ROLE OF GLYCOLYSIS IN THE TENSION DEVELOPMENT UNDER ANOXIA IN GUINEA PIG TAENIA COLI

Tetsuyuki NASU*, Kazumi YUI, Hideyuki NAKAGAWA** and Yukio ISHIDA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi, Tokushima 770, Japan and **Department of Health Sciences, Faculty of Education, University of Tokushima, Minami-josanjimacho, Tokushima 770, Japan

Accepted: October 14, 1981

Abstract—The role of glycolysis in the tension development under anoxic conditions in a high-K medium was studied in the intestinal smooth muscle of guinea pig taenia coli. After exposure to the high-K medium (isotonic, 60 mM) under normal oxygen for 30 min, the muscles were exposed to a high-K medium bubbled with N₂ gas. The tonic contraction decreased gradually to about 10% of the original level. Glucose was then cumulatively added to the high-K medium under anoxia. The maximum tension was observed following the addition of the higher concentrations of glucose. The muscle tension which developed in the high-K medium with a high concentration of glucose under anoxia was dependent on the external Ca²⁺ and was inhibited by iodoacetic acid (IAA). The addition of glucose to a high-K medium under anoxia also increased lactate release from the muscle. Pretreatment with 1 mM IAA decreased the lactate release from the muscle. In a Ca²⁺-free medium under anoxia, the addition of glucose did not increase the muscle tension although there was a significant increase in the lactate release. In summary, it is considered that the smooth muscle of taenia coli develops tension utilizing energy produced by the glycolytic pathway under anoxia in a high-K medium.

It has been reported (1, 2) that a high-K (hypertonic or isotonic, 40 mM) medium produces a tonic contraction in intestinal smooth muscle of guinea pig taenia coli. The high-K medium induced a phasic response after pretreatment of the muscle with 2,4-dinitrophenol (DNP), nitrogen gas bubbling, removal of glucose from the medium (1, 2). During the phasic response to the high-K medium, ⁴⁶Ca uptake and the tissue calcium content did not change from the control values (1, 3). Saito et al. (4) reported that the use of high-K caused an increase in oxygen consumption accompanying the tension development. During the tonic response to the high-K medium, the calcium uptake and tissue calcium content increases (1, 3) and DNP, anoxia and glucose removal are known to nullify this high-K induced tonic response as well as the calcium uptake in taenia coli (5, 6). It is suggested that the high-K induced phasic phase is the result of a Ca²⁺ release from some cellular site, whereas the tonic response is maintained by a Ca²⁺ influx from the external medium and is dependent on the aerobic metabolism in guinea pig taenia coli.
It has been shown that the tissue calcium content of taenia coli rises with cooling and anoxia (7), and the rate of calcium loss from taenia coli was largely controlled by a physical process (8). This suggests a possible Ca-extrusion mechanism which is dependent on metabolic energy.

Although the tonic phase induced by the high-K was greatly attenuated by anoxia, about 10% of the original tension that developed in the high-K was resistant to anoxia. The present experiment was done to clarify the nature of the tension maintained in a high-K medium even under anoxia in taenia coli.

**MATERIALS AND METHODS**

Strips of taenia coli were freshly dissected from male Hartley strain guinea pigs weighing about 400 g, and the strips were immersed in Tyrode solution bubbled with 95% O₂ and 5% CO₂ gas mixture at 37°C. The solution contained (mM): NaCl 136.8, KCl 2.7, CaCl₂ 2.5, MgCl₂ 1.0, NaH₂PO₄ 0.4, NaHCO₃ 11.9 and glucose 5.5. A gas mixture of 95% N₂ and 5% CO₂ was used to produce the anoxic condition.

High-K (isotonic, 60 mM) medium was made by replacing 60 mM NaCl by equimolar KCl.

**Contraction:** Contractile responses were recorded isometrically by a strain-gauge transducer (Nihon Kohden). The muscles were equilibrated under a resting tension of 0.6 g.

