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AEROBIC AND ANAEROBIC METABOLISM IN SMOOTH MUSCLE CELLS OF TAENIA COLI IN RELATION TO ACTIVE ION TRANSPORT

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SUMMARY

1. The O_2 consumption and lactic acid production of the guinea-pig's taenia coli have been studied in relation to the active Na-K transport, in order to estimate the ratio: active Na extrusion/active K uptake/ATP hydrolysis.

2. By applying different procedures of partial metabolic inhibition, it was found that a reactivation of the active Na-K transport in K-depleted tissues could occur in an anaerobic medium, provided glucose was present and in an aerobic medium free of added metabolizable substrate. The active Na-K transport was rapidly blocked in an anaerobic-substrate free medium.

3. Readmission of K to K-depleted tissues under aerobic conditions stimulates both O_2 consumption and lactic acid production. While the O_2 consumption creeps up slowly and requires 50 min to reach control values, the aerobic lactic acid production increases to a maximum within 10 min and decreases again during the next 50 min to its steady-state value.

4. A reactivation of the Na-pump in K-depleted cells in a N_2-glucose medium causes an immediate increase of the lactic acid production, which decreases to its control value after 60 min.

The maximal increase in anaerobic lactic acid production during reactivation of the Na-K pump is a function of $[K]_o$. The system can be described with first order kinetics having a $V_{max} = 0{\cdot}72$ μmole.g^{-1} f.wt. min^{-1} and a $K_m = 1{\cdot}1$ mM.

5. By varying the glucose concentration or $[K]_o$ during reactivation of the Na-K pump, different Na-K pumping rates can be obtained. The ratios net Na extrusion/ATP or net K accumulation/ATP amount to $-1{\cdot}32 \pm 0{\cdot}19$ (36) and $1{\cdot}02 \pm 0{\cdot}11$ (36), in the experiments with different glucose concentrations. Taking into account the interference by net

passive fluxes, one can estimate a ratio: active Na transport/active K transport/ATP, of 1·7/0·8/1. This ratio is not very different from the values observed in other tissues.

INTRODUCTION

The first question of importance about the relationship between active Na-K transport and metabolism in taenia coli of the guinea-pig is whether the ATP that drives this transport comes mainly from oxidative phosphorylation or from anaerobic glycolysis. Secondly, we have to investigate the influence of the pumping rate on the metabolism and to try and assess the energy consumption of the pump. Both aspects can be studied using K-depleted tissues (Casteels, Droogmans & Hendrickx, 1971*a*). Such cells are devoid of all active Na-K exchange and they can consequently be considered as a control condition to estimate the additional energy consumption required by active Na-K transport. Readmitting K to such tissues initiates the active Na extrusion at a rate which is initially some 3 times higher than the rate in normal Krebs solution.

Several experimental procedures can be used to study the energy requirements of active Na-K transport. The most direct approach consists in comparing the ATP turnover measured with radioactive tracers during active ion transport and during experimental conditions without ion transport. This procedure depends on the use of isotopes such as $^{32}PO_4$ or $H_2{}^{18}O$. However, the rapid turnover of ATP, the low permeability of cell membranes for PO_4 and the lack of knowledge on the exchange reactions between $H_2{}^{18}O$ and ATP make this method difficult to use and decrease appreciably its reliability (Mommaerts, 1969). Another possibility is to inhibit the reactions responsible for the rephosphorylation of ADP and to follow the decrease of the ATP content in tissues under different experimental conditions (Dydynska & Harris, 1966). This method too has certainly in smooth muscle some severe drawbacks. Because of the rather high energy consumption in taenia coli cells, inhibition of the metabolism will lead to such a rapid decrease of the ATP levels that a further active Na-K exchange becomes impossible. Moreover prolonged metabolic depletion leads to the development of an unspecific leakiness of the cell membrane (Casteels, van Breemen & Wuytack, 1972). We have therefore tried to determine the ATP consumption of ion transport in an indirect way by measuring the O_2 consumption or the anaerobic lactic acid production under different experimental conditions. These experiments have also revealed some interesting characteristics of the oxidative phosphorylation and the anaerobic glycolytic phosphorylation in smooth muscle cells. Some of the results have already been communicated in a preliminary form (Wuytack & Casteels, 1972; Wuytack, Raeymaekers & Casteels, 1973; Casteels, Raeymaekers & Wuytack, 1973).

METHODS

Taenia coli strips were cut from the caecum of male guinea-pigs weighing 300–400 g. They were mounted isometrically on Teflon rods and transferred to a Krebs solution containing (mM): Na^+, 137; K^+, 5·9; Mg^{2+}, 1·2; Ca^{2+}, 2·5; Cl^-, 134; $H_2PO_4^-$, 1·2; HCO_3^-, 15·5 and glucose, 11·5. This solution was bubbled with a gas mixture of 95% O_2 and 5% CO_2 and had a pH of 7·35 at 35° C.

Solutions. K-free solution was prepared by replacing KCl by an equivalent amount of NaCl. Na-deficient solutions were prepared by substituting sucrose, LiCl, choline chloride or Tris chloride for NaCl. If choline chloride was used, atropine sulphate 10^{-5} g/ml. was added.

Muscle extracts. Tissues weighing between 10 and 30 mg were quickly removed from the solution and plunged in isopentane cooled with liquid N_2. They were ground in a stainless-steel cartridge by means of a ball mixer during three periods of 15 sec, interrupted by immersion in liquid N_2 for cooling. 1 ml. ice cold 0·5 N-$HClO_4$ was then added to the tissue powder and the content of the cartridge allowed to thaw during mixing. The fluid was decanted into a polypropylene tube. The cartridge was rinsed with 0·5 ml. $HClO_4$ solution and this liquid added to the tube. The tubes were centrifuged for 20 min at 40,000 *g* in a refrigerated centrifuge (Sorvall RC 2B) at 0° C. One ml. of the supernatant was neutralized with 2·5 N-K_2CO_3 containing 0·5 mM-EGTA. The precipitated $KClO_4$ was removed by centrifugation and the supernatant was stored at −20° C for analysis the next day.

