EFFECTS OF FACTORS INHIBITING TENSION DEVELOPMENT ON OXYGEN CONSUMPTION OF GUINEA PIG TAENIA COLI IN HIGH K MEDIUM

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Hypertonically added high-potassium induced a tension development in the guinea pig taenia coli, which was composed of two distinct parts, a phasic response and a tonic one (1, 2). The tonic response was dependent on the aerobic breakdown of carbohydrate and was abolished by substrate removal, anoxia, a decrease in temperature, 2, 4-dinitrophenol (DNP), lithium substitution and ouabain. These factors had little or no effect on the phasic response. From these data it was suggested that the phasic response is a passive process, whereas the tonic response is an active one depending on metabolism. A part of this concept is supported by the finding that the application of high K caused an increase in oxygen consumption that accompanies the muscle tension change (3).

In the present paper, effects of factors inhibiting tension development, viz. glucose removal, low oxygen, decrease in temperature, DNP and ouabain, on oxygen consumption in high-potassium medium were studied.

METHODS

Strips of taenia coli, weighing about 20–30 mg, isolated from white male guinea pigs were quickly suspended in the chamber of the apparatus to record simultaneously Po2 in the medium and muscle tension changes. The sputtered platinum film electrode invented by Saito (4) was employed to measure polarographically the Po2 of the medium. Tension change in the muscle was recorded by a strain gauge. Tyrode solution equilibrated with a gas mixture of 95% O2, 5% CO2 and maintained at 37°C was perfused at a constant rate of 6.47 ml/hr except when otherwise stated, from a reservoir through the bath.

Apparatus and method have been described in detail earlier (3).

The Tyrode solution used was of the following composition (mm): NaCl, 136.8; KCl, 5.4; CaCl2, 2.5; MgCl2, 1.0; NaH2PO4, 0.4; NaHCO3, 11.9 and glucose, 5.5. In some experiments, a Tyrode solution containing 2.7 mm KCl was used. High K (40 mm) solution was prepared by substituting KCl for NaCl in equivalent amounts. DNP (1 × 10−4m)
or ouabain \((2.5 \times 10^{-6} \text{M} \text{ or } 1 \times 10^{-5} \text{M})\) was dissolved in Tyrode solution. Low oxygen supply was done by aeration with a gas mixture of 5% \(\text{O}_2\), 90% \(\text{N}_2\) and 5% \(\text{CO}_2\).

**RESULTS**

When normal medium was exchanged with high K medium, the muscle showed a tension development accompanied by a decrease in \(\text{P}_0_2\) level in the medium. Values of \(\text{Q}_0_2\) (\(\mu\text{d} \text{O}_2/\text{g} \cdot \text{hr}\)) in normal and high K media were more variable than the values shown in the previous paper (3).

Glucose removal: Fig. 1 shows the effect of glucose removal on \(\text{P}_0_2\) level in the medium and muscle tension changes. When removal of glucose from the high K medium was performed 30 minutes after the application of high K, a gradual decrease in the muscle tension was seen and it coincided with an increase in \(\text{P}_0_2\) level in the medium. About

![Fig. 1](image)

**Fig. 1.** Effects of glucose removal on \(\text{P}_0_2\) in medium and muscle tension of taenia coli following application of high K in normal medium.

Upper curve shows \(\text{P}_0_2\) level (mmHg), lower one, muscle tension (g).

The dot line indicates the \(\text{P}_0_2\) level without the muscle in the chamber.

Time (in minutes) represents the time after the muscle was suspended in the chamber.

The wet weight of taenia coli was 28.8 mg.

At A: Normal medium changed to high K (isotonic, 40 mm) medium.

At B: High K medium changed to glucose (-) high K medium.