**Lactate release:** Muscle strips were incubated with 3 ml of desired medium for 60 min, and the lactate contents in the medium were determined by the lactic dehydrogenase method described by Lundholm et al. (9). A perchloric acid (0.39 ml) was added to the medium. The medium was centrifuged at 3,000 rpm for 10 min at 2°C. Then 0.3 ml of the supernatant fraction was added to the 0.5 M glycine buffer (pH 9.0) containing 27 mM nicotinadenine dinucleotide (NAD, oxidized form), 0.02 ml lactic dehydrogenase (2 mg enzyme protein/ml), 0.4 M hydrazine, and the reaction solution (total volume: 2.52 ml) was left for 60 min at 25°C. The absorbance of NAD (reduced form) in the reaction solution was measured at 630 nm by a spectrophotometer (Hitachi, 207).

**RESULTS**

**Effects of glucose on the tension under anoxia:** When the muscle was treated with the high-K (isotonic 60 mM), it developed a tension of about 10 g which gradually fell within the first 20 min and then remained at a steady level of about 7 g. Strips incubated in the high-K medium for 30 min under O₂ gas (normoxia) were then exposed to a high-K medium bubbled with N₂ gas. The tonic contractions were gradually reduced to about 10% of the original tension level after 60 min of N₂ gas. Increasing concentrations of glucose were cumulatively added to the high-K medium under anoxia. The tension became progressively greater as the glucose concentration increased (Figs. 1 and 2).

The responses of taenia coli to graded changes in the tonicity of the extracellular medium were studied. The addition of sorbitol and sucrose, both unable to penetrate into the smooth muscle cell membrane (10, 11), did not develop tension when similar experiments were performed (data not shown). From these results, it is considered that the tension developed by the addition of glucose under anoxia in the high-K medium is not the result of changes in osmolarity.

**Characteristics of tension developed by glucose under anoxia:** The role of external Ca²⁺ in the contraction developed by 50 mM glucose under anoxia in a high-K medium was studied. After a 60 min incubation under anoxia in the high-K medium, Ca²⁺ was removed from the external medium, and the
tension decreased to base line levels. After a 30 min incubation in a Ca\(^{2+}\)-free high-K medium under anoxia, 50 mM glucose was added, but no change in muscle tension was noted (Fig. 3).

It was suggested that D-600 inhibited the Ca\(^{2+}\) influx across the cell membrane in taenia coli (12). The glucose-induced contraction under anoxia was inhibited by 1 × 10^{-6} M D-600 (Fig. 3). These results suggest that a Ca\(^{2+}\) influx is required to maintain contraction induced by the glucose under anoxia.

To understand the mechanisms responsible for the tension caused by glucose addition under anoxia, the effects of metabolic inhibitors on this tension were studied. Iodoacetic acid (IAA) (1 × 10^{-3} M) was administered after 60 min under anoxia in a high-K medium. After 30 min, 50 mM glucose was added. In the presence of the IAA, tension did not develop. In another experiment, 30 min after the addition of 50 mM glucose to the high-K medium under anoxia, 1 × 10^{-3} M IAA was administered. The tension developed by 50 mM glucose was inhibited by the IAA. On the other hand, the tension developed by 50 mM glucose under anoxia in a high-K medium was not inhibited by dinitophenol (DNP) at a concentration (1 × 10^{-4} M) sufficient to suppress the contractile response to the high-K medium under normoxia (Fig. 4).

As a measure of a series elastic element stiffness, the quick release method was used.
by Bose and Bose (13). The rapid decrease in length of the muscle strip (about 1.5 cm length) by 1 or 2 mm was done in the contracted state with the high-K under normoxia. A rapid decrease in tension was then seen and tension redevelopment followed, which is indicative of an active state. Furthermore, the quick release was done in the relaxed state under anoxia or the contracted state after 50 mM glucose addition. In both cases, a rapid decrease followed by tension was seen (Fig. 5).

Table 1). The result suggests that the tension developed by glucose under anoxia in a high-K medium is an active state.

