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Reviews

'The physiology of the smooth muscle: an interdisciplinary review – Part II'

Possibility of metabolic control of membrane excitation

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Introduction

Energy metabolism in smooth muscles has been analyzed in some detail, but the analyses are mostly concerned with vascular and tracheal smooth muscles[23,28]. There is also some work on intestinal smooth muscles, but the main object has been to study the relationship between contraction and the energy supply, and only very little research has been done in relation to the electrophysiology of the membrane.

In addition to the activation of the contractile machinery itself, muscle contraction depends on many factors which control the intracellular Ca concentration; these include the Ca permeability of the plasma membrane, Ca release from intracellular stores, and Ca sequestering or extrusion mechanisms. It is not well understood how these processes are correlated with the energy metabolism of the smooth muscle, particularly in relation to drug actions. It has been shown that in the guinea pig taenia coli, the spontaneous electrical and mechanical activities are transiently suppressed when glucose is readmitted after prolonged treatment with glucose-free Krebs solution[2,3]. The inhibition by metabolic reactivation through readmission of glucose is similar to inhibition by adrenaline which is also associated with an increase in energy-rich phosphate compounds[6], although oxygen consumption of the tissue is apparently not correlated with the adrenaline action[9]. α-Adrenergic receptors in the taenia coli mediate hyperpolarization of the membrane accompanied by a decrease in membrane resistance. This is explained by an increase in K conductance and Ca is considered to be involved in this action[11,12]. On the other hand, β-adrenergic receptors in the taenia coli mediate suppression of spontaneous spike activity with a small hyperpolarization[11,13] and this is probably linked with activation of a Ca-pump in the plasma membrane[8].

It is reasonable to assume that adrenaline affects several biochemical processes, and it is necessary to understand more about the properties of the metabolic pathways which support cellular functions, e.g. spontaneous activity, in order to clarify the mechanisms underlying the relaxation caused by adrenaline. In this respect, regulation of the intracellular concentration of free Ca ion through the plasma membrane, as well as active transport of Na and Ca by the plasma membrane would be most interesting subjects to investigate. In this review article, our recent work on the relationship between cellular metabolism and contraction in relation to the membrane potential in the guinea pig taenia coli will be mainly described.

K contracture and its metabolic support

It is generally assumed that depolarization of the smooth muscle membrane increases Ca conductance and Ca influx, causing contracture. In the guinea pig taenia coli, the tension developed in excess K solution can be maintained at a more or less constant level for more than 1 h. This K contracture is highly dependent on the external Ca concentration and it quickly disappears on Ca removal, suggesting that a continuous influx is necessary for its maintenance. In the nerve membrane, the Na conductance is increased by depolarization, but it is rapidly inactivated when the membrane is kept depolarized. Thus, it seems that the inactivation process for the Ca conductance in the smooth muscle of the guinea pig taenia coli is different from that for the Na conductance in the nerve fiber, because the Ca conductance can be sustained for a long time in excess K solution.

Based on the measurements of ^{45}Ca uptake and the total tissue Ca content, it has been postulated that the early

phasic part of a K contracture is mainly due to Ca released from some cellular site while the late tonic component is due to Ca entry through the plasma membrane which is maintained during the contracture under metabolic control[32]. The idea that Ca influx during the tonic contracture is linked with metabolism is supported by the observations that the tonic phase is selectively suppressed by glucose removal, anoxia, reduced temperature (16 °C), and metabolic inhibition (DNP, 0.1 mM)[3,25,32].

In a recent report, however, it has been shown that the cellular content of Ca, measured with the lanthanum method, increases from 69.9 ± 1.58 μmoles/kg to 130.7 ± 8.69 μmoles/kg on exposure to excess (45.4 mM) K in hypoxic conditions, in which the tonic component of K contracture is reduced to less than 20%[19,22]. Furthermore, when the tension has recovered to 65% of the control after increasing glucose concentration from 5.5 to 50 mM, no significant change in cellular Ca was found. Thus, the previous idea that reduction of tension under energy-depleted conditions results from a decrease in Ca influx through the plasma membrane can be questioned[19]. It will, however, be difficult to correlate functional changes with changes in Ca content until we have developed more reliable methods for detecting actual transmembrane Ca fluxes and the free intracellular Ca concentration.