At C: Glucose (-) high K medium changed to high K medium.
FIG. 2. Effects of high K application on Po$_2$ in medium and muscle tension of taenia coli under low oxygen (5% O$_2$).
Refer to the note on Fig. 1.
The wet weight of taenia coli was 22.5 mg.
At A & A': Normal medium changed to high K (isotonic, 40 mm) medium.
At B & B': High K medium changed to normal medium.

one hour after glucose removal, the tension reached almost the original level and Po$_2$ nearly the level in the normal medium. When glucose was replaced, Po$_2$ level immediately decreased and the tension gradually increased to 5 g. In six experiments, glucose removal abolished the already developed tension in high K medium within two hours, and decreased the increased oxygen consumption to the level in normal medium in four out of six experiments, though the glucose removal diminished the oxygen consumption to about half in the other two experiments.

Low oxygen: When the Tyrode solution was bubbled with 5% O$_2$ gas mixture, the muscle appeared to consume an extremely small amount of oxygen. An application of high K induced a small phasic contraction but did not show significant changes in Po$_2$ level in the medium (Fig. 2).
Low temperature: Lowering the temperature of the bath from 37°C to 12°C greatly reduced oxygen consumption of the muscle in the normal medium. An exchange of the normal Tyrode solution with a high K one induced a typical phasic contraction but had no effect on the Po₂ level (Fig. 3).

**Fig. 4.** Effect of high K on Po₂ in medium and muscle tension of taenia coli pretreated with 1 x 10⁻⁵M ouabain.

Refer to the note on FIG. 1.
The wet weight of taenia coli was 31.4 mg.
At A: Normal medium changed to ouabain (1 x 10⁻⁵M) medium.
At B: Ouabain medium changed to ouabain-high K (40 mm, isotonic) medium.
At C: Ouabain-high K medium to ouabain medium.

**Fig. 5.** Effect of ouabain on Po₂ in medium and muscle tension of taenia coli following application of high K to normal medium.

Refer to the note on FIG. 1.
The wet weight of taenia coli was 34.0 mg.
At A: Normal medium changed to high K (isotonic, 40 mm) medium.
At B: High K medium changed to ouabain (2.5 x 10⁻⁶M) high K medium.
At C: Ca was depleted from ouabain (2.5 x 10⁻⁶M) high K medium and 0.1 mm EGTA was added.
At D: Ca was replaced to the medium and 0.1 mm EGTA was depleted.
**Fig. 6.** Effect of DNP on $P_{O_2}$ in medium and muscle tension of taenia coli following application of high K in normal medium.

Refer to the note on Fig. 1.

The wet weight of taenia coli was 26.9 mg.

At A: Normal medium changed to high K (isotonic, 40 mm) medium.
At B: High K medium changed to DNP ($1 \times 10^{-4}$M) high K medium.
At C & C': Ca was depleted from DNP ($1 \times 10^{-4}$M) high K medium and 0.1 mM EGTA was added.
At D: Ca was replaced to the medium and 0.1 mM EGTA was depleted.

![Graph showing effect of DNP on $P_{O_2}$ and tension](image)

**Fig. 7.** Effects of DNP and ouabain on the $Q_{O_2}$ and the tension after the application of high K in taenia coli.

Values of $Q_{O_2}$ (µl O$_2$/g-hr) and tension (for 15 minutes) from the 15th minute to the 30th minute after high K application are expressed as 100%.

The unshaded bars represent the mean percentage value of changes in $Q_{O_2}$ during the 15 minutes and the shaded bars represent those in tension.

A: High K (in 2.7 mm K Tyrode solution).
B: DNP ($1 \times 10^{-4}$M), (in 2.7 mm K Tyrode solution).
C: Ouabain ($2.5 \times 10^{-4}$M), (in 2.7 mm K Tyrode solution).

$T_1$: 0 to 15th minute after treatment in high K medium; $T_2$: 15th to 30th minute; $T_3$: 30th to 45th minute; $T_4$: 45th to 60th minute.
Oxygen Consumption in Taenia Coli

Ouabain: Pretreatment with $1 \times 10^{-5}$M ouabain showed a transient tension development accompanied by a decrease in $P_{O_2}$ level. When the $P_{O_2}$ level returned to normal, high K was added. The muscle then showed a phasic contraction and a corresponding phasic decrease of $P_{O_2}$ in the medium (Fig. 4). After glucose removal, an application of high K showed results similar to ouabain treatment. Reproducibility of these changes was poor when high K application was repeated. When the muscle was pretreated with $2.5 \times 10^{-6}$M ouabain, an application of high K induced a phasic response followed by a small tonic contraction and a corresponding change in $P_{O_2}$ level.

