J. Physiol. (1984), **347**, *pp.* 149–159
With 5 text-figures
Printed in Great Britain

TENSION MAINTENANCE, CALCIUM CONTENT AND ENERGY PRODUCTION OF THE TAENIA OF THE GUINEA-PIG CAECUM UNDER HYPOXIA

BY YUKISATO ISHIDA*, KEIKO TAKAGI* AND NORIMOTO URAKAWA†

*From the * Department of Pharmacology, Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan and the † Department of Veterinary Pharmacology, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

(*Received 22 April 1983*)

SUMMARY

1. When potassium (45·4 mM) was applied to isolated taenia of guinea-pig caecum, the muscle developed a rapid phasic and sustained tonic tension during aerobic conditions bubbled with 95% O_2:5% CO_2. Under hypoxic conditions bubbled with 95% N_2:5% CO_2, the taenia lost its ability to respond to high potassium with sustained tonic contraction, although it still showed rapid phasic contraction.

2. Raising the glucose concentration from 5·5 to 55·5 mM in the presence of high potassium during hypoxia caused development of a sustained contraction which was 50% that of the muscle in aerobic conditions.

3. In the presence of high potassium, the ATP content of the taenia decreased in hypoxia, but increased with increasing glucose concentration.

4. When the taenia was exposed to hypoxic conditions, the amount of lactate released from the muscle increased. Raising the glucose concentration caused a further increase in lactate release in the presence of high potassium under hypoxia.

5. Good correlations ($\gamma > 0{\cdot}9$) were observed between tension development, the ATP content and lactate release of the taenia in the presence of high potassium under hypoxia when the glucose concentration was varied between 5·5 and 55·5 mM.

6. The total calcium content was increased by the presence of high potassium under aerobic conditions and the increase was abolished when the muscle was exposed to hypoxic conditions. Under hypoxia the total calcium content was not increased by raising the glucose concentration in the presence of high potassium.

7. The cellular calcium content of the taenia, determined by the lanthanum method, was increased in the presence of high potassium under aerobic and hypoxic conditions, but the content was smaller in hypoxic conditions than in aerobic conditions. Under hypoxic conditions, raising the glucose concentration in the presence of high potassium did not affect the cellular calcium content.

8. These results suggest that under hypoxic conditions the potassium-induced sustained contraction of the taenia is increased by raising the glucose concentration owing to increased ATP production through the glycolytic pathway, but not through mechanisms increasing the intracellular Ca concentration.

INTRODUCTION

Raising the concentration of potassium from 5·4 to 45·4 mM causes a rapid phasic and sustained tonic contraction of the taenia of guinea-pig caecum during aerobic conditions when substrate (glucose) is present (Chujo & Holland, 1963; Urakawa & Holland, 1964). This potassium-induced contraction is accompanied by an increase in the rate of oxygen consumption of the taenia (Saito, Sakai, Ikeda & Urakawa, 1968; Karaki, Suzuki, Urakawa, Ishida & Shibata, 1982). The contractile responses to histamine and acetylcholine are also accompanied by increase in oxygen consumption (Bülbring, 1953; Saito, Sakai, Ikeda & Urakawa, 1971). These results suggest that the contractile response of the taenia is dependent on oxidative energy metabolism.

Conditions suppressing metabolism, such as hypoxia, the presence of dinitrophenol (DNP) or glucose depletion, have been reported to cause greater inhibition of the sustained tonic component than of the phasic component of potassium-induced contraction of the taenia (Born & Bülbring, 1955; Urakawa & Holland, 1964; Pfaffman, Urakawa & Holland, 1965; Ganeshanandan, Karaki, Ikeda & Urakawa, 1969). Earlier, West, Hadden & Farah (1951) reported that during hypoxic conditions the intestine developed a phasic contraction without the sustained tonic component when exposed to agonists. More recently, carboxylic ionophores, such as X537A and monensin, were shown to cause selective inhibition of the tonic contractile response to high potassium, apparently by suppressing oxidative energy metabolism in the taenia (Ishida & Shibata, 1982; Kishimoto, Ozaki, Karaki, Urakawa & Ishida, 1982). Therefore, the large sustained tonic contraction of the taenia seems to depend on aerobic breakdown of carbohydrates to carbon dioxide and water.

Recently, Nasu, Yui, Nakagawa & Ishida (1982) reported that in hypoxic conditions the sustained tonic contractile response to high potassium was reduced to one-tenth of that during aerobic conditions and that an increase in glucose concentration during hypoxia allowed development of a significant contractile response to high potassium that was accompanied by an increase in lactate release from the taenia, suggesting that the tension is maintained by anaerobic glycolytic activity.

