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Changes in K^+-induced contractions in ileal longitudinal smooth muscle of guinea-pig, induced by monoiodoacetic acid

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Abstract—The ileal responses to 40 mM $[K^+]_o$ were changed in the period after administration of monoiodoacetic acid (IAA). The phasic contraction to K^+ appeared in the period before the development of a rigor, after the administration of 1 mM IAA. However, the response to K^+ was absent during the development of a rigor to IAA during decreasing tissue ATP concentrations.

Monoiodoacetic acid (IAA) (Lowy & Mulvany 1973; Butler et al 1976; Nasu et al 1990) and dinitrofluorobenzene (DFB) (Nasu et al 1991) cause a rigor with decreasing tissue ATP concentrations in guinea-pig isolated ileal longitudinal smooth muscle or taenia coli. After pretreatment with the Ca^{2+} antagonist, gallopamil, the time of onset of the ileal rigor to IAA was prolonged, but, the maximal response of the rigor was not affected (Nasu et al 1990).

The phasic response to $[K^+]_o$ (40 mM or more) in taenia coli is due to the release of Ca^{2+} from the cell membrane, the tonic response mainly to an increase in the membrane permeability to Ca^{2+} through voltage operated channels as a result of membrane depolarization (Urakawa & Holland 1964; Shimo & Holland 1966; Karaki et al 1984). The inhibitors of oxidative phosphorylation in mitochondria, such as N_2 gas (Pfaffman et al 1965; Karaki et al 1969; Nasu et al 1982) or KCN (Nasu & Ishida 1990) preferentially inhibit the tonic response in taenia coli. Thus, the tonic tension to 40 mM K^+ is maintained by energy produced during oxidative metabolism. The present experiments were designed to study (i) the changes in the ileal responses to 40 mM K^+ under conditions of low tissue ATP concentrations and (ii) the extent of the rigor, at various periods after the administration of IAA in the presence or absence of $[Ca^{2+}]_o$.

Materials and methods

Strips of longitudinal smooth muscle were isolated from ileum of male Hartley guinea-pigs, 400 g (body weight), and were immersed in Tyrode's solution bubbled with 95% O_2–5% CO_2 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. A Ca^{2+}-free solution was prepared by omitting $CaCl_2$ from the normal Tyrode's solution and ethylenediaminetetraacetic acid disodium salt (0·1 mM EDTA) added. The 40 mM K^+ solution was prepared by adding an appropriate amount of 2 M KCl solution to Tyrode's solution. The muscle strips were suspended under a resting tension of 0·6 g and allowed to equilibrate for 40 min with several changes of normal Tyrode's solution. Isometric contractions of the muscle were measured by a strain gauge transducer (Nihon Kohden, RM-6000).

The ATP concentration in muscle was determined by the method of Ishida et al (1984) as modified by Strehler & McElroy (1957). The muscle was removed from the bath at the end of each experiment and boiled in test tubes containing 1 mL water for 5 min. The ATP concentration in the extracts was determined using a luminometer (Lumac, M1070) with luciferine-luciferase reagent.

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Results and discussion

When strips of ileal longitudinal muscle were treated with hypertonic, 40 mM K^+ medium, the phasic tension was followed by a sustained tonic contraction (Fig. 1a). When 1 mM IAA was administered to the muscle, after a lag of 15·8 ± 0·7 min (n = 20) there was an increase in tension which reached 27% of the tonic contraction observed in response to 40 mM K^+. The tension then decreased slowly within 65 min to the initial level (Fig. 1b). After incubation in a Ca^{2+}-free (plus 0·1 mM EDTA) medium for 30 min, the lag period for tension development to IAA was prolonged to 19·2 ± 0·6 (n = 18) min although the maximal tension did not change (Fig. 1c). This suggests that $[Ca^{2+}]_o$ 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. It has been demonstrated that a rigor development of skinned taenia coli in ATP-free solution is independent of Ca^{2+} (Arner & Rüegg 1985) and is not associated with myosin phosphorylation (Arner et al 1987).

Changes in ileal responses to 40 mM K^+ were investigated at various times after pretreatment with IAA. After 10 min pretreatment with 1 mM IAA, 40 mM K^+ caused a steep phasic contraction and was followed by the redevelopment of a biphasic contraction, after which the tension decreased slowly to the initial level (Fig. 2a). After 15 min pretreatment with 1 mM IAA, 40 mM $[K^+]_o$ induced a phasic contraction and the same force contraction as that produced by 1 mM IAA acting alone (Figs 1b,c, 2b).

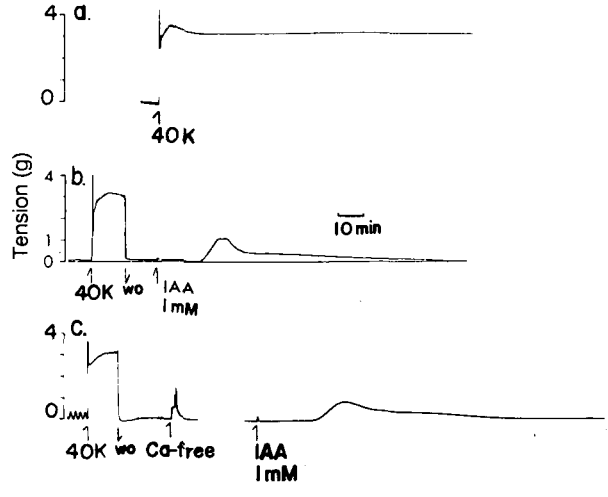

FIG. 1. The effects of monoiodoacetic acid (IAA) on the responses of ileal longitudinal muscle in the presence and absence of external Ca^{2+}. (a) The response to high-K^+ (40 mM). (b) The response to 1 mM IAA in normal medium. (c) After the muscle had been incubated in Ca^{2+}-free medium containing 0·1 mM EDTA for 30 min, 1 mM IAA was administered. Each response is from a different preparation.

