Decrease in Muscle Tension and Reduced Pyridine Nucleotides of the Guinea Pig Ileal Longitudinal Smooth Muscle in High K⁺,Na⁺-Deficient Solution

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ABSTRACT — In the present experiment, we studied the inhibitory mechanism of Na⁺ depletion on high K⁺-induced contraction by simultaneously measuring reduced pyridine nucleotides (PNred) or oxidized flavoproteins (FPox) fluorescence and contractile tension of the guinea pig ileal longitudinal muscle. Tension, PNred and FPox were all reversibly increased by the addition of hyperosmotic 65 mM KCl (H-65K⁺). A high K⁺,Na⁺-deficient (Iso-154K⁺) solution induced a contraction followed by a gradual relaxation and gradually decreased PNred fluorescence. A hyperosmotic addition of NaCl to the Iso-154K⁺ solution prevented the decreases in tension and PNred fluorescence. Addition of pyruvate or oxaloacetate restored the decrease in Iso-154K⁺-induced contraction, but not the decrease in PNred fluorescence. In contrast to the PNred fluorescence, an application of the Iso-154K⁺ solution increased the FPox fluorescence which was not significantly changed by an addition of NaCl, pyruvate or oxaloacetate. These results suggest that the inhibitory mechanism of Na⁺ depletion on the Iso-154K⁺-induced contraction is an inhibition of glucose utilization.

An application of hyperosmotically added high K⁺ solution produced a sustained contraction, while an isosmotically substituted high K⁺,Na⁺-deficient (Iso-154K⁺) solution induces a contraction followed by a gradual relaxation in various smooth muscle preparations (1-7). In the taenia coli (6), urinary bladder (2) and vas deferens (7), we have suggested that the inhibition of contraction by the high K⁺,Na⁺-deficient solution is mainly due to the inhibition of glucose utilization resulting from Na⁺ deficiency in the medium. On the other hand, fluorometric measurements of reduced pyridine nucleotides (PNred) have been applied to isolated skeletal (8-11) and cardiac muscles (12, 13). Recently, Ozaki et al. (14) have reported that PNred and oxidized flavoproteins (FPox) were measured fluorometrically in the intestinal smooth muscle strip of guinea pig taenia coli simultaneously with contractile tension. In the present experiment, we studied the inhibitory mechanism of Na⁺ depletion in the Iso-154K⁺-induced contraction by simultaneously measuring PNred or FPox fluorescence and contractile tension in the guinea pig ileal longitudinal muscle.
MATERIALS AND METHODS

Male guinea pigs weighing 300 to 400 g were killed by a blow on the head and bled to death. After exsanguination, the abdomen was opened, and the ileum was removed. The lower part of the ileum, a 10-cm stretch from the opening of the caecum, was discarded. The ileal longitudinal smooth muscle preparations were made as described by Paton and Aboo Zar (15). Physiological salt solution (PSS) contained: 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl₂, 1.0 mM MgCl₂, 11.9 mM NaHCO₃, and 5.5 mM glucose. The solution was continuously bubbled with 95% O₂, 5% CO₂ at 37°C and pH 7.2. High K⁺ (65.4 mM) (H₆₅K⁺) solution was made by increasing the KCl concentration in the PSS. Isosmotic 154.1 mM K⁺ solution was made by substituting an equimolar amount of K⁺ for Na⁺ in the PSS. NaCl (25 mM), pyruvate (5.5 mM) or oxaloacetate (5.5 mM) was added to Iso-154K⁺ solution hyperosmotically.

Simultaneous recordings of PNred or FPox fluorescence and force development were carried out by the method of Ozaki et al. (14). To detect PNred fluorescence, 470-nm emission wavelength and 340-nm excitation wavelength were employed; and to detect FPox fluorescence, 530-nm emission wavelength and 450-nm excitation wavelength were employed. These experiments were performed with a fluorimeter specially designed to measure the fluorescence of living tissues (CAF-100, Japan Spectroscopic). The muscle strip was held horizontally in a temperature controlled 7-ml volume organ bath. One end of the muscle strip was connected to a strain-gauge transducer to monitor the mechanical activity. The time constant of the optical channels was 0.25 sec. The fluorescence intensity obtained under the basal incubation conditions was deliberately chosen as 0% and that with H-65K⁺ solution, as 100% and changes were expressed as a percent of this value.