Nucleotide, creatine and lactic acid measurements. The nucleotides and lactic acid were determined by an enzymic fluorometric technique using the Farrand Ratio Fluorometer. ATP was measured according to the technique of Greengard (1965), ADP and AMP according to Jaworek, Gruber & Bergmeyer (1970) and lactic acid with a method described by Passonneau (1970). The total creatine and the phosphorylcreatine were determined as described by Ennor & Rosenberg (1952).

O_2 consumption. The O_2 consumption was measured in a continuous perfusion system using a Clark type electrode (Yellow Springs Instruments Model 5331) connected to the Biological Oxygen Monitor. Warmed and oxygenated Krebs solution was pumped at a constant rate of 6·01 ml./hr by means of a syringe pump (Braun perfusor) around a large piece of tissue (50–100 mg) and then past the electrode. The oxygen tension in the perfusate and the mechanical activity developed by the tissue were recorded simultaneously.

Lactic acid production. The lactic acid production by the tissue was measured by determining its concentration in Krebs solution that was pumped past the tissues (40–60 mg) at a constant rate of 1–2 ml./min by means of a peristaltic pump (Harvard apparatus model 1201). Fractions of this superfusate were collected at 1 min interval with a fraction collector (LKB Ultrorac-7000). An alternative method consisted in transferring 10–20 mg tissues which were mounted isometrically on Teflon rods, through a series of test tubes at 5, 10 or 15 min interval. The tubes were kept at constant temperature (35° C) and bubbled with the appropriate gas mixture. In order to make these solutions anaerobic, they were already equilibrated with N_2 before transferring the tissues.

Ion concentrations, extracellular space, dry wt./wet wt. ratio and Na fluxes were estimated as described by Casteels (1969).

RESULTS

The effect of metabolic inhibition on the active Na-K transport

We have first investigated whether in taenia coli, as in most other tissues, oxidative phosphorylation is essential for the active Na-K transport, or whether this active transport can also proceed normally under anaerobic conditions. The reactivation of the active Na-K exchange obtained by readmission of 5·9 mM-$[K]_o$ to K-depleted cells was therefore studied during different types of partial metabolic inhibition. It was found in preliminary experiments that K depletion did not modify the tissue content of ATP, ADP or AMP, but that it decreases the phosphorylcreatine content from $3·25 \pm 0·30$ (15) $2·01 \pm 0·26$ (14) μmole.g^{-1} f. wt.

Total ion content, dry wt./wet wt. ratio and [^{14}C]sorbitol space of K-depleted cells were measured after different exposure times (1) to a normal Krebs solution (5·9 mM-K^+) bubbled with O_2, (2) to a normal Krebs solution bubbled with N_2, and (3) to a Krebs solution containing sorbitol instead of glucose and bubbled with O_2. The K-depleted tissues were first incubated for 30 min in a K-free solution of the same type before transferring them to the corresponding K-containing solution. It is found that under all these experimental conditions the cells can support the active Na and K transport at least for 2 hr and that their $[Na]_i$ and $[K]_i$ change in exactly the same way as described previously by Casteels *et al.* (1971*b*). Even tissues which are depleted of glycogen by incubation for 20 min in N_2-sorbitol medium followed by 4 hr O_2-sorbitol (Bueding, Bülbring, Gercken, Hawkins & Kuiryama, 1967) show the same or a slightly lower pumping rate on readmission of 5·9 mM-K^+ in O_2-sorbitol medium. Apparently some substrate different from glycogen can be oxidized to supply energy for the active Na-K transport.

In contrast when tissues, after incubation in K-free O_2-glucose solution were transferred to a N_2-sorbitol solution containing 5·9 mM-K^+ they could only extrude Na and reaccumulate K during the first 20 min. Thereafter the pump activity ceased and K leaked again out of the cells and was replaced by Na (Table 1). During this metabolic inhibition the ATP and phosphorylcreatine content of the tissues declined rapidly while the AMP content increased. These changes are not significantly different from the rates observed during metabolic inhibition of K-depleted cells. It is therefore not possible to estimate the energy requirements of the active Na-K transport in taenia coli by comparing the rate of decrease of ATP and phosphorylcreatine in metabolically inhibited tissues with and without active Na-K transport. Moreover the rapid changes of the content of phosphorylated compounds might already modify the characteristics of the ion pump during the initial 20 min of this metabolic inhibition.

TABLE 1. Total ion content (m-mole.kg wet wt.) and adenine nucleotide content (μmole.g wet wt.) given as mean ± S.E. of mean during reaccumulation of K in a N_2-sorbitol medium containing 5·9 mM-K^+

Time of recovery (min)	Na^+	K^+	ATP	ADP	AMP
0	150 ± 1 (4)	1·0 ± 0·5 (4)	2·01 ± 0·27 (6)	1·07 ± 0·13 (6)	0·12 ± 0·02 (6)
5	131 ± 2 (3)	14 ± 0·1 (3)	1·99 ± 0·16 (6)	0·92 ± 0·07 (6)	0·14 ± 0·03 (6)
10	130 ± 2 (4)	21 ± 2 (4)	1·48 ± 0·10 (6)	1·12 ± 0·09 (6)	0·26 ± 0·05 (6)
20	114 ± 2 (4)	33 ± 2 (4)	1·25 ± 0·27 (5)	1·25 ± 0·26 (5)	0·36 ± 0·09 (5)
30	116 ± 1 (3)	31 ± 2 (3)	0·69 ± 0·18 (6)	1·09 ± 0·17 (6)	0·55 ± 0·09 (6)
60	134 ± 6 (4)	20 ± 2 (4)	—	—	—

Influence of the Na-K pump on the metabolism

The effect of the active Na-K transport on the O_2 consumption and lactic acid production can be determined by an inhibition of this transport in tissues maintained otherwise in physiological conditions or by the reactivation of this transport in K-depleted tissues.