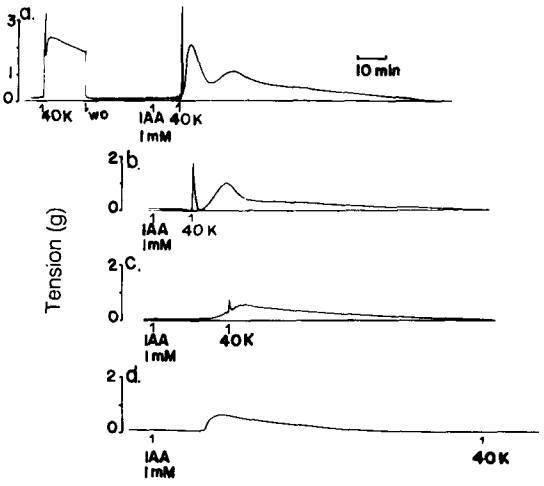

FIG. 2. The ileal responses to 40 mM $[K^+]_o$ at various periods after the administration of 1 mM IAA. 40 mM $[K^+]_o$ was added 10 min (a) and 15 min (b) after the exposure to 1 mM IAA. In another series of experiments, 40 mM $[K^+]_o$ was added early in the response to 1 mM IAA (c) or after the decline of the response to 1 mM IAA (d). Each response is from a different preparation.

Experiments were done in $[Ca^{2+}]_o$-free conditions to determine the extent to which external Ca^{2+} contributed to the tension changes to 40 mM K^+ in the presence of IAA. Depletion of $[Ca^{2+}]_o$ abolishes the 40 mM $[K^+]_o$-evoked increase in tension and O_2 consumption in taenia coli (Urakawa et al 1968). After the muscle was incubated in $[Ca^{2+}]_o$-free (0·1 mM EDTA) medium for 30 min, 40 mM K^+ was added in the presence of 1 mM IAA in the same experimental protocol as in Fig. 2a. Incubation in Ca^{2+}-free medium abolished both the steep phasic contraction and the subsequent second small contraction seen in response to medium containing 40 mM K^+, 2·5 mM Ca^{2+} and IAA (Fig. 3). This suggests both parts of the contraction seen in the presence of $[Ca^{2+}]_o$ in Fig. 2a were elicited by Ca^{2+} mobilized by 40 mM $[K^+]_o$. Furthermore, when 40 mM $[K^+]_o$ was added in the presence of 1 mM IAA to $[Ca^{2+}]_o$-free medium, using the same protocol as in Fig. 2b, no phasic contraction was seen (data not shown).

In other experiments, 40 mM $[K^+]_o$ was added at the point at which a rigor began to be elicited by 1 mM IAA. A small contraction alone was seen superimposed on the rigor response to 40 mM K^+ (Fig. 2c). When 40 mM $[K^+]_o$ was added after a rigor contraction to 1 mM IAA had declined to the initial level, no further contraction was evident (Fig. 2d). The excitation-contraction coupling by K^+ was disrupted during a rigor.

IAA decreased tissue ATP concentrations in a concentration dependent manner (Nasu et al 1990). The ileal tissue ATP concentration in normal solution was $1·52 \pm 0·14$ (n = 12) mmol (kg wet wt)$^{-1}$. This did not change significantly until 10 min after

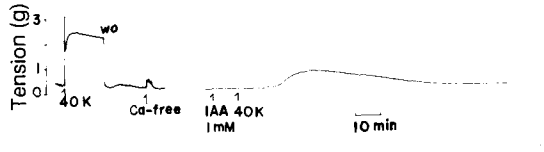

FIG. 3. Responses to 40 mM $[K^+]_o$ in the presence of IAA in Ca^{2+}-free medium. 40 mM $[K^+]$ was added 10 min after the administration of 1 mM IAA following incubation in Ca^{2+}-free medium containing 0·1 mM EDTA for 30 min.

1 mM IAA treatment, when it rapidly fell to approximately 57 and 32% of the original level after 15 and 20 min, respectively, and after 30 and 60 min slowly reduced to 25 and 13%, respectively. Neither incubation in Ca^{2+} medium for 30 min nor pretreatment with 1 mM IAA for 10 min followed by addition of 40 mM $[K^+]_o$ changed the time course of the decrease in tissue ATP concentration produced by 1 mM IAA.

In conclusion, the measured ileal response to 40 mM $[K^+]_o$ depended on the length of time it was examined after the administration of IAA. If the ileal tissue ATP concentration did not decline within 10 min of the addition of 1 mM IAA, both a phasic contraction and part of a tonic contraction to 40 mM K^+ was observed. However, the response to 40 mM K^+ was absent during the development of rigor to IAA and the resulting decrease in the concentrations of tissue ATP.

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