On the other hand, strong metabolic inhibition, such as by a combination of glucose depletion and hypoxia (Bauer, Goodford & Hüter, 1965) or treatment with DNP plus iodoacetic acid (Casteels & van Breemen, 1975) has been reported to increase calcium uptake by the taenia. However, by determination of the ATP content of the taenia, these conditions were shown to cause more damage to the muscle than a single treatment with hypoxia or glucose depletion (Bose & Bose, 1975; Knull & Bose, 1975). van Breemen, Hwang & Siegel (1977) also reported that complete metabolic inhibition caused membrane leakiness.

In the present experiments we examined whether the augmentation by glucose of high potassium-induced tension of the taenia during hypoxia was due to increased energy production or activation of mechanisms increasing the intracellular free calcium concentration in the taenia. For this we measured the ATP content, lactate release and total and cellular calcium contents and the tension of the muscle under various conditions.

METHODS

Contractile responses

Male guinea-pigs weighing 300–400 g were stunned and bled. The taenia was dissected from the caecum and cut into strips about 15 mm long. Strips were suspended in a tissue bath containing 20 ml physiological salt solution of the following composition (mM): NaCl, 136·8; KCl, 5·4; $CaCl_2$, 2·5; $MgCl_2$, 1·0; $NaHCO_3$, 11·9 and glucose, 5·5. The solution was maintained at 37 °C and bubbled with 95 % O_2 : 5 % CO_2 for aerobic conditions or 95 % N_2 : 5 % CO_2 for hypoxic conditions. The pH of the solution was 7·2. High-potassium (45·4 mM) solution was made by adding an appropriate amount of 2 M-KCl solution hyperosmotically.

Contractile responses of the muscle were recorded isometrically with a force-displacement transducer (Toyo-Baldwin T-7-30, Japan) and displayed on a polygraph (Nihon Kohden RM-6000, Japan). The muscle was equilibrated in physiological salt solution for more than 60 min before each experiment.

ATP content

The amount of adenosine triphosphate (ATP) in the muscle was determined as reported previously (Ishida & Shibata, 1982) by a modification of the method of Strehler & McElroy (1957). Muscles were weighed (about 5 mg) on an electronic balance (Sauter AR-1014, West Germany) and incubated in test solutions for the desired period after more than 60 min equilibration in normal solution. Then ATP was extracted by boiling the muscle in 3 ml water for 5 min. The muscle was then promptly cooled to 0 °C. The amount of ATP in the extract was determined using a photon counter (Lumac Biocounter M2010, the Netherlands) with purified luciferin–luciferase reagent (Lumit) supplied by Lumac. The quantity of ATP in the muscle was calculated and expressed in mmol/kg wet weight.

Assay of lactate release

The amount of lactate released from the muscle was determined by a combination of reactions of lactate dehydrogenase (LDH) and the bacterial luciferase system. Muscles (about 10 mg) were incubated in 2 ml test solutions for 60 min after equilibration in the normal solution for at least 60 min. Then 0·2 ml of the solution was treated with LDH to obtain NADH as described in the manual of Boehringer Mannheim (West Germany). The amount of NADH was determined by measuring the light emitted by the coupled reaction of NADH : FMN oxidoreductase and bacterial (*Vibrio*) luciferase using a photon counter. After the treatment, a 20 μl sample of the solution containing LDH was diluted 20 times with 150 mM-HEPES (final pH of the solution, about 7) and 200 μl of the diluted solution was introduced into the dark chamber of the apparatus (Lumac Biocounter 2010). 100 μl of a mixture of NADH : FMN oxidoreductase, luciferase and myristic aldehyde (all from Lumac) was injected and the emitted light was integrated for 30 s. The amount of lactate was calculated and expressed in mmol/h . kg wet weight.

Calcium content

The calcium content of the muscle was determined as reported previously (Ishida & Shibata, 1982). Muscles (about 10 mg) were equilibrated for at least 60 min, incubated in test solutions for another 60 min, and then blotted and ashed with 0·1 ml 60 % perchloric acid in a quartz tube at 500 °C for 8 h. The ashed samples were dissolved in 2 ml solution containing 0·4 % EDTA and 0·2 % $SrCl_2$. Then the amount of calcium was determined using an atomic absorption spectrophotometer (Varian AA-175, Australia), and the calcium content was expressed in mmol/kg wet weight.