Suppression of the tonic component of K contracture by removal of glucose is shown in figure 1. After equilibrating the preparation in normal solution for more than 1 h, the solution was changed to glucose-free, Ca-free, high K (106 mM) solution containing 20 mM Na. In this solution, a large transient contraction was produced, but the preparation relaxed completely within 10–20 min due to the lack of Ca. When 2.4 mM Ca was readmitted 30 min after the exposure to this solution, the tension developed quickly as shown in the lower trace of figure 1, but in the absence of glucose, it was not maintained, as previously reported[33]. When Ca readmission for 20 min was repeated at 30-min intervals the mechanical response became gradually smaller and more transient, and the tonic phase of the contracture had nearly disappeared by the 3rd or the 4th application of Ca.

The rate of oxygen consumption (Q O_2) measured simultaneously with the tension development is shown in the upper trace of figure 1. In this preparation, the Q O_2 in normal solution was 0.61 μmole/min/g wet wt, and it decreased to 0.35 μmoles/min/g after the first exposure to glucose-free, Ca-free, excess K solution. The average Q O_2 in normal solution was 0.53 ± 0.12 μmole (11.9 μl)/min/g wet wt (n = 58), close to those that have previously been reported (0.45–0.89 (9); 0.45 (27); 0.50 (17); 0.62 μmole/min/g (20)).

The Q O_2 increased on Ca readmission, but the increase became less as the mechanical response to repeated Ca application decreased. Thus, there is some correlation between the tension development and O_2 consumption. Since ATP production depends mainly on oxidative phosphorylation, it can be concluded that the tension development is also correlated with the rate of ATP production. In support of this, when the tissue contents of ATP and creatine phosphate (CrP) were measured, it was found that the high energy phosphate compounds decreased roughly in parallel with the tension response.

The Q O_2 during the relaxed state in Ca-free solution also decreased between each successive Ca readmission. However, the Q O_2 remained at about 35–40% (0.20–0.24 μmole/min/g) of the control in normal solution (0.53 μmole/min/g). It was also found that the tissue contents of ATP and CrP were still more than 25% of the control values, even when the tonic component of the mechanical response had nearly disappeared after the 3rd Ca application in glucose-free, high K solution. Therefore, although

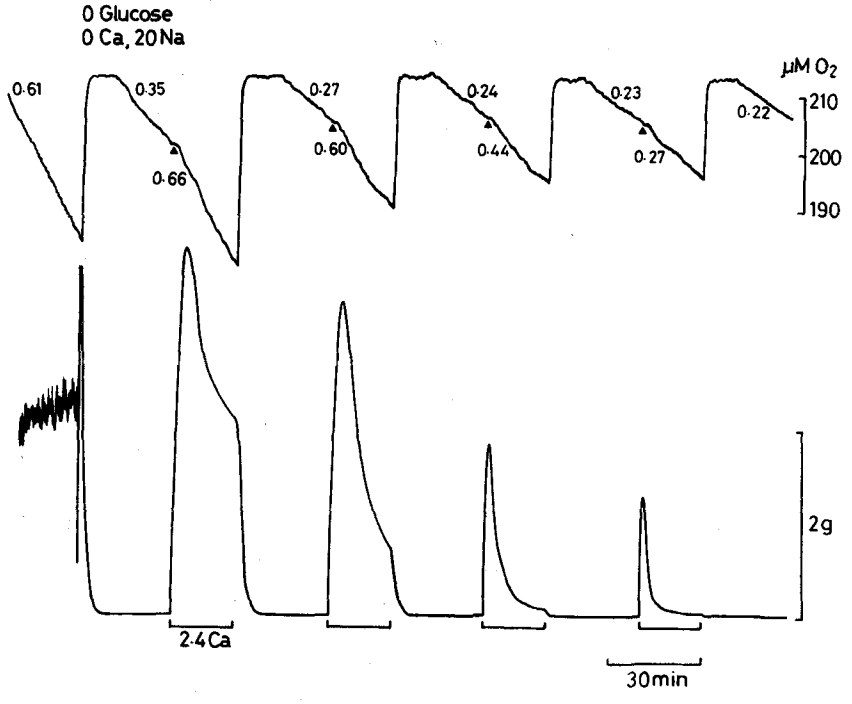

Figure 1. Oxygen concentration in a closed chamber containing a preparation (upper trace) and muscle tension of guinea pig taenia coli (lower trace). After equilibration in normal solution, exposure to excess K solution (106 K, 0 glucose, 0 Ca, 20 mM Na). 2.4 mM Ca was applied repeatedly as indicated at the bottom (and triangles in upper trace). The values in the upper record are O_2 consumption rate (μmole/min/g wet wt) obtained from the slope of the decrease in O_2 concentration. Increases in O_2 concentration of the medium to the original level were due to exchange of the solution with fresh Ca-free solution.