1. *Influence of the Na-pump on the metabolism in normal tissues*

If the ATP splitting reaction driving the pump and the processes rephosphorylating ADP are coupled, we should find that an inhibition of the pump is accompanied by a decrease of the rate of rephosphorylation. Because of the relation between the rephosphorylation and the O_2 consumption or the lactic acid production we have studied the effect of inhibition of the Na pump on both metabolic parameters.

In normal Krebs solution the O_2 consumption of taenia coli amounts to 11·2 ± 1·01 (7) μl. g^{-1} f. wt. min^{-1}. This value is similar to the one found by Bülbring & Golenhofen (1967). It is, however, difficult to assess which part of this total O_2 consumption is used for the spontaneous mechanical activity of the muscle under these conditions. We have therefore compared the O_2 consumption of tissues presenting spontaneous mechanical activity and tissues that are mechanically quiescent, either spontaneously or by adding D 600 to the bathing solution. These results show that the O_2 requirement of the mechanical activity represents only a limited fraction (less than 20 %) of the total.

After K-depletion the cells maintain a rather high level of O_2 consumption (6·4 ± 0·51 (10) μl. g^{-1} f. wt. min^{-1}) in a K-free solution in spite of the absence of mechanical activity and of active Na-K exchange.

Because of the uncertainty on the ADP/O ratio in smooth muscle (Somlyo & Somlyo, 1968) and because of the well-known stoichiometry between lactic acid production and ADP rephosphorylation we have used

the anaerobic lactic acid production as a measure of the ATP requirement of the Na-pump. This approach is further justified by our finding that the Na-pump activity and the ATP content of the cells are well preserved under anaerobic conditions. The phophorylcreatine content, however, decreases from $3{\cdot}7 \pm 0{\cdot}14$ (6) to $1{\cdot}6 \pm 0{\cdot}47$ after 120 min anaerobic incubation (μmole.g^{-1} f. wt.; mean $\pm$ S.E. of mean). A further advantage of working under anaerobic conditions is that the tissues do not present spontaneous mechanical activity and that consequently we have not to take into account the unknown energetic requirements of contraction. We have determined the anaerobic lactic acid production of tissues in which the Na pump was inhibited by prolonged exposure to a K-free solution. Such K-depleted cells produce less lactic acid ($0{\cdot}73 \pm 0{\cdot}02$ (20) μmole.g^{-1}.min^{-1}) than tissues incubated for the same period in normal Krebs solution ($1{\cdot}03$ (6) μmole.g^{-1}.min^{-1}).

Inhibiting the active Na-K exchange by adding 10^{-5} M ouabain or by incubating the tissues for 30 min in a Na-free solution causes a variable decrease of the anaerobic lactic acid production. This decrease is more pronounced in a Ca-free solution than in a similar solution containing 2·5 mM-Ca^{2+} (Table 2).

TABLE 2. The anaerobic lactic acid production of taenia coli amounts to $0{\cdot}86 \pm 0{\cdot}01$ (25) μmole.g^{-1} f. wt. min^{-1} (mean $\pm$ S.E. of mean) after 60 min exposure to a Ca-free Krebs solution and to $0{\cdot}89 \pm 0{\cdot}02$ (29) μmole.g^{-1}.min^{-1} after 60 min exposure to a Krebs solution containing 2·5 mM-Ca^{2+}. The modification of the anaerobic lactic acid production by the experimental conditions mentioned in the first column of the Table has been determined after 60 min exposure to the respective Ca-free and Ca-containing solutions and should be compared to the above mentioned reference values. Phosphate was omitted from all solutions and Tris buffer was used instead of the $HCO_3^- - CO_2$ buffer

Experimental conditions	Ca-free	2·5 mM-Ca^{2+}
10^{-5} M ouabain	$0{\cdot}50 \pm 0{\cdot}03$ (4)	$0{\cdot}57 \pm 0{\cdot}03$ (7)
Sucrose substitution	$0{\cdot}58 \pm 0{\cdot}03$ (6)	$0{\cdot}73 \pm 0{\cdot}05$ (6)
Choline substitution	$0{\cdot}53 \pm 0{\cdot}02$ (6)	$0{\cdot}65 \pm 0{\cdot}02$ (6)
Tris substitution	$0{\cdot}28 \pm 0{\cdot}01$ (3)	$0{\cdot}62 \pm 0{\cdot}02$ (4)
K substitution	$0{\cdot}47 \pm 0{\cdot}02$ (6)	$0{\cdot}56 \pm 0{\cdot}02$ (6)

2. *Changes of the O_2 consumption and lactic acid production during Na-extrusion from K depleted tissues*

If the Na-pump is reactivated under anaerobic conditions (N_2-glucose Krebs solution) by adding K to the external medium, the anaerobic lactic acid production increases rapidly and reaches its maximum within 10 min (Fig. 1). This value is not maintained and the lactic acid production decreases during the next 60 min towards its control value. The maximal

increase of lactic acid production as observed 10 min after the reactivation of the pump is a function of $[K]_o$. The relation between these two variables can be described as a system with first order kinetics, having a V_{max} = 0·72 μmole.g^{-1}.min^{-1} and a K_m = 1·1 mM (Fig. 2). This K_m value is of the same magnitude as the values given in the literature for the Na-K ATPase (Skou, 1965). The ATP content of the tissues is not modified by

