred to as 'rigor' because it increases with decreasing tissue ATP concentration in ileum.

INTRODUCTION

Smooth muscle contractions are generally associated with a continuous chemical energy supply (Lynch and Paul, 1985). However, the facts show that metabolic inhibition causes contractions in many types of smooth muscles. Previous reports have indicated that hypoxia causes contractions in guinea-pig taenia coli (Bose and Bose, 1975; Nasu *et al.*, 1983), saphenous and pulmonary vessels (Vanhoutte, 1976) and tracheal muscles (Kroegar and Stephens, 1971; Bose, 1976) in glucose-free media.

It has been demonstrated that the application of iodoacetic acid (IAA; glyceraldehyde-3-phosphate dehydrogenase inhibitor in glycolysis) to skeletal muscle causes an irreversible breakdown of adenosine triphosphate (ATP). The breakdown parallels the onset and extent of rigor characterized by a decrease in extensibility of the muscle (Bendall, 1951; Sandow and Schneyer, 1955). It was also found to increase in tension by IAA in guinea-pig taenia coli (Lowy and Mulvany, 1973; Butler *et al.*, 1976). To understand more about the mechanism of the onset in rigor in ileal smooth muscle, the changes of the contraction and tissue ATP concentration to IAA were studied.

MATERIALS AND METHODS

Strips of longitudinal smooth muscle were isolated from the ileum of male Hartley strain guinea-pigs, 400 g, using the method of Rang (1964), and immersed in a Tyrode solution bubbled with 95% O_2 and 5% CO_2, unless stated otherwise, at 37°C. The solution contained (mM): NaCl 136.8, KCl 2.7, $CaCl_2$ 2.5, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 5.5. The muscle strips were suspended under a resting tension of 0.6 g and allowed to equilibrate for 40 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, RM-6000). The contractile responses were recorded isotonically using a light lever (magnification 7:1, tension 0.1 g). A gas mixture of 95% N_2 and CO_2 was used to produce the hypoxic condition.

The ATP concentration in the muscle was determined by the method described by Ishida *et al.* (1984) with the slight modification of Strehler and McElroy (1957). The muscle was removed from the bath at the end of experiments, boiled in a test tube containing 1 ml water for 5 min. The ATP concentration in the extract was determined using a luminometer (Lumac, M1070) with luciferine–luciferase reagent.

RESULTS

The effects of IAA on muscle contraction

The ileal longitudinal muscle developed a tension after a delay in response to IAA. The threshold concentration for contraction was approximately 0.1 mM. When 1 mM IAA was administered to the muscle, after a delay of approximately 15 min, there was an increase in tension and after reaching a peak which was approximately 27% of the maximal tonic tension development to 40 mM K^+, the developed tension decreased slowly to the initial tension level within 90 min. As the IAA concentration was increased (0.1–10 mM), the delay of the onset of the response and the duration of the tension development reduced and the maximal tension (peak at 1 mM IAA) increased (Fig. 1). Following the disappearance of the contraction to IAA in each concentration, IAA was again administered after wash with a normal solution for 30 min. However, the IAA-induced response was irreversible.

The duration of the shortening to 1 mM IAA with a light load (0.1 g) was the same as the duration of the tension development (Fig. 2).

The effects of the aerobic metabolic inhibitors on the IAA-induced contractions were investigated.