O_2 consumption and high energy phosphates are decreased in glucose-free solution, their change is clearly less than the decrease in the mechanical response.

Rates of energy consumption

As pointed out for vascular smooth muscle by Paul[23] and Hellstrand and Paul[18], the ATP reserve is very limited and immediate production is necessary to meet the energy demand. In the taenia coli, the Q O_2 is about 0.5 µmole/min/g. From this value, ATP production and, accordingly, ATP consumption to maintain equilibrium, is estimated to be about 3 µmole/min/g, based on a typical carbohydrate metabolism for simplicity. This production rate is quite high compared with the content of high energy phosphate compounds which is only 3–5 µmole/min/g[1,6,21]. This means that ATP turnover rate is remarkably high and that the intracellular ATP would be depleted very quickly whenever the ATP production was inhibited.

The glycogen content of the taenia coli was about 3 µmoles glucose-unit/g, and 6 moles of O_2 are utilized for aerobic metabolism of 1 mole of glucose. In glucose-free solution, the Q O_2 decreases to about 0.20–0.24 µmole/min/g (35–40% of the value in normal solution), but continues at this level for several hours. If this O_2 consumption is entirely linked with oxidative phosphorylation of glucose, about 0.03–0.04 µmole of glucose/min are being consumed, and the total glycogen would be depleted within 100 min. Actually, the glycogen content was reduced to less than 3% of the control in glucose-free, high K solution containing 2.4 mM Ca after 1 h incubation[1]. This result is very similar to that obtained after 70 min exposure to 70 mM K medium[21].

Recovery of tension development by substrate readmission

Figure 2 shows a similar experiment to that shown in figure 1. In this preparation, Ca readmission was also repeated 4 times at 30-min intervals until the mechanical response to Ca became transient. The recording in this figure starts with the response to the 3rd application of Ca, and after observing the response to the 4th application of Ca, glucose (11.8 mM) was readmitted in the