These results indicate the possibility that the tension induced by glucose under anoxia in a high-K medium is maintained by energy supplied by glycolysis.

Effect of glucose on lactate release: It has been shown (14) that lactate is the end product of glycolysis and readily diffuses through the cell membrane. The lactate in the suspension medium was determined under aerobic or anaerobic conditions. The lactate contents that diffused from taenia coli were determined during an incubation period of 60 min in each medium. In the muscles incubated with normal Tyrode solution under normoxia, the lactate concentration was low. When the muscles were

Table 1. Effect of quick release. States A, B and C are the same as in Fig. 5. The (a) series is the fall of tension which decreased by rapid reduction by 1 or 2 mm in muscle length. The (b) series is the redevelopment of tension for 30 sec which was followed by a rapid increase of 1 or 2 mm in muscle length.

|          | State A          | State B          | State C          |
|----------|------------------|------------------|------------------|
| 1 mm     | (a) 3.45±0.37 (12) g | 1.23±0.17 (12)     | 1.48±0.11 (12)     |
| Release  | (b) 1.95±0.27 (12) | 0.60±0.08 (12)     | 0.88±0.09 (12)     |
| 2 mm     | (a) 5.93±0.35 (6)  | 1.42±0.31 (6)      | 2.13±0.21 (6)      |
| Release  | (b) 3.02±0.10 (6)  | 0.73±0.13 (6)      | 1.42±0.12 (6)      |
incubated in the high-K under normoxia, the lactate was undetectable in the medium. However, when they were incubated in a high-K medium under anoxia in the normal glucose (5.5 mM) medium, this caused a marked increase in lactate release from the muscle (77±6 μM/g/hr, n=12). In a high-K medium under anoxia, the addition of 30, 50 or 100 mM glucose for 60 min caused a marked increase in the lactate levels and the amounts of lactate released 94±7 (n=8), 125±6 (n=8) or 144±8 μM/g/hr, (n=10), respectively. In the muscle strip pretreated with IAA under anoxia in a high-K medium, there was no tension and the lactate release was unchanged in spite of the addition of 50 mM glucose. In the Ca²⁺-free, high-K medium under anoxia, the addition of 50 mM glucose did not develop tension. However, there was a significant increase in the lactate release (74±4 μM/g/hr, n=10) (Fig. 6).

DISCUSSION

It has been reported that the nature of the tension, Ca movement and energy metabolism during a high-K induced tonic response are changed by the ratios of external potassium and sodium concentrations in taenia coli (15–17). In the tonic phase of 60 mM K medium (normal Na⁺), the membrane spike activity ceased and the muscle remained depolarized at a high level (18). Furthermore, the tonic phase is dependent on energy metabolism (18). In the present experiment, a high-K medium was made by the subtraction of 60 mM NaCl from normal Tyrode solution and by the addition of 89 mM sodium was always contained in the high-K medium.

The tonic response induced by the 60 mM K medium in taenia coli was inhibited by anoxic conditions, however, about 10% of the original tension remained even with anoxia (Fig. 1). The tension which developed following the addition of glucose to a high-K medium under anoxia was dependent on the external glucose concentration and reached a plateau when the glucose concentration was greater than 70 mM (Figs. 1 and 2). Arnqvist (19, 20) has studied the membrane transport of glucose in the rabbit colon and in the bovine...
mesenteric arteries and found that glucose uptake under normoxia increased when the concentration of glucose in the medium was raised. Lundholm et al. (14) investigated the glucose uptake under anoxia in the bovine mesenteric artery and reported that when the oxygen concentration in the gas phase was reduced from 95% to 0%, the glucose uptake was increased by 50%. As shown in Figs. 1 and 2, the muscle tension induced by the addition of glucose under anoxia in the high-K medium was smaller than the high-K induced tension under normoxia in a normal glucose medium. This will be explained by the fact that two moles of ATP are generated when one mole of glucose is converted to lactate in glycolysis while 36 moles of ATP are produced per mole of glucose when pyruvate is oxidized in the Krebs cycle (14).