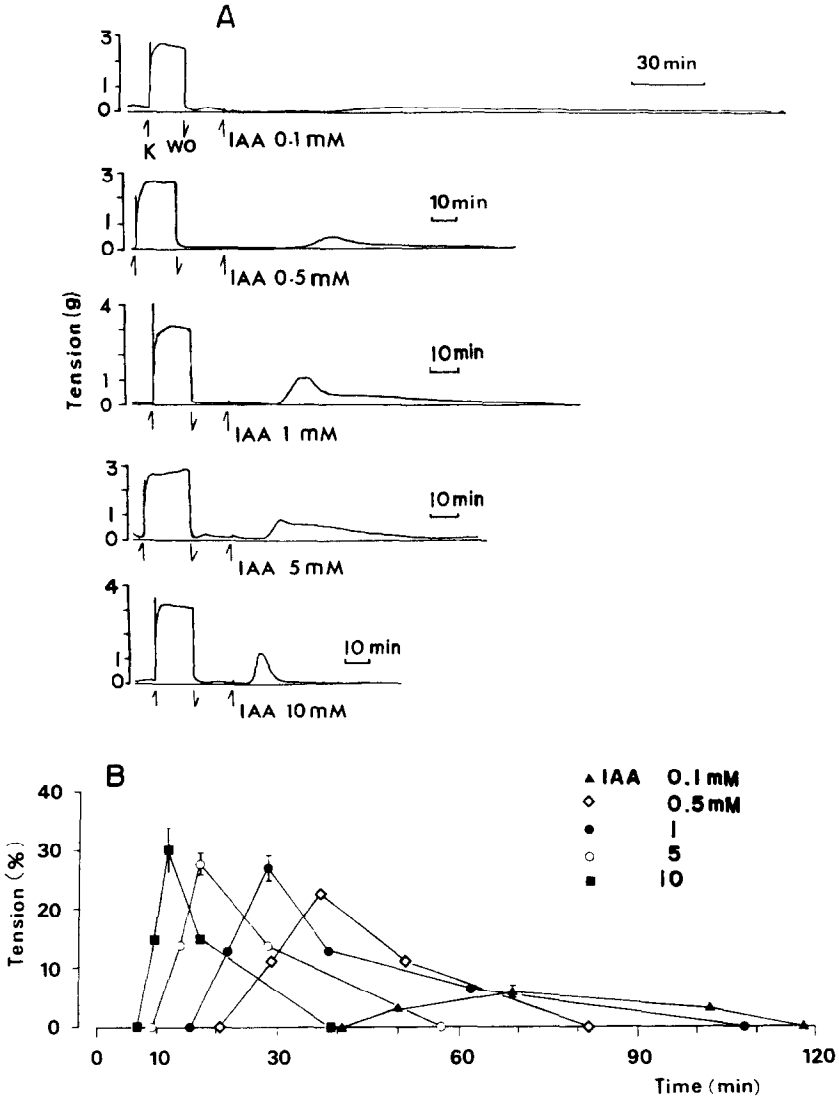

Fig. 1. The effects of iodoacetic acid (IAA) on the responses of ileal longitudinal muscle. (A) Each concentration (0.1, 0.5, 1, 5, 10 mM/l) of IAA was added to the normal medium aerated with 95% O_2 and 5% CO_2 following wash after exposure to high-K^+ (40 mM). (B) Tension changes after IAA application in various concentrations calculated as per cent of maximal tonic tension [2.5 ± 0.1 g (n = 45)] developed by high-K^+. Each point of this figure represents the mean of eight experiments (mean ± SE).

Both the N_2 gas bubbling and uncoupler, 0.1 mM dinitrophenol (DNP) markedly reduced the delay of the onset of IAA-induced contraction (Fig. 3A). In another experiment, ileal muscles were incubated in glucose-free, 40 mM K^+ solution for 60 min and glucose-free solution for 60 min. However, there was no differences in the delay of the response compared with IAA-induced contraction in normal medium. When the muscle was incubated in glucose-free, 40 mM K^+ solution for 4.5 h and glucose-free solution for 2 h, a contraction developed abruptly after the addition of 1 mM IAA (Fig. 3B).

Figure 4 illustrates the effects of metabolic intermediates on the response to IAA. The response to

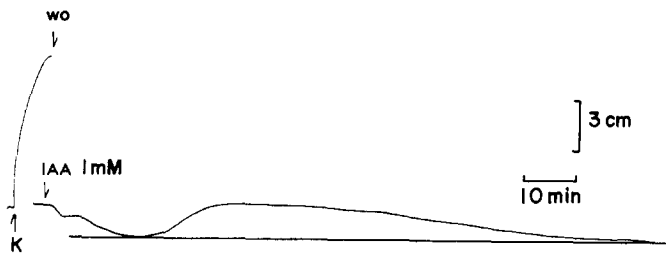

Fig. 2. The effects of 1 mM IAA on the isotonic response of ileal longitudinal muscle.