RESULTS

Changes in tension and PNred or FPox fluorescence in high K⁺-treated muscles

H-65K⁺ solution: An application of H-65K⁺ produced a transient contraction followed by a sustained one. PNred fluorescence increased simultaneously with or immediately after the initiation of muscle contraction. When H-65K⁺ was removed, the fluorescence began to decrease 5–10 sec after the initiation of relaxation, and the fluorescence was still decreasing toward the resting level when the muscle was completely relaxed (Fig. 1A). In contrast to the PNred fluorescence, the FPox fluorescence started to increase just before the initiation of the H-65K⁺-induced contraction. When the muscle strip was relaxed by removal of H-65K⁺, the fluorescence began to decrease after a latency of 1–5 sec (Fig. 1B). Tension, PNred and FPox fluorescence were all reversibly increased by the addition of H-65K⁺. On the initiation of H-65K⁺-induced contraction in the ileal muscle, PNred fluorescence increased immediately after the onset of contraction, while FPox fluorescence increased before the initiation of contraction in the same manner as in taenia coli (14).

Iso-154K⁺ solution: An application of Iso-154K⁺ solution induced a large contraction followed by gradual relaxation. The contraction at 20 min after the application of Iso-154K⁺ solution was 35.2 ± 4.2% (n = 12) of that by H-65K⁺. As shown in Fig. 1A, the level of PNred fluorescence (upper trace) in the presence of Iso-154K⁺ solution gradually decreased to 22.2 ± 5.2% (n = 6) of that by H-65K⁺ at 20 min. At 20 min after treatment with the Iso-154K⁺ solution, the maximum level of the FPox fluorescence was 98.5 ± 3.2% (n = 6) of that by H-65K⁺ (Fig. 1B). Because an exchange of Iso-154K⁺ solution with the PSS caused a small stimulus artifact and the fluorescence change comes very fast, the initiation of change in PNred or FPox fluorescence was unclear in the Iso-154K⁺-treated muscle.
Fig. 1. Changes in PNred fluorescence (A), FPox fluorescence (B) and muscle contraction in the ileal muscle stimulated by hyperosmotically added 65.4 mM K\(^+\) (H-65K\(^+\)) or isosmotically added 154.1 mM K\(^+\) (Iso-154K\(^+\)) solution. After the treatment, H-65K\(^+\) or Iso-154K\(^+\) solution was exchanged with the normal solution. An artifact of the solution exchange disturbed a transient rise of fluorescence level of PNred or FPox. The vertical bars present the percent change in fluorescence, taking the basal fluorescence as 0% and that with H-65K\(^+\) solution as 100%.

**Effect of a hyperosmotic addition of Na\(^+\) to the Iso-154K\(^+\) treated muscle**

The decrease in tension induced by the Iso-154K\(^+\) solution in the ileal muscle was gradually reversed by a hyperosmotic addition of Na\(^+\) at a concentration of 25 mM (Fig. 2A). When 25 mM Na\(^+\) was applied to the muscle, the decrease in PNred fluorescence due to Iso-154K\(^+\) solution was gradually restored, although FPox fluorescence was not affected (Fig. 2B). The concentration-response relationship between Na\(^+\) concentration and muscle tension or PNred fluorescence of the tissue in Iso-154K\(^+\) solution is shown in Fig. 3A. Increasing Na\(^+\) concentration induced an increase in the tension or the level of PNred fluorescence. A correlation was obtained between the tension development and the PNred fluorescence (Fig. 3B). The regression line calculated from the data was \(Y = 1.2X - 23.3\) with a correlation index (\(\gamma\)) of 0.997.

**Effect of adding pyruvate or oxaloacetate to the Iso-154K\(^+\) treated muscle**

It is well-known that the sustained contraction induced by high K\(^+\) solution in guinea pig intestine is dependent on the external glucose in the presence of Na\(^+\). Both pyruvate and oxaloacetate, substrates for oxidative metabolism, are as effective as glucose on the contraction even in the absence of Na\(^+\) (6). When the muscle strip was relaxed by the sub-
stitution of Iso-154K+ solution, addition of 5.5 mM pyruvate restored the tension to 95.4 ± 3.8% (n = 10) of that by H-65K+, but not the PNred fluorescence, although the level of FPox fluorescence in the presence of Iso-154K+ solution plus pyruvate (5.5 mM) was 96.4 ± 4.9% (n = 4) of that by H-65K+, which is similar to those in Iso-154K+ solution (98.5 ± 3.2%, n = 6) (Fig. 4, A and B). On the changes in muscle tension, PNred or FPox fluorescence, oxaloacetate (5.5 mM) showed similar effects to those of pyruvate (Fig. 5, A and B).