Cellular calcium content

The cellular calcium content of the muscle was determined by the modified lanthanum method described by van Breemen, Farinas, Gerba & McNaughton (1972). Strips of the taenia were incubated in test conditions with ^{45}Ca (1 μCi/ml) for 60 min and then exposed to 10 mM-lanthanum solution without calcium for 45 min. The muscles were then blotted, placed in scintillation vials and solubilized overnight at 50 °C with 0·2 ml solubilizer (Soluene 350, Packard, U.S.A.). Radioactivity was counted in a liquid scintillation spectrophotometer (Packard, Tri-Carb 3380).

The physiological salt solution and lanthanum solution employed were buffered with 24 mM-Tris HCl, pH 7·2 at 37 °C, instead of with $NaHCO_3$, and were bubbled with O_2 or N_2 without CO_2. The contractile response in the Tris-buffered solution was the same as that in HCO_3/CO_2 buffered solution.

RESULTS

When the taenia of the guinea-pig was exposed to a high concentration (45·4 mM) of potassium in aerobic conditions it developed a rapid phasic and a sustained tonic contraction (Fig. 1). The phasic component showed a tension of $10·4 \pm 1·18$ g ($n = 8$) and the tonic component at 20 min after the application of high potassium showed a tension of $10·7 \pm 1·26$ g ($n = 8$). During hypoxic conditions bubbled with 95% N_2:5% CO_2 instead of 95% O_2:5% CO_2, exposure to high potassium caused a transient

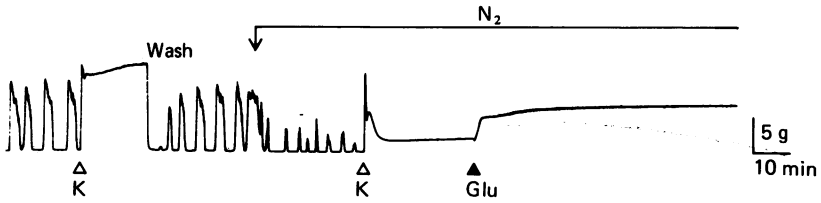

Fig. 1. Contractile response to potassium (45·4 mM) during aerobic and hypoxic conditions and the effect of glucose (50 mM) on the response in the taenia of the guinea-pig caecum. Potassium (K) was applied at △. Glucose (Glu) was applied at ▲. Hypoxic conditions (N_2) were made by bubbling the solution with 95% N_2:5% CO_2; aerobic conditions, 95% O_2:5% CO_2.

phasic contraction of about normal size ($8·3 \pm 0·76$ g, $n = 8$) followed by a small sustained contraction of $1·5 \pm 0·13$ g ($n = 8$) (Fig. 1). It seems that hypoxic conditions cause a more selective inhibition of the tonic than phasic contraction of the taenia. Fig. 1 also shows that when a high concentration (50 mM, total 55·5 mM) of glucose was applied in the presence of high potassium under hypoxic conditions, the taenia developed a significant sustained contraction of $5·3 \pm 0·45$ g ($n = 8$). Exposure of the muscle to sorbitol (50 mM) in the presence of high potassium under hypoxia did not cause sustained contraction and the muscle had a tension of $1·6 \pm 0·09$ g ($n = 4$) 60 min after application of sorbitol.

When the taenia, already contracted by potassium (45·4 mM), was exposed to hypoxic conditions, its tension decreased (Fig. 2*A*). Fig. 2*B* shows the time course of tension change in four tissues. During aerobic conditions, tension developed by high potassium decreased only slightly during 90 min. Exposure to hypoxic conditions caused rapid decrease in the tension and after 10 min the contractile response to high potassium became steady at about 10% of the maximum response in aerobic conditions.

Fig. 2*B* also shows the time course of change in the ATP content of the muscle ($n = 6$). In aerobic conditions, the ATP content decreased only slightly in the presence of potassium (45·4 mM). When the muscle was exposed to hypoxic conditions in the presence of high potassium, its ATP content gradually decreased and after

30 min it became steady at about half that in the aerobic conditions, as reported by Karaki, Suzuki, Ozaki, Urakawa & Ishida (1982). A difference was observed between the time courses of change in tension and ATP content during the first 30 min of hypoxia. Therefore, in the following experiments, the parameters of tension, ATP content, lactate release and total and cellular calcium contents were determined 60 min after the application of test conditions, when steady-state levels of these parameters had presumably been reached.