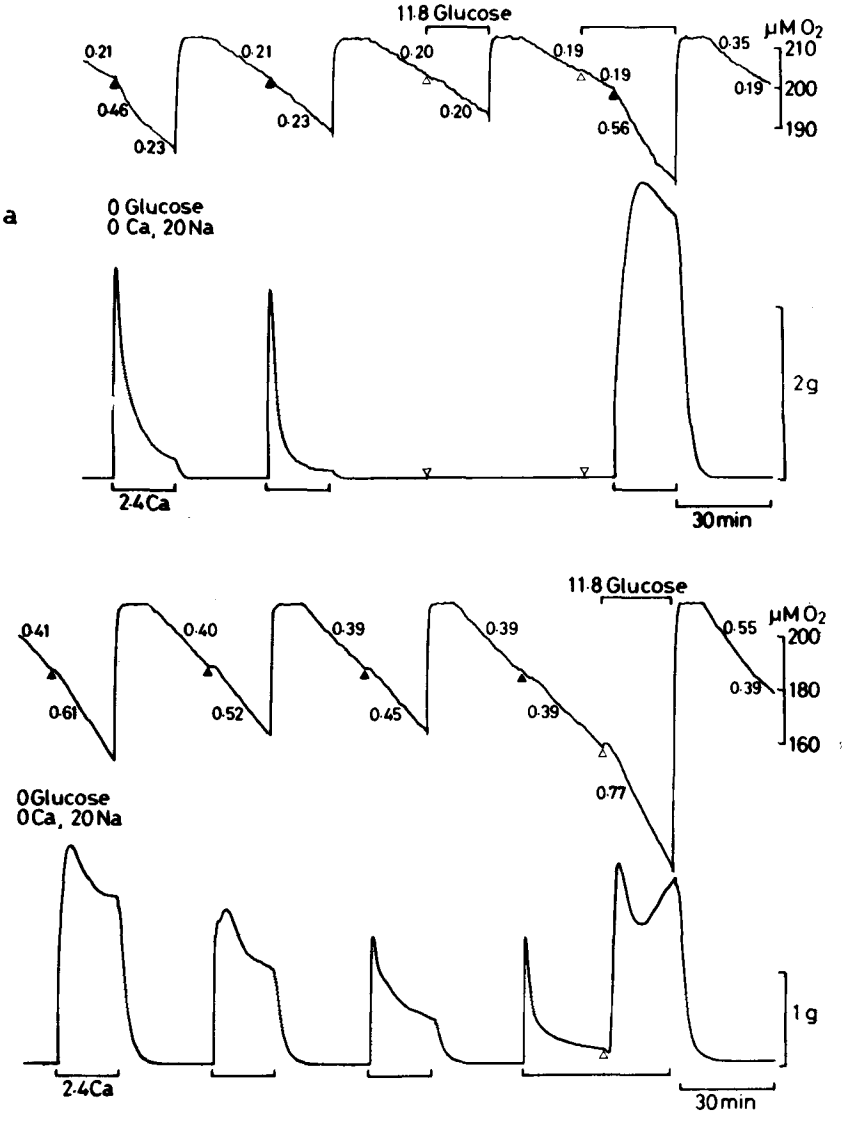

Figure 2. Effects of readmission of glucose (upper trace: oxygen concentration of medium; lower trace: muscle tension). Experimental procedure as figure 1. *a* After the 3rd and 4th applications of 2.4 mM Ca for 20 min at 30-min intervals, glucose (11.8 mM) was readmitted (open triangles) for 20 min. 10 min after the 2nd application (for 30 min) of glucose, 2.4 mM Ca was given for 20 min in the presence of glucose. *b* After observing responses to readmission of 2.4 mM Ca 4 times, 11.8 mM glucose was readmitted, 26 min after the 4th Ca application, still in the presence of Ca. Closed triangles in upper trace indicate the time of Ca application.

absence of Ca. This produced neither a mechanical response nor an increase in Q O_2. On the other hand, Ca application in the presence of glucose evoked a large tonic contraction accompanied by a clear increase in Q O_2.

In the experiment shown in figure 2b, glucose (11.8 mM) was readmitted in the presence of Ca, when the tonic component of contracture had become small after the 4th application of Ca. The addition of glucose in the presence of Ca produced a quick increase in tension similar to a response to Ca application in the presence of glucose, and increased the Q O_2. A small relaxation usually preceded the increase in tension. When β-hydroxybutyrate (βHB, 11.8 mM), which can be utilized as a substrate through the tricarboxylic acid (TCA) cycle, was used instead of glucose, nearly identical results were observed.

Readmission of glucose (11.8 mM) or βHB (11.8 mM) produced a partial recovery of both ATP and CrP contents. 15 min after the 3rd application of Ca in glucose-free high K solution, the high energy phosphate compounds (ATP + CrP) were 0.90 µmole/g wet wt. This increased to 1.39 µmole/g with glucose and 1.37 µmole/g wet wt with βHB, when measured 15 min after readmission of substrate in the presence of Ca. Thus, there was some correlation between the content of high energy phosphate compounds and the tension development, independent of the type of substrate. This suggests that the amount or the rate of ATP production is probably the main determining factor in the recovery of tension development and a glycolytic process may not be oligatory for this recovery.

Membrane potential and readmission of substrate

The removal of glucose from normal Krebs solution is known to increase the spontaneous electrical activity and a varying degree of inhibition of spike activity is observed on returning glucose[2,3]. The membrane is gradually depolarized in glucose-free solution, although the precise amount is difficult to measure if the sucrose-gap method is used. The spike activity finally stops after 4–8 h of glucose removal, accompanied by depolarization of the membrane. According to Bueding and Hawkins[7], depletion of tissue glycogen is very slow and significant amounts (18–28% of the original value) of glycogen can still be detected even after 6 h in glucose-free Krebs solution. Further experiments should be carried out to evaluate more precisely the relationship between energy metabolism and membrane potential in normal Krebs solution. In the following experiments preparations in which glycogen was depleted nearly completely were used. In this condition, no spontaneous spike activity was observed, but the resting potential was in the same range (55–60 mV) as observed in normal Krebs solution without glycogen depletion.

Figure 3 shows the effect of glucose readmission on the membrane potential, measured with an intracellular microelectrode, in the presence of 20 mM K. The preparation was first treated similarly to that shown in figure 1 in order to deplete glycogen, and then exposed to glucose-free Krebs solution containing normal K (5.9 mM) and Ca (2.4 mM). 20 mM K produced depolarization of about 20 mV and spike activity only during the early phase of depolarization. When glucose (11.8 mM) was readmitted in the presence of 20 mM K a transient repolarization of about 10 mV was produced, but the membrane gradually depolarized again beyond the previous level, accompanied by spike activity. Although the electrophysiological experiments were carried out in separate experiments using different preparations from those for the mechanical experiments and only 20 mM K, the changes in membrane potential were in accord with