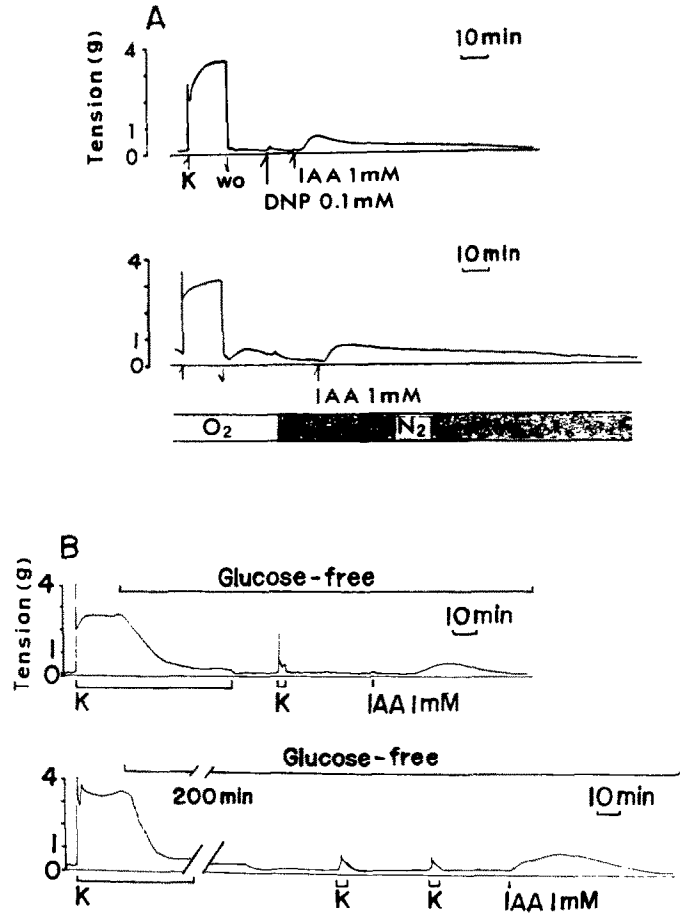

Fig. 3. The effects of metabolic inhibitors on IAA-induced contractions. (A) Following exposure to 0.1 mM DNP or 95% N_2 and 5% CO_2 gas, the muscle was exposed to 1 mM IAA. (B) Following 1 (upper) or 4.5 (lower) h of exposure to glucose-free, high-K^+ medium, the muscle was further incubated in a glucose-free medium for 1 (upper) or 2 (lower) h. A dose of 1 mM IAA was administered to the glucose-free medium.

1 mM IAA was greatly reduced in the presence of 5.5 mM lactate or 5.5 mM pyruvate.

The calcium antagonist, D-600 (gallopamil) did not affect the maximal tension to 1 mM IAA, although the delay of the response slightly prolonged to 21.8 ± 0.3 ($n = 15$) min. Further, 1 mM IAA also elicited a tension after an exposure to Ca^{2+}-free solution for 30 min (Fig. 5).

The effects of IAA on tissue ATP concentrations

The tissue ATP concentrations of ileal muscles after IAA treatment are shown in Fig. 6. The tissue ATP concentration in normal solution was 1.49 ± 0.15 mM/kg wet wt and the value did not change significantly until 10 min after 1 mM IAA treatment. The ATP concentration of ileal muscle was rapidly reduced to approximately 57% and 32% of the control value 15 and 20 min after administration of 1 mM IAA and slowly reduced thereafter. When muscles were treated with 10 mM IAA, the tissue ATP concentration reduced rapidly.

There was a greater decrease in the tissue ATP concentration after treatment with 1 mM IAA in bubbling 95% N_2 compared with normoxia. However, the tissue ATP concentration remained unchanged with the control level in the case of the administration of 1 mM IAA in the presence of 5.5 mM lactate.

DISCUSSION

After administration of IAA on ileal longitudinal muscle, the tissue ATP concentrations decreased before the tension development. When the ATP concentration fell below approximately 60–55% of the control level, there was an increase in tension. As the IAA concentration increased (0.1–10 mM), the tissue ATP concentrations decreased rapidly and the delay of the tension onset became to shorten (Figs 1 and 6). The ileal tension to IAA is referred to as 'rigor' because it increases with decreasing tissue ATP concentration.