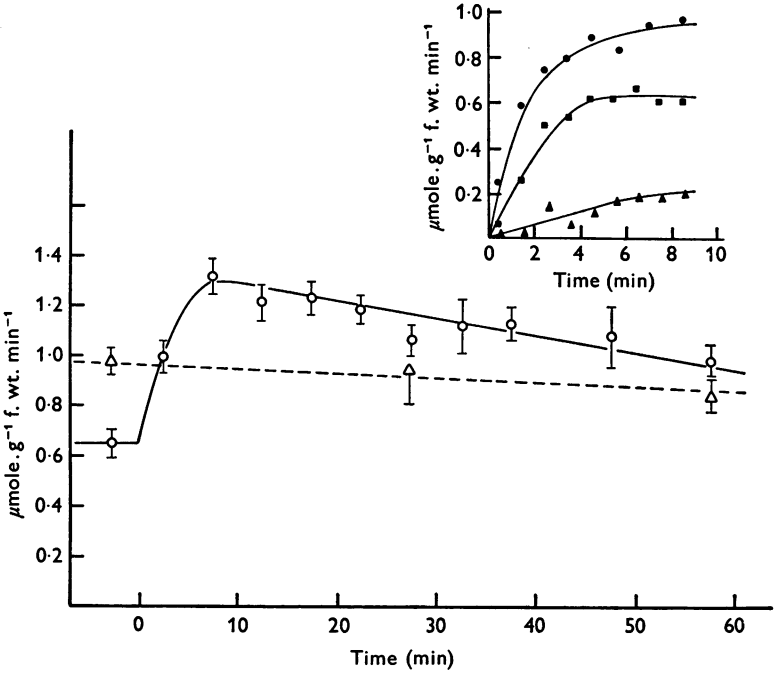

Fig. 1. The amount of lactic acid produced by taenia coli (μmole.g^{-1} f. wt. min^{-1}) is given as a function of time for different experimental conditions: anaerobic lactic production in normal Krebs solution (△); and anaerobic lactic acid production (○) induced in K-depleted cells by adding 5·9 mM-K to the external medium. This modification of the external solution is introduced at time 0. All values are mean ± 1 S.E. of mean (six observations). In the inset: the increase of the anaerobic lactic acid production by K-depleted cells after adding K is represented as a function of time for three different $[K]_o$: ▲, 0·59; ■, 5·9; ●, 23·6 mM.

this stimulation of the Na pump. Addition of 10^{-5} M oubain prior to the readmission of K in the external medium prevents a reactivation of the pump and blocks completely the increase of the lactic acid production.

The activation of the Na pump by external K in an O_2-glucose medium is accompanied by a slow increase of the O_2 consumption, which reaches its maximum only after 50 min (Fig. 3). In order to elucidate this discrepancy between the rate of O_2 consumption in aerobic medium and the rate of

lactic acid production in anaerobic medium for a similar activation of the Na pump we have also measured the lactic acid production during reactivation of the Na pump under aerobic conditions. Here too the lactic acid production was found to increase rapidly and to reach its maximal value at about the same time as in an anaerobic medium. This maximal increase of the lactic acid production ($0{\cdot}43 \pm 0{\cdot}02$ (6) μmole.g^{-1}.min^{-1})

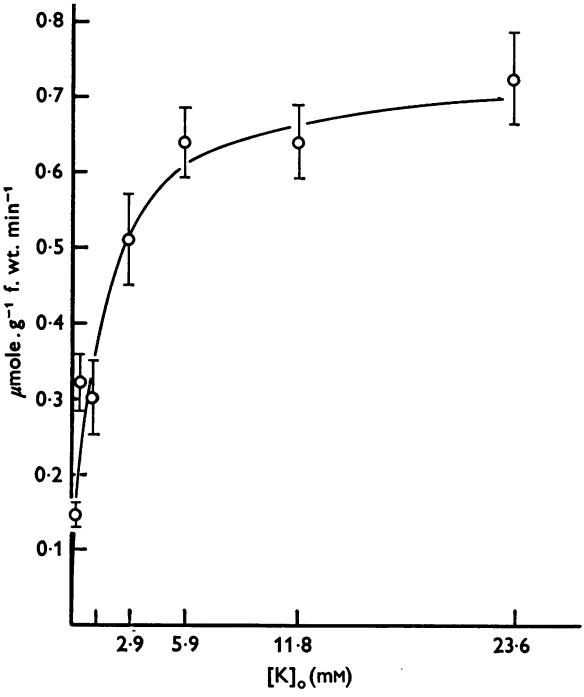

Fig. 2. The maximal increase in anaerobic lactic acid production of K-depleted tissues after reactivation of the Na pump at different $[K]_o$. The curves are fitted to a first order system by means of a non-linear least-square method.

remains, however, lower than the value measured under anaerobic conditions ($0{\cdot}64 \pm 0{\cdot}03$ (6) μmole.g^{-1}.min^{-1}) and it is followed by a more pronounced decrease than under anaerobic conditions. After 50 min of exposure to a K-containing solution the aerobic lactic acid production has decreased to its steady-state value of 0·1 μmole.g^{-1} f. wt. min^{-1} in Krebs solution (Fig. 3).

The relation between ATP-hydrolysis and the active Na-K pump

The amount of Na and K ions pumped for each ATP that is hydrolysed has been estimated by comparing the modification of the intracellular Na and K content of the tissues and the supplementary amount of lactic acid

produced by K-depleted cells during reactivation of the Na-K pump. Different pumping rates have been obtained either by varying the external glucose concentration at a constant value of 5·9 mM-$[K]_o$ or varying $[K]_o$ at the optimal glucose concentration of 11·5 mM. For the first group of experiments, K-depleted cells were incubated for 30 min in a K-free solution bubbled with N_2 and containing 0, 2·9, 5·8, 11·5, 23 or 46 mM glucose.