It has been shown that IAA inhibits glycolysis by inactivating 3-phosphoglyceraldehyde dehydrogenase. On the other hand, DNP (uncoupling agent) inhibits oxidative phosphorylation in mitochondria. As shown in Fig. 4, the tension developed by the addition of glucose under anoxia was inhibited by IAA, but not by DNP. This data suggests that the tension developed by glucose addition under anoxia is dependent on glycolytic energy metabolism. Furthermore, it is suggested that the tension developed by glucose addition under anoxia in a high-K medium was an active process as shown by the quick release method.

The lactate production in vascular smooth muscles has been reviewed by Lundholm et al. (14). In bovine mesenteric artery, the lactate release was about 3.5 \( \mu \)M/g/hr in normoxia (95% O\(_2\)) and about 14 \( \mu \)M/g/hr in anoxia (14). In taenia coli, it was found the lactate release was about 3.9 \( \mu \)M/g/hr in normoxia and 18 \( \mu \)M/g/hr in anoxia in unstimulated muscle. The lactate release was not noted in the high-K medium under normoxia in taenia coli. In taenia coli at normal oxygen levels, the glucose degradation may not stop at the lactate stage, but its product may be further oxidized.

As shown in Fig. 6, there is a marked increase in lactate release in a high-K medium under anoxia (normal glucose). The lactate release from cells in taenia coli increased when higher concentrations of glucose were added to the high-K medium under anoxia. In the bovine mesenteric artery under anoxia, epinephrine or high-K produced a 3 to 5 fold increase in the lactate release following a rise in muscle tension (21). When the high-K stimulated taenia coli was exposed to nitrogen for 60 min in a normal glucose medium, about 10% of the original tension was resistant to anoxia (Fig. 1). The ATP and creatine phosphate contents in the taenia coli were maintained at approximately 23 or 15% of the original levels in taenia coli, respectively (22). It seems that the ATP produced in glycolysis has an important role in the maintenance of the tension developed under anoxia in a high-K medium.

In the Ca\(^{2+}\)-free, high-K medium under anoxic conditions, glucose addition did not develop tension in spite of lactate production (Fig. 6). However, the release of lactate in the Ca\(^{2+}\)-free medium was smaller than that seen in a normal Ca\(^{2+}\) (2.5 mM), high-K medium under anoxia. This fact illustrates that taenia coli smooth muscle does not contract even if lactate is produced in the high-K medium under anoxia when Ca ions are not present in the external medium. The actual mechanism by which the lactate release is affected during the Ca\(^{2+}\)-free, high-K medium under anoxia as compared with a Ca\(^{2+}\)-containing medium has not been explained. The muscle contraction supported by glucose under anoxia in high-K was inhibited by the Ca antagonist, D-600 (Fig. 3). These results suggest that external Ca\(^{2+}\) is required to maintain the tension.
induced by the glucose addition under anoxia.

In summary, the smooth muscle of taenia coli, when in a high-K medium and under anoxic conditions, develops tension utilizing energy produced in the glycolytic pathways.

REFERENCES

1) Urakawa, N. and Holland, W.C.: Ca\(^{415}\) uptake and tissue calcium in K-induced phasic and tonic contraction in taenia coli. Am. J. Physiol. 207, 873–876 (1964)

2) Pfaffman, M., Urakawa, N. and Holland, W.C.: Role of metabolism in K-induced tension changes in guinea pig taenia coli. Am. J. Physiol. 208, 1203–1205 (1965)

3) Karaki, H., Ikeda, M. and Urakawa, N.: Movement of calcium during tension development induced by barium and high-potassium in guinea pig taenia coli. Japan. J. Pharmacol. 19, 291–299 (1969)

4) Saito, Y., Sakai, Y., Ikeda, M. and Urakawa, N.: Oxygen consumption during potassium induced contracture in guinea pig taenia coli. Japan. J. Pharmacol. 18, 321–331 (1968)