When ouabain ($2.5 \times 10^{-4}$M) was added to the high K medium, the tension was decreased from 7 g to about 1 g within one hour. In contrast to the marked change in tension, the decreased $P_{O_2}$ level in the high K medium was not significantly influenced by ouabain application (Fig. 5 and 7-B). A removal of external calcium reversibly abolished the residual tension and increased the $P_{O_2}$ level to that in normal medium.

DNP: When DNP ($1 \times 10^{-4}$M) was added to the high K medium, the muscle tension developed fell to the original level, and $P_{O_2}$ was either decreased further or maintained at the same level (Fig. 6 and 7-C). This decreased $P_{O_2}$ level did not show any significant change by removal of external calcium.

DISCUSSION

It was reported that a dissociation of electrical and mechanical activity of taenia coli was produced by exposing the muscle to glucose-free medium (5). The activity of the membrane persisted for several hours in the absence of glucose but the tension response declined more rapidly, generally disappearing in 1~2 hours. Timms (6) described that after only two or three minutes exposure to a glucose-free medium, the glucose available within the taenia coli was reduced to a very low level (about 25% of normal level) and this level declined further but at a very slow rate (only further 10 percent in next 30 minutes). And it was extremely difficult to deplete glycogen from taenia coli merely by removing external glucose. In fact, after one hour in glucose-free medium under aerobic condition, the glycogen level fell by between 10 and 40 percent and even after six hours it was possible to demonstrate the presence of significant amounts of glycogen. But the slow breakdown of glycogen under glucose-free conditions may be insufficient to maintain the link to the contractile processes. In this experiments, application of high K induced an increase in oxygen consumption accompanying tension development of the smooth muscle and removal of glucose from the medium gradually decreased both the developed tension and the increased oxygen consumption, though these changes were not completely reversible. These results show that the tonic contraction may be closely related to an increase in oxygen consumption of the muscle which utilizes glucose in medium and confirms the concept that the tonic contraction is an active process depending on aerobic carbohydrate breakdown (1, 2).

The muscle pretreated with ouabain and maintaining normal respiration, showed a phasic contraction accompanying a transient rise of oxygen consumption on the appli-
cation of high K. The muscle when under low oxygen supply or low temperature showed an extremely small oxygen consumption rate in the normal medium, and under these conditions high K induced a phasic contraction without changes in oxygen consumption. The data show that the phasic contraction does not depend on changes in muscle cell respiration and therefore supports the concept that the phasic contraction is a passive process 

Urakawa et al. (7) reported that Ca entering smooth muscle treated with high K probably increases oxygen consumption, independent of an increase in muscle tension. In the phasic contraction induced by high K sufficient Ca was thought to be released from a cellular site to initiate contraction and then to be rapidly rebound (1), and this proposal was supported by other experiments (8). Calcium released from a cellular site during the phasic contraction is assumed to increase transiently the oxygen consumption of muscles maintaining normal respiration, although no change in oxygen consumption occurs in the muscle under greatly depressed respiratory conditions.

It was proposed that in the tonic contraction induced by high K, enough Ca crossed the membrane to maintain contraction and that this Ca movement was dependent on carbohydrate breakdown (1, 2). The explanation of this phenomenon may be that Ca entering the muscle through the membrane depolarized by high K stimulates its metabolic process(es), resulting in an increased oxygen consumption, and the energy liberated by phosphate metabolism coupled with aerobic carbohydrate breakdown is probably utilized to maintain the process of Ca entry into the fiber which sustains contracture.

It is known that DNP increases the oxygen consumption of whole animals and of isolated tissues. According to Born and Bülbring (9), $1 \times 10^{-6}$ M DNP decreased tension and ATP content, but showed a slight increase in the rate of oxygen consumption in taenia coli. In the above experiments it was found that the increased oxygen consumption of the muscle treated with high K was further increased or maintained at the same level by adding $1 \times 10^{-6}$ M DNP while the already developed tension disappeared.