Oxidative metabolism produces most of the ATP in intact smooth muscle (Lynch and Paul, 1985). DNP effects the mitochondrial function by uncoupling the oxidative phosphorylation and N_2 bubbling inhibits aerobic metabolism of mitochondria in guinea-pig taenia coli (Bueding *et al.*, 1967). The combination of these aerobic metabolic inhibitors and IAA caused rapid decrease in ATP concentration compared with

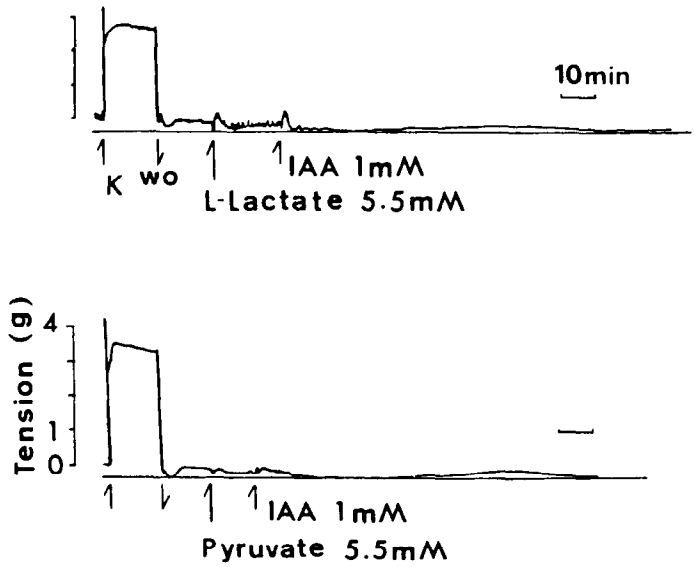

Fig. 4. The effects of preincubation with lactate or pyruvate on a 1 mM IAA-induced contraction.

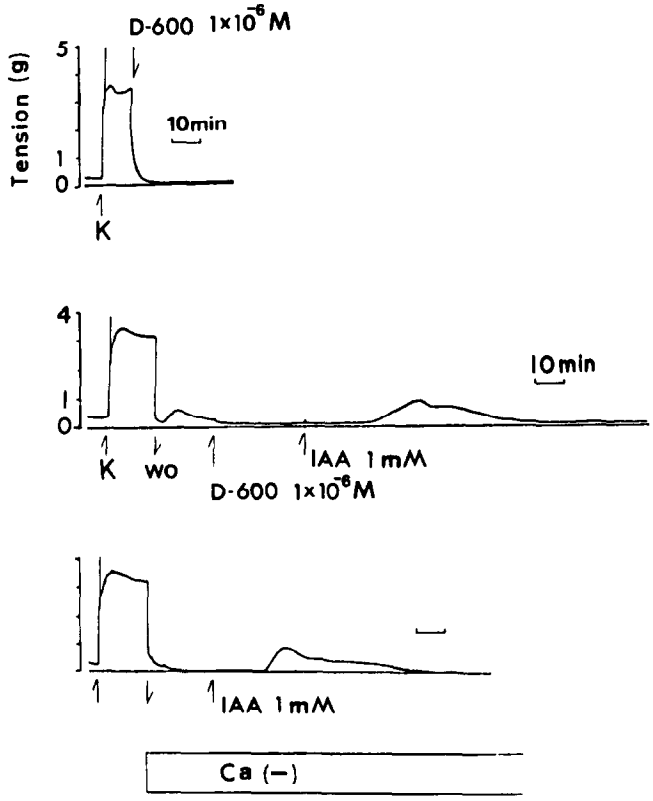

Fig. 5. The effects of preincubation with D-600 or Ca^{2+}-free medium on a 1 mM IAA-induced contraction.

incubation with only IAA and the rapid onset of the rigor tension was observed (Figs 3 and 6).