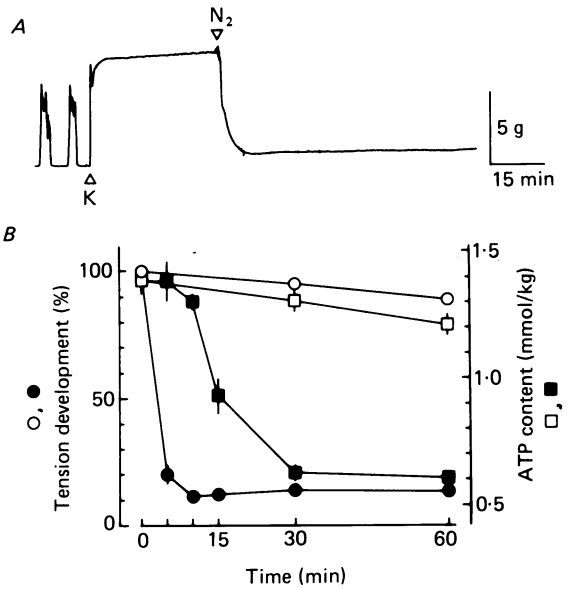

Fig. 2. Effect of hypoxic conditions (N_2) on the potassium (K)-induced sustained tonic contraction and ATP content of the taenia. *A*, a typical contractile response. *B*, the statistical results: ○, contractile response under aerobic conditions (O_2) ($n = 4$); ●, contractile response under hypoxic conditions (N_2) ($n = 4$); □, ATP content under aerobic conditions ($n = 6$); ■, ATP content under hypoxic conditions ($n = 6$). Muscles were pre-treated with potassium (45·4 mM) for 30 min in aerobic conditions and then at time 0 min subjected to hypoxic conditions as shown in *A*.

Raising the glucose concentration in the presence of potassium (45·4 mM) in hypoxic conditions caused a concentration-dependent increase in tension of the taenia (Table 1). The maximum tension was obtained with 16·5 mM-glucose, and was about 35 % of the tension developed with 5·5 mM-glucose in the presence of high potassium in aerobic conditions. The tension developed (t, %) was linearly correlated with the glucose concentration (x, mM) between 5·5 and 16·5 mM, giving the equation: $t = 1{\cdot}80x + 1{\cdot}234$ (correlation coefficient, $\gamma = 0{\cdot}968$).

Table 1 also shows that the ATP content of the muscle increased with increase in glucose concentration in the presence of potassium (45·4 mM) during hypoxia. The maximum value was obtained with 16·5 mM-glucose. The ATP content (a, mmol/kg wet weight) was also linearly correlated with the glucose concentration (x, mM) between 5·5 and 16·5 mM: $a = 0{\cdot}032x + 0{\cdot}400$ ($\gamma = 0{\cdot}992$).

The amount of lactate released from the taenia was also determined (Table 1). At 5·5 mM-glucose in the presence of high potassium the taenia released a small amount of lactate (11·2±0·8 mmol/h.kg wet weight, $n = 6$) during aerobic conditions, but during hypoxia the release of lactate increased greatly to 72·8±10·3 mmol/h.kg

TABLE 1. Effect of glucose concentration on the tension, ATP content and lactate formation of the taenia in the presence of potassium (45·4 mM) under hypoxia. Muscles were incubated in test conditions for 60 min and then tension, ATP content and lactate release were measured. Tension is expressed as a percentage of the response to potassium under aerobic conditions. Values are means ±S.E. of means; $n = 4$ for tension, $n = 6$ for ATP content and lactate. n.d., not determined

Conditions	Glucose (mM)	Tension (%)	ATP content (mmol/kg wet wt.)	Released lactate (mmol/h.kg wet wt.)
Aerobic	5·5	100	1·34±0·03	11·2±0·8
Hypoxic	5·5	12·7±0·75	0·57±0·03	72·8±10·3
Hypoxic	11·0	18·1±1·79	0·75±0·02	145·3±12·2
Hypoxic	13·8	25·7±1·53	0·87±0·01	n.d.
Hypoxic	16·5	32·7±4·27	0·91±0·05	208·2±14·5
Hypoxic	30·5	34·0±3·29	1·04±0·06	188·2±8·3
Hypoxic	55·5	33·3±4·70	1·05±0·04	219·5±11·5