5) Urakawa, N., Karaki, H. and Ikeda, M.: Effects of ouabain and metabolic inhibiting factors on Ca distribution during K-induced contracture in guinea pig taenia coli. Japan. J. Pharmacol. 20, 360–366 (1970)

6) Karaki, H., Ikeda, M. and Urakawa, N.: Effects of ouabain and 2,4-dinitrophenol on calcium distribution and exchange in guinea pig taenia coli in high-potassium solution. Japan. J. Pharmacol. 20, 530–535 (1970)

7) Bauer, H., Goodford, P.J. and Hütter, J.: The calcium content and \(^{45}\)Ca uptake of the smooth muscle of the guinea-pig taenia coli. J. Physiol. 176, 183–179 (1965)

8) Goodford, P.J.: The loss of radioactive \(^{45}\)Ca from the smooth muscle of the guinea-pig taenia coli. J. Physiol. 176, 180–190 (1965)

9) Lundholm, L., Lundholm, E.M. and Vamos, N.: Lactic acid assay with L(+)lactic acid dehydrogenase from rabbit muscle. Acta physiol. scand. 58, 243–249 (1963)

10) Goodford, P.J. and Leach, E.H.: The extracellular space of the smooth muscle of the guinea-pig taenia coli. J. Physiol. 186, 1–10 (1966)

11) Krejci, I. and Daniel, E.E.: Effect of contraction on movements of calcium 45 into and out of rat myometrium. Am. J. Physiol. 219, 256–262 (1970)

12) Mayer, C.J., Breemen, C.V. and Casteels, R.: The action of lanthanum and D600 on the calcium exchange in the smooth muscle cells of the guinea-pig taenia coli. Pflügers Arch. 337, 333–350 (1972)

13) Bose, D. and Bose, D.: Mechanics of guinea pig taenia coli smooth muscle during anoxia and rigor. Am. J. Physiol. 229, 324–328 (1975)

14) Lundholm, L., Anderson, R.G.G., Arnegqvist, H.J. and Lundholm, E.M.: Glycolysis and glycoenolysis in smooth muscle. In The Biochemistry of Smooth Muscle. Edited by Stephens, N.L., p. 159–207, Univ. Press, Baltimore, London and Tokyo (1977)

15) Urakawa, N., Karaki, H. and Ikeda, M.: \(^{43}\)Ca uptake and tissue Ca of guinea pig taenia coli in isotonic high-K/Na-deficient medium. Japan. J. Pharmacol. 18, 294–298 (1968)

16) Karaki, H., Ganeshanandon, S.S., Ikeda, M. and Urakawa, N.: Changes in tension, Ca movement and metabolism of guinea pig taenia coli in varying concentration of external Na and K. Japan. J. Pharmacol. 19, 569–577 (1969)

17) Breemen, C.V., Aaronson, P. and Loutzenhiser, R.: Sodium-calcium interaction in mammalian smooth muscle. Pharmacol. Rev. 30, 167–208 (1979)

18) Shimo, Y. and Holland, W.C.: Effects of potassium on membrane potential, spike discharge, and tension in taenia coli. Am. J. Physiol. 211, 1299–1304 (1966)

19) Arnegqvist, H.J.: Characteristics of monosaccharide permeability in arterial tissue and intestinal smooth muscle; effect of insulin. Acta physiol. scand. 85, 217–227 (1972)

20) Arnegqvist, H.J.: Effects of increasing glucose concentrations on the glucose metabolism in arterial tissue and intestinal smooth muscle. Acta physiol. scand. 88, 481–490 (1973)

21) Lundholm, L. and Lundholm, E.M.: Energetics of isometric and isotonic contraction in isolated vascular smooth muscle under anaerobic conditions. Acta physiol. scand. 64, 275–282 (1965)

22) Knull, H.R. and Bose, D.: Reversibility of mechanical and biochemical changes in smooth muscle due to anoxia and substrate depletion. Am. J. Physiol. 229, 328–333 (1975)