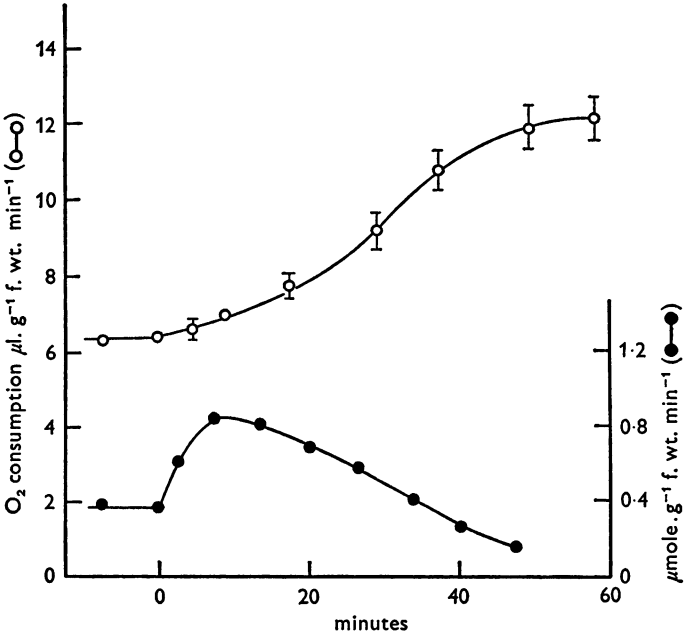

Fig. 3. ○, O_2 consumption (left ordinate) after 4 hr incubation in K-free solution and after readmission of 5·9 mM-$[K]_o$ at time 0. ●, the concomitant aerobic lactic acid production (right ordinate) under the same experimental conditions.

They were then transferred for 15 min to a first tube containing 10 ml. of this solution and thereafter for the same period of time to a second tube containing also 10 ml. of a similar solution, but with 5·9 mM-$[K]_o$. Both tubes were bubbled with N_2. The amount of lactic acid produced by tissues without active Na-K transport was estimated in the first tube, and the supplementary lactic acid production due to an activation of the pump was estimated in the second tube. After the final 15 min the tissues were blotted, weighed and prepared for Na and K flame photometry. From these data the intracellular Na and K content (μmole.g^{-1} f. wt.) after these 15 min of active transport, and the supplementary amount of lactic acid produced during the same period (μmole.g^{-1} f. wt.) have been calculated. It is found that during K-reaccumulation the changes of

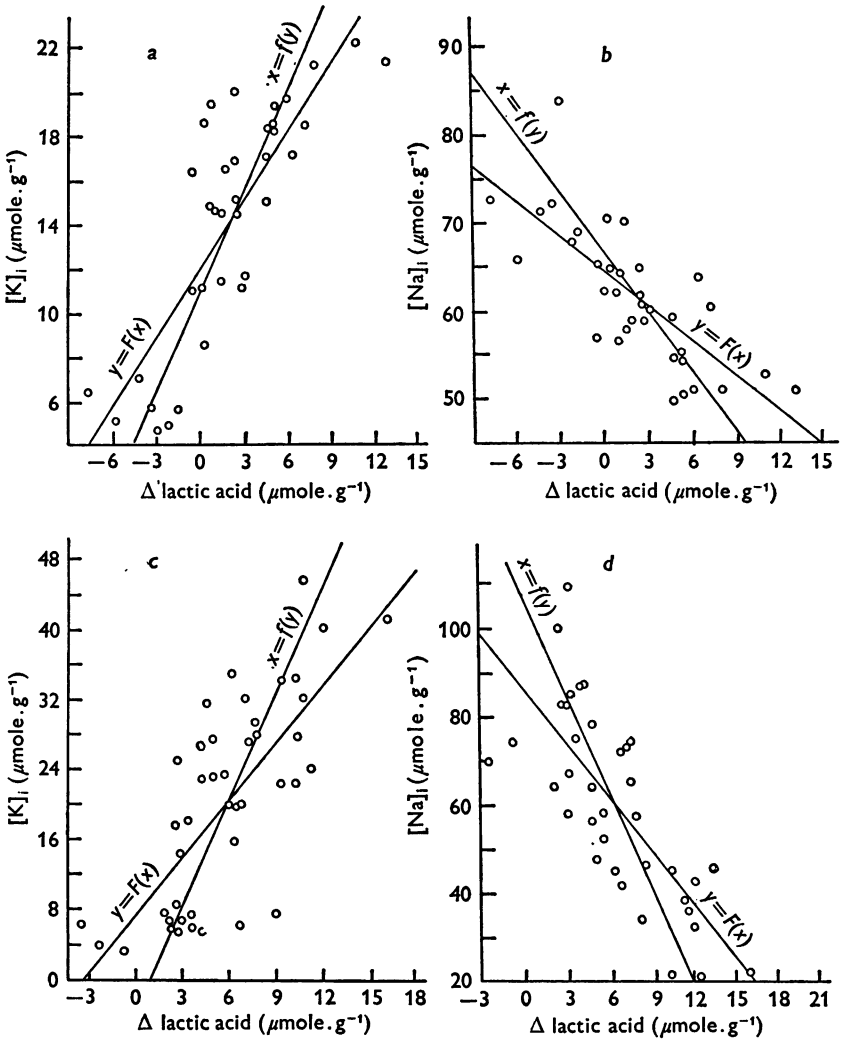

Fig. 4. The values of intracellular K and Na content obtained after 15 min reactivation of the Na pump expressed in μmole.g^{-1} wet wt. and the supplementary amount of anaerobic lactic acid produced (μmole.g^{-1} wet wt.), during this 15 min period are plotted as a function of each other for conditions with changing concentration of glucose at $[K]_o$ of 5·9 mM (graph *a* and *b*) and for conditions with different $[K]_o$ at a glucose concentration of 11·5 mM (graph *c* and *d*). The regression lines $y = F(x)$ and $x = f(y)$ have been calculated for the four graphs. The correlation coefficients and regression coefficients are given in Table 3. The scales on the abscissa of graph *c* and *d* are different from the scales used in graph *a* and *b*.

intracellular Na and K are linearly correlated to the supplementary amount of lactic acid produced (Table 3, Fig. 4). Moreover these experiments indicate that this lactic acid production in K-free solution is a function of the external glucose concentration and that the usual glucose concentration of 11·5 mM allows a maximal production of lactic acid.