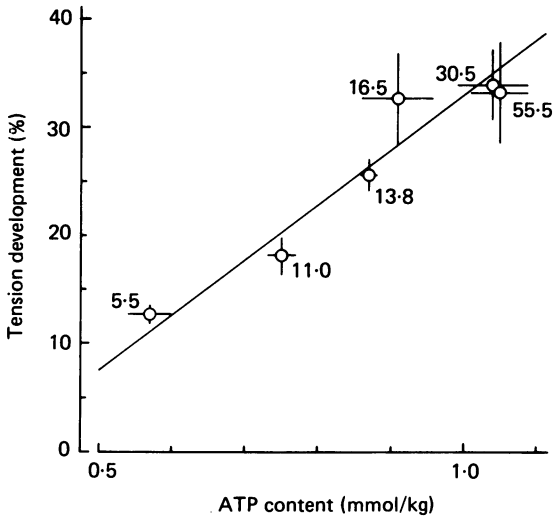

Fig. 3. Correlation between ATP content and tension development of the taenia in the presence of potassium (45·4 mM) under hypoxic conditions. The glucose concentration was changed between 5·5 and 55·5 mM as indicated by numbers in the Figure. Values are taken from Table 1. The unweighed regression line drawn from the mean values fits $t = 472a - 14·7$ ($\gamma = 0·962$), where t is the tension developed, a the ATP content and γ the correlation coefficient. Sustained tension developed by potassium under aerobic conditions was served as 100% contraction.

($n = 6$), indicating a marked Pasteur effect. Raising the glucose concentration in the presence of high potassium during hypoxia also increased lactate release and a maximum release of about 200 mmol/h.kg was obtained at 16·5 mM-glucose. The lactate release (l, mmol/h.kg) was also linearly correlated with the glucose concentration (x, mM) between 5·5 and 16·5 mM: $l = 12·3x + 6·70$ ($\gamma = 0·999$).

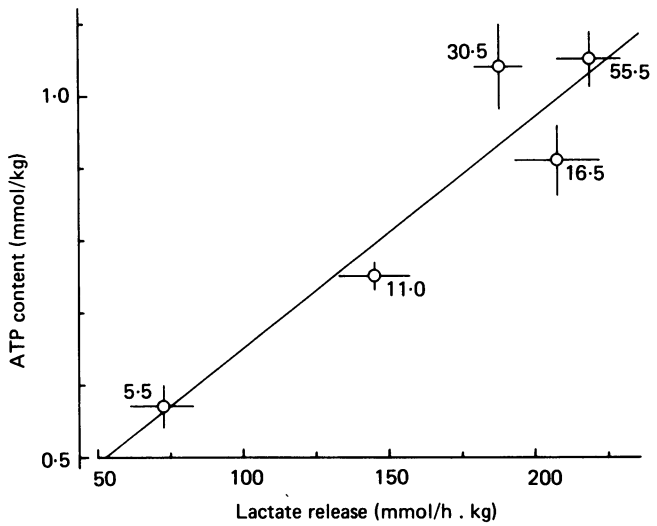

Fig. 4. Correlation between amount of lactate released from the taenia and the ATP content in the presence of potassium (45·4 mM) under hypoxic conditions. Values were taken from Table 1. Numbers in the Figure indicate glucose concentrations (5·5–55·5 mM). The regression line fits $a = 0{\cdot}0032l + 0{\cdot}331$ ($\gamma = 0{\cdot}934$), where a is the ATP content and l the amount of lactate released.

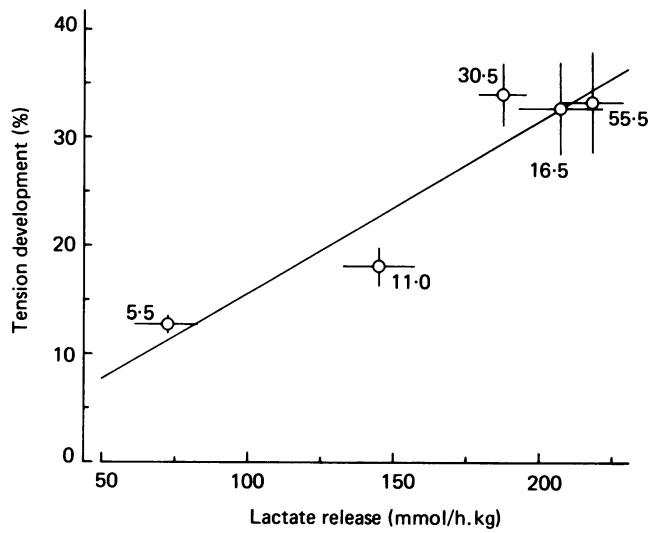

Fig. 5. Correlation between amount of lactate released from the taenia and tension development in the presence of potassium (45·4 mM) under hypoxia. Values were taken from Table 1. Numbers in the Figure indicate glucose concentrations (5·5–55·5 mM). The regression line fits $t = 0{\cdot}158l - 0{\cdot}221$ ($\gamma = 0{\cdot}942$), where t is the tension development and l the amount of lactate released.