Schatzmann found that strophanthin did not affect oxygen consumption or lactic acid production but prevented the active movements of sodium and potassium in red cell (10). Later ouabain was reported to inhibit activity respirations when rat brain cortex slices (11, 12) and rabbit desheathed cervical vagus nerve (13) were electrically stimulated. Nissan et al. (14) have also reported that the addition of NaCl increased oxygen consumption in the isolated rat hemidiaphragm and ouabain ($1 \times 10^{-6}$ M) depressed this increased oxygen consumption. In rabbit gall bladder, ouabain ($1 \times 10^{-5}$ M) inhibited about half the activity oxygen consumption but not the basal oxygen consumption (15). In the present experiments, $2.5 \times 10^{-6}$ M ouabain did not alter the increased oxygen consumption of the smooth muscle induced by high K, but almost abolished the already developed tension.

The addition of DNP or ouabain to high K medium increased further or maintained the increased oxygen consumption although they abolished the developed tension. That is, DNP and ouabain dissociated the increased oxygen consumption from tension. In
the previous paper (7), the substitution of Sr for Ca in the medium elicited a rise in oxygen consumption without an accompanying rise in tension of the muscle treated with high K. From these data it may be said that tension development does not regulate oxygen consumption of the muscle in high K medium.

Application of ouabain did not effect the increased oxygen consumption of the muscle in the high K medium, however, removal of Ca from the medium reversibly reduced this increase in oxygen consumption to the level in normal medium. The data suggest the possibility of Ca playing an essential role in the increased oxygen consumption of the muscle treated with high K and ouabain. On the other hand, the activity respiration of the muscle treated with high K was further increased or maintained at the same level by DNP and this increase was not modified by Ca depletion. Yabu reported that when 10 mM DNP was applied to the Ca-deficient sartorius muscle of frog, the oxygen consumption, measured manometrically, increased as in the case of normal muscle (16). These results suggest that the change in oxygen consumption caused by DNP is probably induced by a different type of mechanism, and it awaits further investigation. A report on the relationship between Ca movement and oxygen consumption in high K medium containing ouabain or DNP is under preparation.

An increase in oxygen consumption accompanied by an increase in tension of the muscle by ouabain itself has not been particularly mentioned or discussed in this paper.

SUMMARY

1. High K (isotonic, 40 mM) induced a tension development accompanied by an increase in oxygen consumption of the smooth muscle of guinea pig taenia coli. Glucose removal from the high K medium reversibly abolished the already developed tension, and this corresponded with a decrease in the elevated oxygen consumption rate.

2. Application of high K on the muscle pretreated with ouabain (1 × 10⁻⁵M) induced a phasic contraction accompanied by a transient increase in oxygen consumption.

3. The muscle showed an extremely small oxygen consumption rate in the normal medium under low oxygen supply (5%) or low temperature (12°C), and high K induced a phasic contraction but an insignificant change in oxygen consumption.

4. Addition of DNP (1 × 10⁻⁴M) to high K medium further increased or maintained the increased oxygen consumption although it abolished the already developed tension. The change in oxygen consumption was not modified by calcium removal. On the other hand ouabain (2.5 × 10⁻⁶M) maintained the increased oxygen consumption in high K medium, but almost abolished the developed tension. Both changes were dependent on external calcium.

5. It may be concluded that tonic contraction induced by high K corresponds with an increase in oxygen consumption and phasic contraction does so only in case of muscles maintaining respiration at normal levels. This confirms the concept that the phasic response is a passive process, whereas the tonic response is an active one depending on metabolism. A relationship between Ca movement and oxygen consumption during high
K induced phasic and tonic contractions is discussed.

6. The data suggest the possibility of Ca playing an essential role in the increased oxygen consumption of the muscle treated with high K and ouabain, and that the change in oxygen consumption caused by DNP is probably induced by a different type of mechanism.

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