TABLE 3. The correlation coefficient (r) and linear regression coefficients a and b (mean ± S.E. of mean for b) have been calculated for the relation between intracellular Na or K content, and the supplementary amount of lactic acid produced during the first 15 min of reactivation of the Na-K pump. The experimental data were obtained (1) in the presence of different glucose concentrations and 5·9 mM-$[K]_0$ and (2) in the presence of different $[K]_0$ and 11·5 mM glucose. a and b are the coefficients of the equation $y = a + bx$ represented in Fig. 4. The coefficients of the graph $x = a' + b'y$ which is also represented in Fig. 4 are not given

Changing parameter	Correlation between the intracellular cation content and Δ lactic acid produced	r	a	b
(1) Glucose concentration	Na	−0·77	64·5	−1·32 ± 0·19 (36)
	K	0·83	11·8	1·02 ± 0·11 (36)
(2) K concentration	Na	−0·75	86·8	−4·2 ± 0·63 (37)
	K	0·74	7·3	2·0 ± 0·30 (43)

In a second series of experiments, the supplementary amount of the lactic acid production and the intracellular Na and K contents were determined in a similar way after 15 min pumping in media with a $[K]_0$ of 0·59, 1·2, 2·9, 5·9, 11·8 and 23·6 mM, but all containing 11·5 mM glucose (Fig. 4, Table 3).

The absolute values of the slopes of the curves representing the intracellular content of Na or K after 15 min pumping as a function of the supplementary amount of lactic acid produced during the same period are significantly different in the two sets of experiments. The slopes obtained in solutions with different $[K]_0$ are steeper than those obtained in solutions with different $[glucose]_0$.

In order to deduce from these results the relation between active Na-K exchange and the lactic acid production (or ATP hydrolysis) we have to take into account that the changes of the intracellular ion content are not only determined by the active Na-K exchange but also by net passive influxes of both ions. These influxes during the first 15 min of the activation of the pump by external K are influenced by the progressive changes of the transmembrane ion gradients and the important modifications of the membrane potential during electrogenic pumping (Casteels *et al.* 1971*b*). The passive influxes affect the intracellular ion content more in experiments with different $[K]_0$ than in the experiments with changing

glucose concentrations. However, the values obtained in the latter experiments still underestimate the ratio: active Na transport/lactic acid production and overestimate the ratio: active K transport/lactic acid production. Using a lactic acid/ATP ratio of 1 and the coefficients b as given in Table 3 for solutions with different $[glucose]_o$, we obtain an uncorrected ratio of 1·3/1/1 for Na extrusion/K uptake/ATP hydrolysis.

The correction factors to convert the net change of ion content into active ion transport are 1·3 for Na and 0·8 for K (see below). The corrected ratio: active Na transport/active K transport/ATP hydrolysis therefore amounts to 1·7/0·8/1.

Calculation of the correction factor used to convert net Na transport and net K transport into active Na transport and active K transport

During the first 15 min of electrogenic Na-pumping the electrochemical gradients of Na and K are both inwardly directed. Later on ($t > 20$ min) when E_k becomes more negative than the membrane potential, the electrochemical gradient for Na remains inwardly directed but that for K is outwardly directed.

Because no significant difference was observed in active Na extrusion and K reaccumulation between tissues exposed to aerobic and anaerobic medium we have used for the following calculations the data obtained previously in aerobic medium.

The concentrations $[Na]_i$ and $[K]_i$ of K-depleted cells as a function of time (in min) of exposure to a Krebs solution containing 5·9 mM-K can be fitted by the equations

$$[K]_i = 2{\cdot}0 + 5{\cdot}51t - 0{\cdot}039t^2, \quad (1)$$

$$[Na]_i = 200 - 6{\cdot}2t - 0{\cdot}05t^2, \quad (2)$$

as described by Casteels, Droogmans & Hendrickx (1973). From these equations the net Na and K fluxes (J_j^{net}; expressed in mole.cm^{-2}.sec^{-1}) can be calculated by

$$J_j^{net} = \frac{d(j)_i}{dt} \frac{V}{A} \frac{1}{60},$$

where j is Na or K and V/A the volume surface ratio of taenia coli (1·4 μm (Casteels, 1969)). The total unidirectional Na efflux is given by

$$J_{Na,\,out} = k.[Na]_i \frac{V}{A} \frac{1}{60},$$

where k is the rate coefficient of Na efflux (counts.min^{-2}/(Σ counts.min^{-1})).

The rate coefficient of ^{22}Na-efflux during K-reaccumulation in a solution containing 5·9 mM-K is represented as a function of time in Fig. 5. The experimental values can be fitted by the quadratic equation:

$$\frac{cpm^2}{\Sigma cpm} \times 10^3 = 41{\cdot}8 + 2{\cdot}16t + 0{\cdot}081t^2,$$

where t is the time after reactivation of the active Na-K transport.