When the glucose concentration was altered between 5·5 and 55·5 mM, good correlations were also found between tension development, the ATP content and lactate release of the taenia in the presence of high potassium under hypoxia. Fig. 3 shows the relation between tension development (t) and the ATP content (a) of the muscle: $t = 47{\cdot}2a - 14{\cdot}7$ ($\gamma = 0{\cdot}962$). The relationship between ATP content (a) and lactate release (l) was $a = 0{\cdot}0032l + 0{\cdot}331$ ($\gamma = 0{\cdot}934$) (Fig. 4) and that between tension (t) and lactate release (l) was $t = 0{\cdot}158l - 0{\cdot}221$ ($\gamma = 0{\cdot}942$) (Fig. 5).

TABLE 2. Effects of excess potassium and hypoxia on the total calcium content of the taenia at low (5·5 mM) and high (55·5 mM) glucose concentrations. Muscles were incubated in test conditions for 60 min. Values are means ±S.E. of means ($n = 6$). An asterisk indicates a significant difference from the other value ($P < 0{\cdot}05$)

Glucose (mM)	Potassium (mM)	Total calcium content (mmol/kg wet wt.)	
		Aerobic	Hypoxic
5·5	5·4	2·23 ±0·07	2·21±0·19
5·5	45·4	2·53*±0·06	2·19±0·06
55·5	5·4	n.d.	2·14±0·05
55·5	45·4	n.d.	2·27±0·10

TABLE 3. Effect of glucose concentration, excess potassium and hypoxia on the cellular calcium content. Muscles were incubated in test conditions for 60 min. Cellular calcium was detetermined by the lanthanum method using ^{45}Ca. Values are means ±S.E. of means ($n = 8$). An asterisk indicates that the value is significantly different from that in normal solution ($P < 0{\cdot}05$)

Glucose (mM)	Potassium (mM)	Cellular calcium content (μmol/kg wet wt.)	
		Aerobic	Hypoxic
5·5	5·4	98·0±4·45	69·9 ±1·58
5·5	45·4	178·6*±13·44	130·7*±8·69
30·5	45·4	n.d.	130·8*±7·10
55·5	45·4	n.d.	136·8*±9·04

Table 2 shows the effects of potassium (45·4 mM) and hypoxic conditions on the total calcium content of the taenia at low (5·5 mM) and high (55·5 mM) concentrations of glucose. In aerobic conditions with 5·5 mM-glucose, the muscle contained 2·23±0·07 mmol calcium/kg wet weight ($n = 6$) in normal solution. Application of high potassium in aerobic conditions with 5·5 mM-glucose caused a significant ($P < 0{\cdot}05$) increase in the total calcium content to 2·53±0·06 mmol/kg ($n = 6$). But during hypoxic conditions, the total calcium content was not affected by the presence of high potassium at low (5·5 mM) or high (55·5 mM) glucose concentration, being the same as that in aerobic conditions in normal solution (Table 2). This suggests that the total calcium content of the taenia is increased by the presence of high potassium only in aerobic conditions.

The cellular calcium content of the taenia was measured by the lanthanum method (van Breemen *et al.* 1972). Table 3 shows the effects of potassium (45·4 mM) and hypoxic conditions on the cellular calcium content of the taenia on raising the glucose

concentration during hypoxia. At 5·5 mM-glucose, the cellular calcium content of the muscle was 98·0 ± 4·45 μmol/kg wet weight ($n = 8$) in aerobic and 69·9 ± 1·58 μmol/kg ($n = 8$) in hypoxic conditions. When high potassium was applied, the cellular contents were increased to 178·6 ± 13·44 μmol/kg ($n = 8$) in aerobic and 130·7 ± 8·69 μmol/kg ($n = 8$) in hypoxic conditions. When the glucose concentration was raised to 30·5 and 55·5 mM in the presence of high potassium under hypoxia, the cellular content remained the same as that at 5·5 mM-glucose, being about 130 μmol/kg. Thus hypoxic conditions reduced the cellular calcium content of the taenia both in the absence and presence of high potassium, although the reduction was greater in the presence of high potassium than in normal solution: the reduction by hypoxia was about 50 μmol/kg in the presence of high potassium and 30 μmol/kg in normal solution. Table 3 also suggests that the cellular calcium content in the presence of high potassium was not affected by raising the glucose concentration during hypoxia.