When the ileum was pretreated with lactate or pyruvate, the IAA-induced tensions were greatly diminished (Fig. 4). Lactate and pyruvate under normoxia is expected to have been utilized as substrate required for the Krebs cycle even in the presence of IAA. In fact, 1 mM IAA failed to decrease in the tissue ATP concentration in the presence of 5.5 mM lactate in ileum.

It is now well established that Ca^{2+} plays a major role as a second messenger in the excitation contraction coupling of intact smooth muscle (Inomata and Kao, 1985; Droogmans and Callewaert, 1986) and the contractile proteins of smooth muscles are regulated by Ca^{2+} in a concentration range of

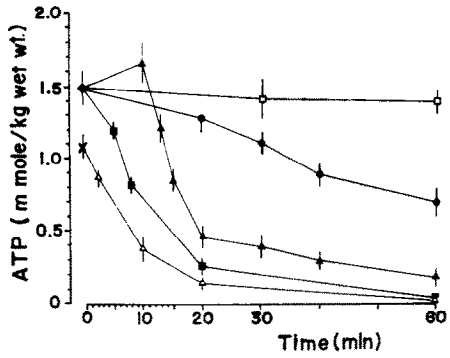

Fig. 6. The effects of IAA on the tissue ATP concentrations in ileal muscles. Each point in this figure represents the mean of 8–12 experiments (mean ± SE). Control (◆), 0.1 mM IAA (●), 1 mM IAA (▲), 10 mM IAA (■), 1 mM IAA in the presence of 5.5 mM lactate (□), 95% N_2 and 5% CO_2, 15 min (×), 1 mM IAA under 95% N_2 and 5% CO_2 bubbling (△).

10^{-8}–10^{-5} mole (Saida and Nonomura, 1978; Kreye *et al.*, 1983; Nasu and Ishida, 1986). For relaxation from contraction associated with an increase in Ca^{2+} influx in intact smooth muscle, the intracellular Ca^{2+} is needed to decrease to the level to initiate relaxation in the condition of sufficient concentration of Mg-ATP (Arheden *et al.*, 1988). However, the role of Ca^{2+} in the rigor state of smooth muscle is uncertain. In rigor to IAA in skeletal muscle, it has been shown that the rate of ^{45}Ca efflux remained at an elevated level (Bianchi, 1963). The increase in Ca efflux by IAA is considered to be due to interference with the rebinding of released Ca^{2+} by sarcoplasmic reticulum. Nauss and Davies (1966) reasoned that the onset by physical changes in skeletal muscle undergoing rigor to dinitrofluorobenzene seems to be at the point of internal liberation of a sufficient amount of Ca^{2+} to initiate an interaction between actin and myosin. On the other hand, ^{45}Ca efflux from canine trachea (Kroegar and Stephens, 1971) and ^{45}Ca uptake in guinea-pig taenia coli (Nasu *et al.*, 1983) also increased during rigor-like contraction in anoxic glucose-free environment. On the basis of these findings, it may be postulated that Ca permeability of the smooth muscle cell membrane increases under low ATP conditions. In the present experiments, a ileal rigor to IAA developed in the presence of D-600 or in Ca^{2+}-free solution, however, the onset of the rigor was prolonged. These results suggest that Ca^{2+} is not essential to induce a rigor to IAA, however, Ca^{2+} may act as a trigger to form a rigor linkage in contractile proteins. Bose *et al.* (1979) have demonstrated that smooth muscle actomyosin shows a progressive loss of Ca^{2+} sensitivity with decreasing ATP concentrations.

Both the ileal tension and shortening to IAA gradually decreased with the same time course after the each peak. The duration of ileal rigor tension or shortening 1 mM IAA was approximately 1.5 h. Lowy and Mulvany (1973) have also demonstrated that the tension of guinea-pig taenia coli to 5 mM IAA fell to the original tension within 2 h and the muscle was about 40-times stiffer than that of a muscle held in Ca^{2+}-free solution. The rigor tension to 1 mM IAA of skeletal muscle (frog sartorius), unlike smooth muscle, persisted for more than 3 h (Sandow and Schneyer, 1955). This may mean that the stiffness in rigor linkage in ileal smooth muscle is weak compared with skeletal muscle.

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