The unidirectional net Na influx, which is assumed to be completely passive is given by

$$J_{Na,\,in}^{pass} = J_{Na}^{net} - J_{Na,\,out}.$$

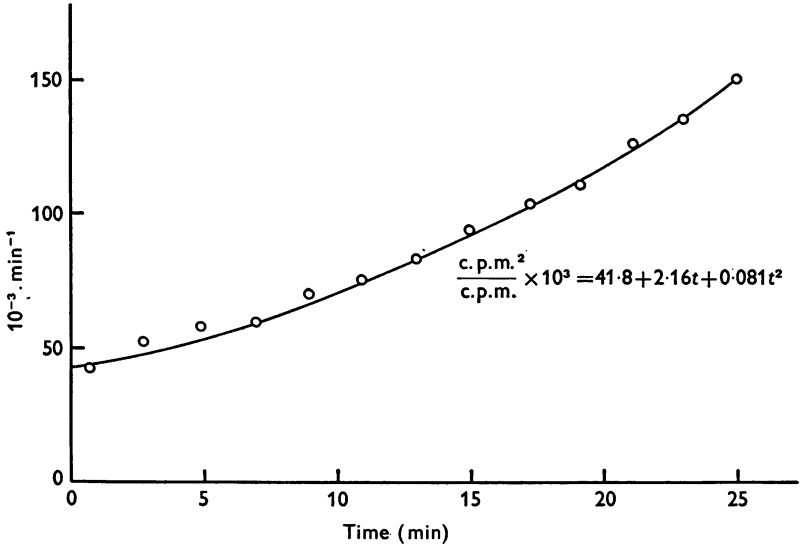

Fig. 5. The rate coefficient of the ^{22}Na efflux from K-depleted cells during the reactivation of the Na-pump in a medium containing 5·9 mM-K.

Using Ussing's unidirectional flux ratio equation the unidirectional passive Na efflux can be calculated:

$$\frac{-J^{\text{pass}}_{\text{Na, out}}}{J^{\text{pass}}_{\text{Na, in}}} = \exp \frac{F(E_{\text{M}} - E_{\text{Na}})}{RT}.$$

The active Na efflux can be calculated by

$$J_{\text{Na,out}} - J^{\text{pass}}_{\text{Na, out}} = J^{\text{act}}_{\text{Na, out}}.$$

The correction factor required to convert the net change of intracellular Na to the amount of actively transported Na is given by the ratio of the time integrals for the period between 0 and 15 min after reactivation of the Na pump as represented in Fig. 6.

$$\frac{\int_0^{15} J^{\text{act}}_{\text{Na, out}}\, dt}{\int_0^{15} J^{\text{net}}_{\text{Na}}\, dt} \approx 1{\cdot}3.$$

The correction factor for the K fluxes can only be estimated indirectly. We obtain the net K uptake by

$$J^{\text{net}}_{\text{K}} = \frac{d\,[\text{K}]_i}{dt} \frac{V}{A} \frac{1}{60},$$

where $d[K]_i/dt = 5{\cdot}5 - 0{\cdot}08t$ as calculated from the quadratic eqn. (1). In order to obtain the active and passive component of this net inward flux we made use of the finding that 20 min after the readmission of 5·9 mM-K to K-depleted tissues $E_m = E_K$ (Casteels *et al.* 1971*b*). At this stage the net passive flux should equal zero and the net flux equals the active flux. We can consequently calculate that at time 20 min the ratio of active K flux/active Na flux equals −0·4. Applying this same ratio to the

calculated active Na efflux, the active K uptake can be obtained for any time between 0 and 15 min.

$$J^{act}_{K,\,in} = -0{\cdot}4 J^{act}_{Na,\,out}.$$

In analogy with the procedure used for calculating the correction factor for the Na flux we obtain the correction factor for the K flux by

$$\frac{\int_0^{15} J^{act}_{K,\,in}\,dt}{\int_0^{15} J^{net}_{K}\,dt} \approx 0{\cdot}8.$$

These corrections are only very rough estimates and they do not take into account the possible interference of Na–Na exchange diffusion.

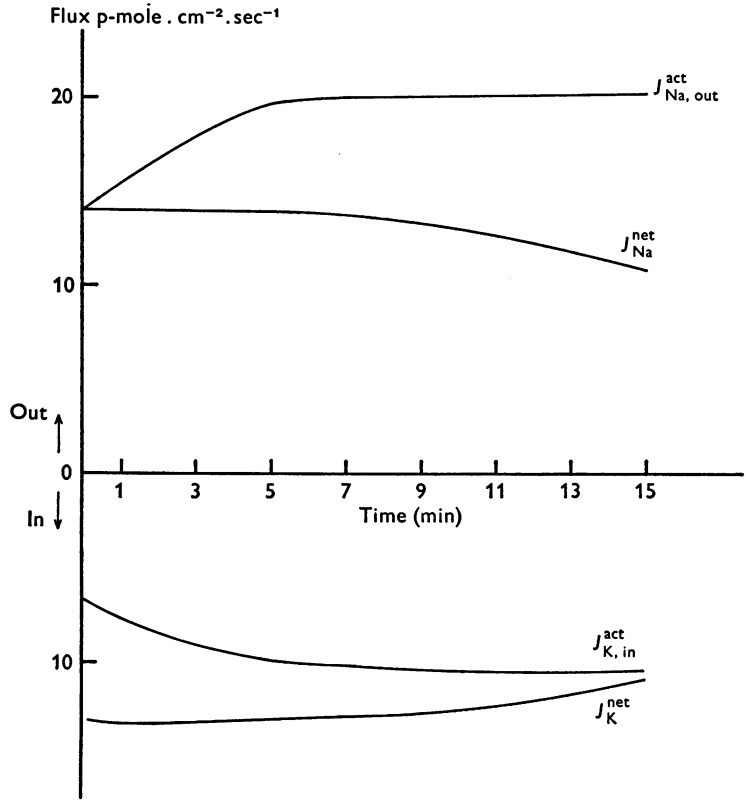

Fig. 6. The active Na efflux ($J^{act}_{Na,\,out}$), active K influx ($J^{act}_{K,\,in}$) and net Na (J^{net}_{Na}) and net K (J^{net}_{K}) movements as a function of time during the first 15 min of reactivation of the Na-K pump in a medium containing 5·9 mM-K.