DISCUSSION

When the taenia was exposed to potassium (45·4 mM) in aerobic conditions, it showed a rapid phasic contraction and then a sustained tonic contraction. Introduction of hypoxic conditions caused selective inhibition of the tonic contractile response to high potassium. The present experiments showed that in hypoxic conditions, increase in the glucose concentration in the presence of high potassium caused a significant increase in the contractile response.

In aerobic conditions, potassium is known to cause contraction by increasing calcium influx through the plasma membrane (Urakawa & Holland, 1964; Bolton, 1979). Therefore, inhibition by hypoxia of the tonic contractile response to high potassium might be caused by alteration of calcium metabolism resulting in decrease in free intracellular calcium, or inhibition of energy production of the muscle, or both these mechanisms.

Hypoxia or glucose depletion and application of DNP have been reported to inhibit the potassium-induced increase in the total calcium content (Urakawa & Holland, 1964; Urakawa, Karaki & Ikeda, 1970) and in the lanthanum-resistant cellular calcium content of the taenia determined using cold lanthanum solution for rinsing the muscle (Karaki, Suzuki, Ozaki, Urakawa & Ishida, 1982). These findings imply that inhibition of the contractile response is attributable to interference with calcium metabolism. However, the total calcium (2 mmol/kg wet weight) and cellular calcium (500 μmol/kg) measured in cold solution are much larger than the expected cellular content (100 μmol/kg) described by van Breemen *et al.* (1977). Since this might interfere with detection of small changes in cellular calcium content, we therefore employed the original lanthanum method using warm lanthanum solution here. By this method, the cellular content of calcium of resting muscle was determined to be about 100 μmol/kg.

In the present experiments both the total and cellular calcium content of the taenia in the presence of high potassium were decreased by hypoxic conditions. The cellular calcium content in the absence of high potassium was also decreased by hypoxia. The difference between the values in the presence and absence of high potassium was about 80 μmol/kg wet weight in aerobic conditions and about 60 μmol/kg in hypoxia,

indicating that the cellular calcium content was increased to almost the same degree in aerobic and hypoxic conditions when the muscle was exposed to high potassium. Recently, Nasu *et al.* (1982) reported that when the taenia was exposed to hypoxia but not to verapamil (a calcium antagonist), increase in the external calcium concentration in the presence of high potassium did not induce significant tension development. Furthermore, the ability of verapamil to cause relaxation of the taenia was reported to be the same in hypoxic and aerobic conditions (Kishimoto *et al.* 1982). These results suggest that the calcium available for tension development is maximally or constantly activated by the presence of high potassium in both aerobic and hypoxic conditions. Thus it is unlikely that reduction of tension in the presence of high potassium induced by hypoxic conditions results from a decrease in calcium influx through the plasma membrane of the muscle.

The ATP content of the taenia in the presence of high potassium was markedly decreased by hypoxic conditions, although hypoxia increased lactate release from the muscle. Thus a large amount of ATP of the taenia seems to be derived from oxidative energy metabolism in aerobic conditions, and inhibition of the contractile response to high potassium in hypoxic conditions is presumably due to suppression of oxidative energy production.

Our experiments showed that increase in the glucose concentration under hypoxic conditions did not increase either the total or cellular calcium content in the presence of high potassium but significantly increased the tension of the taenia. Concomitantly with this increase in muscle tension, the ATP content and lactate release from the taenia also increased in parallel. Therefore, under hypoxia maintenance of tension in the presence of high potassium seems to depend on anaerobic glycolytic activity of the taenia, and the increase in tension induced by glucose is probably due to increased ATP production through the glycolytic pathway. High potassium solution probably initiates the same increase in calcium influx in aerobic and hypoxic conditions, even when the glucose concentration is raised under hypoxia.

The authors wish to thank Mrs Yoshiko Murakami and Mrs Misayo Nakai for typing the manuscript.

REFERENCES

BAUER, H., GOODFORD, P. J. & HÜTER, J. (1965). The calcium content and ^{45}calcium uptake of the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **176**, 163–179.

BOLTON, T. B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.* **59**, 606–718.

BORN, G. V. R. & BÜLBRING, E. (1955). The effect of 2:4-dinitrophenol (DNP) on the smooth muscle of the guinea-pig's taenia coli. *J. Physiol.* **127**, 626–635.

BOSE, D. & BOSE, R. (1975). Mechanics of guinea pig taenia coli smooth muscle during anoxia and rigor. *Am. J. Physiol.* **229**, 324–328.