DISCUSSION

Fluorescence techniques have been applied to measurements of the redox state of PNred in a number of intact tissue preparations. Skeletal muscle was the first tissue in which this method was successfully applied (8), and intensive fluorometric studies helped to elucidate the energy metabolism of the heart muscle (12, 13). On the other hand, Ozaki et al.
have reported that PNred and FPox were measured fluorometrically in the intestinal smooth muscle of guinea pig taenia coli simultaneously with contractile tension. That is, in the K+-depolarized muscle, the increase in PNred was decreased when glycolysis was inhibited by removing glucose and adding 2-deoxyglucose, but not when oxidative metabolism was inhibited by N\textsubscript{2} bubbling or by NaCN. In contrast to this, the increase in the FPox by K\textsuperscript{+}-depolarization was decreased by N\textsubscript{2} bubbling or NaCN, but not by the inhibition of glycolysis. From these results, they have suggested that the major portion of PNred measured fluorometrically in their study exists in the cytoplasm and the amount is regulated by glycolysis.

It is known that hyperosmotically added high K\textsuperscript{+} induces a contraction in the intestinal smooth muscle, which is maintained at a high and steady level but inhibited by glucose removal, suggesting that the contraction is supported by an energy supply from aerobic glycolytic metabolism (16-18). On the other hand, a high K\textsuperscript{+},Na\textsuperscript{+}-deficient solution induces another type of contraction which is followed by a gradual decline in tension level. The decreased tension is restored by the addition of Na\textsuperscript{+} to the medium containing glucose or by the addition of pyruvate or oxaloacetate which can penetrate through the cell membrane without external Na\textsuperscript{+}. From these data, we have proposed that Na\textsuperscript{+} deficiency in high K\textsuperscript{+} solution inhibits the contraction due to inhibition of glucose utilization in the muscle (6). From the biochemical approach, O\textsubscript{2} consumption and ATP content of the muscle was measured during the 154K\textsuperscript{+}-induced contraction. The Na\textsuperscript{+}-deficiency decreased the increased O\textsubscript{2} consumption and ATP content of the muscle treated with high K\textsuperscript{+} (18). However, these measurements were performed at only one or two points of the experimental procedure. In the present experiments, we simultaneously recorded the changes in the muscle tension and PNred or FPox fluorescence during the whole course of the experiment in the ileal muscle.

An application of Iso-154K\textsuperscript{+} solution to the tissue induced a large contraction, then gradually decreased the muscle tension, and it remarkably decreased the PNred fluorescence. This decrease in both the tension and PNred fluorescence were prevented by the hyperosmotic addition of NaCl, but not affected by the substitution of pyruvate or oxaloacetate for glucose in Iso-154K\textsuperscript{+} solution. On the other hand, an application of Iso-154K\textsuperscript{+} solution to the tissue increased FPox fluorescence, which was not influenced by hyperosmotic addition of NaCl, whereas the contraction was markedly restored. Moreover, addition of 5.5 mM pyruvate or oxaloacetate did not significantly change the FPox fluorescence of the muscle in Iso-154K\textsuperscript{+} solution. The reason why pyruvate or oxaloacetate does not increase
FPox fluorescence of the tissue in Iso-154K+ solution is assumed to be as follows: this FPox fluorescence has been kept at a maximal level by a compensatory effect on glycolysis inhibition that is represented by the decreased in PNred fluorescence of the muscle in Iso-154K+ solution; and accordingly, pyruvate or oxaloacetate can not induce any increase in FPox fluorescence under this condition. This assumption is probably supported by the data reported by Paddle (19) that pyruvate had no effect on FPox fluorescence in the rat diaphragm when it was added under conditions in which glycolysis was inhibited.

It is well-known that intracellular Ca++ ion ([Ca^{2+}]i) regulates tension development and metabolic processes in intestinal smooth muscles. To assess the possibility that a change in [Ca^{2+}]i is involved to the relaxation of the ileal muscle in Iso-154K+ solution, we measured simultaneously muscle tension and [Ca^{2+}]i level using fura-2 by the method devised by Ozaki et al. (14). In fura-2-loaded muscle strips, application of Iso-154K+ solution increased tension and [Ca^{2+}]i level, followed by a gradual relaxation without a decrease in [Ca^{2+}]i level (K. Shimizu et al., unpublished data). Therefore, the inhibition of Iso-154K+-induced contraction is probably not limited by the change in [Ca^{2+}]i level.

In Fig. 3 (A and B), it was shown that the muscle tension and PNred fluorescence of the muscle in high K+ solution are dependent on the external Na+ concentration and that the muscle tension has a very close relationship to the PNred fluorescence at all the Na+ concentrations in high K+ solution.

From these data, it is suggested that the Na+ deficiency inhibits the glucose symport through the cell membrane, resulting in a decrease in an intermediate of the glycolytic pathway, PNred, in the cytoplasm. Accordingly, these results probably confirm our proposal that the decrease in the developed tension in Iso-154K+ solution is caused by the inhibition of glucose utilization which involves a Na+ deficiency, as determined by measuring the change in PNred.

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