DISCUSSION

Metabolic inhibition of taenia coli muscles by exposure to a N_2-sorbitol medium or to a solution containing DNP-IAA induces a rapid decrease of the cellular K-content and an increase of the Na-content (van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973). Similar changes of the ion content have been observed during exposure to solutions containing 10^{-6} M ouabain (Casteels, 1966) or to K-free solutions (Casteels *et al.* 1971*a*). These findings suggest the existence of a Na pump, which depends on metabolism and which is ouabain-sensitive.

As in most other tissues the glycolytic phosphorylation cannot completely compensate in taenia coli cells for the loss of oxidative phosphorylation under anaerobic conditions. This is indicated by the limited but significant decrease of the phosphorylcreatine content. The phosphorylation of ADP under these anaerobic conditions remains, however, sufficient to maintain a normal Na-pumping rate and is even sufficient for the important Na-pumping induced by exposing K-depleted cells to a K-containing solution. Because in most tissues part of the O_2 consumption (Whittam, 1961; Baker & Connelly, 1966) or of the lactic acid production (Whittam & Ager, 1965; Dydynska & Harris, 1966) is proportional to the active Na-transport, these metabolic parameters have been used to assess the energetic requirements of the active Na-K transport. An activation of the Na-K pump induced in K-depleted cells by readmission of external K increases the anaerobic lactic acid production as a function of $[K]_o$. However, under aerobic conditions the relation between this active transport and the supplementary O_2 consumption is less clear. The poor relationship between Na extrusion and O_2 consumption is obvious during the initial stimulation of the Na extrusion from K-depleted cells. The additional energy required by the intensive ion exchange is initially supplied by the anaerobic glycolysis while the O_2 consumption creeps up rather slowly. Another argument against using the O_2-consumption as an estimate of the energy requirements of the ion pump is that the ADP/O ratio is not accurately known for smooth muscle (Somlyo & Somlyo, 1968; Stephens & Wrogemann, 1970).

Therefore the most attractive procedure for calculating the energy requirements of the Na-K pump is the measurement of the anaerobic lactic acid production. The procedure is in addition to be preferred because in an anaerobic medium the spontaneous mechanical activity is inhibited and the corresponding energy consumption has not to be taken into account.

Because of the importance of K ions for the normal functioning of anaerobic glycolysis (Bygrave, 1967) and of the oxidative phosphorylation (Blond & Whittam, 1965) one could argue that the changes of the

metabolism during ion transport are not necessarily directly coupled to this active transport. They could as well be induced by concomitant changes of the intracellular ion content. However, in our experiments several findings indicate that the value of $[K]_i$ is rather unimportant for the anaerobic lactic acid production. Firstly the anaerobic lactic acid production stays at a high level in K-depleted cells and the stimulation of the Na-K exchange in such cells causes a maximal increase of the anaerobic lactic acid production at a stage where $[K]_i$ is still low. Secondly an increase of $[K]_i$ in K-depleted cells obtained by exposure to 23·6 mM-$[K]_o$ in the presence of 10^{-5} M ouabain does not cause a change of the lactic acid production.

The Na pump can easily be blocked by 10^{-5} M ouabain, but the effect of this substance on the respiration and the anaerobic lactic acid production of taenia coli is very variable. This is not unexpected, because in other tissues an inhibition of the Na pump by ouabain can be accompanied by an increase of oxygen consumption (Ruscak & Whittam, 1967). This effect of ouabain on respiration might be Ca-dependent. Also an inhibition of the Na pump by using Na-free solutions decreases the lactic acid production. Here again the effect becomes more obvious if the experiments are performed in Ca-free solution. These findings indicate that the secondary effects of some procedures for inhibition of the Na-K exchange cannot be neglected. Most of these experimental conditions increase the Ca uptake of taenia coli as measured with the La method (Casteels, Goffin, Raeymaekers & Wuytack, 1973). If this corresponds to an increase of the cellular Ca, the energy requirements of the cell might be affected and thereby mask the consequences of the inhibition of the Na-K pump.

Our results also show that during any inhibition procedure of the Na pump more than half of the energy supply of the cells as measured by the O_2 consumption or by the anaerobic lactic acid production does not seem to be related to the active Na-K exchange or to mechanical activity.

During the first 15 min of Na extrusion the net amount of Na or K transported is found to be proportional to the amount of supplementary produced lactic acid. This relation can, irrespective of the method used to change the rate of Na extrusion (changing the external glucose concentration or $[K]_o$) be described by a linear equation $y = a + bx$.

To estimate the ratio: active cation transport/ATP hydrolysis we have accepted a ADP/lactic acid ratio of 1, because an important break-down of glycogen, which could imply an ADP/lactic acid ratio of 1·5, can be excluded. This conclusion is deduced from the low glycogen content of taenia coli (Bueding & Hawkins, 1964) and from our finding that the lactic acid production and the rate of active ion transport are a function of the external glucose concentration.

The correction factors we have used to obtain the active efflux of Na and influx of K can only be considered as a first approximation. However, they clearly indicate that during the first 15 min of reactivation of the Na pump the active Na transport is larger and the active K transport is lower than the values deduced from the net changes in intracellular ion content. Therefore the only safe conclusion is that the estimated value of the ratio: active Na transport/active K transport/ATP hydrolysis for taenia coli falls in the range reported for other tissues (Thomas, 1972).

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