BÜLBRING, E. (1953). Measurements of oxygen consumption in smooth muscle. *J. Physiol.* **122**, 111–134.

CASTEELS, R. & VAN BREEMEN, C. (1975). Active and passive Ca^{2+} fluxes across cell membranes of the guinea-pig taenia coli. *Pflügers Arch.* **359**, 197–207.

CHUJO, N. & HOLLAND, W. C. (1963). K-induced contracture and Ca exchange in the guinea-pig's taenia coli. *Am. J. Physiol.* **205**, 94–100.

GANESHANANDAN, S. S., KARAKI, H., IKEDA, M. & URAKAWA, N. (1969). Mechanical response of guinea-pig taenia coli in high-K/Na-deficient medium under anoxia. *Jap. J. Pharmac.* **19**, 329–330.

ISHIDA, Y. & SHIBATA, S. (1982). Relaxing and metabolic inhibitory action of X537A (Lasalocid) on the taenia of the guinea-pig caecum. *J. Physiol.* **333**, 293–304.

KARAKI, H., SUZUKI, T., OZAKI, H., URAKAWA, N. & ISHIDA, Y. (1982). Dissociation of K^+-induced tension and cellular Ca^{2+} retention in vascular and intestinal smooth muscle in normoxia and hypoxia. *Pflügers Arch.* **394**, 118–123.

KARAKI, H., SUZUKI, T., URAKAWA, N., ISHIDA, Y. & SHIBATA, S. (1982). High K^+, Na^+-deficient solution inhibits tension, O_2 consumption, and ATP synthesis in smooth muscle. *Jap. J. Pharmac.* **32**, 727–733.

KISHIMOTO, T., OZAKI, H., KARAKI, H., URAKAWA, N. & ISHIDA, Y. (1982). The inhibitory effect of monensin on high-K-induced contraction in guinea-pig taenia coli. *Eur. J. Pharmacol.* **84**, 25–32.

KNULL, H. R. & BOSE, D. (1975). Reversibility of mechanical and biochemical changes in smooth muscle due to anoxia and substrate depletion. *Am. J. Physiol.* **229**, 329–333.

NASU, T., YUI, K., NAKAGAWA, H. & ISHIDA, Y. (1982). Role of glycolysis in the tension development under anoxia in guinea pig taenia coli. *Jap. J. Pharmac.* **32**, 65–71.

PFAFFMAN, M., URAKAWA, N. & HOLLAND, W. C. (1965). Role of metabolism in K-induced tension changes in guinea pig taenia coli. *Am. J. Physiol.* **208**, 1203–1205.

SAITO, Y., SAKAI, Y., IKEDA, M. & URAKAWA, N. (1968). Oxygen consumption during potassium induced contracture in guinea pig taenia coli. *Jap. J. Pharmac.* **18**, 321–331.

SAITO, Y., SAKAI, Y., IKEDA, M. & URAKAWA, N. (1971). Effect of histamine on oxygen consumption in guinea pig taenia coli. *Jap. J. Pharmac.* **21**, 605–611.

STREHLER, B. L. & MCELROY, W. D. (1957). Assay of adenosine triphosphate. In *Methods in Enzymology*, vol. 3, ed. COLOWICK, S. P. & KAPLAN, N. O., pp. 871–873. New York: Academic Press.

URAKAWA, N. & HOLLAND, W. C. (1964). Ca^{45} uptake and tissue calcium in K-induced phasic and tonic contraction in taenia coli. *Am J. Physiol.* **207**, 873–876.

URAKAWA, N., KARAKI, H. & IKEDA, M. (1970). Effects of ouabain and metabolic inhibiting factors on Ca distribution during K-induced contracture in guinea pig taenia coli. *Jap. J. Pharmac.* **20**, 360–366.

VAN BREEMEN, C., FARINAS, B. R., GERBA, P. & MCNAUGHTON, E. D. (1972). Excitation–contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. *Circulation Res.* **30**, 44–54.

VAN BREEMEN, C., HWANG, O. & SIEGEL, B. (1977). The lanthanum method. In *Excitation–Contraction Coupling in Smooth Muscle*, ed. CASTEELS, R., GODFRAIND, T. & RÜEGG, J. C., pp. 243–252. Amsterdam: Elsevier/North-Holland.

WEST, T. C., HADDEN, G. & FARAH, A. (1951). Effect of anoxia on response of the isolated intestine to various drugs and enzyme inhibitors. *Am. J. Physiol.* **164**, 565–572.