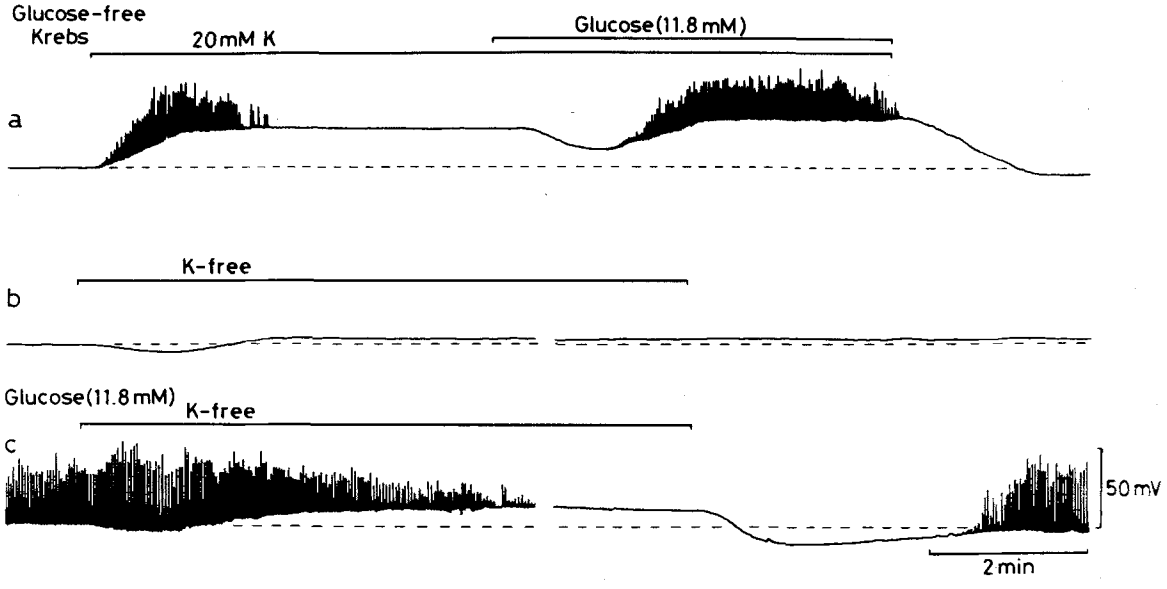

Figure 3. *a* Effects of glucose readmission in the presence of 20 mM K on the membrane potential at a glycogen-depleted taenia coli. The preparation was pretreated as shown in figure 2 to deplete glycogen, and then exposed to glucose-free Krebs solution. *b* Effects of K-removal for 10 min in the absence of glucose. *c* Effect of K-removal for 10 min in the presence of glucose (11.8 mM). Glucose was readmitted 10 min before K-removal and this produced spontaneous spike activity with a delay of about 5 min. Continuous intracellular recording, the interval between records is 20 min. See text for further explanation.

the mechanical response shown in figure 2b; i.e. the early transient spike activity corresponds to the phasic contracture to Ca readmission and the transient hyperpolarization followed by depolarization with spike activity to the transient relaxation followed by the increase in tension.

When glucose was readmitted at 5.9 mM K, the hyperpolarization of the membrane was less than at 20 mM K, being usually less than 5 mV, and the membrane potential returned very slowly to the level before glucose application. Spontaneous spike activity started with a delay of more than 5 min after glucose readmission. It is probable that during the silent period before the generation of spike activity, conditions for excitation are being prepared by a metabolic process, such as proposed by Tomita and Watanabe[29] and Bülbring and Kuriyama[10].

The responses produced by βHB (11.8 mM) application were much the same as those by glucose (11.8 mM) both at 5.9 mM and at 20 mM K. Therefore, the amount of ATP produced may be the important factor for the electrical response independent of the metabolic pathway and glycolysis does not seem to have a special role.

Na-pump and metabolic support

In order to investigate a possible contribution of Na-pump activation to the hyperpolarization caused by substrate readmission, the effect of K-removal and K-readmission were compared, since K-readmission is known to produce hyperpolarization by activating an electrogenic Na-pump in this tissue[15,30]. In the experiment shown in figure 3b, K was removed for 10 min in the absence of glucose. The membrane was slightly depolarized after a transient weak hyperpolarization, and when K was readmitted, no response was observed. On the other hand, in the presence of glucose, which produced spontaneous spike activity, the depolarization in K-free solution was larger and a marked hyperpolarization was produced by K readmission, typical for activation of the electrogenic Na-pump (c). The time course of hyperpolarization was similar in the response to glucose readmission in the presence of 20 mM K (a) and in that to K readmission in the presence of glucose (c).

The same degree of hyperpolarization was observed on K readmission when βHB (11.8 mM) was used as a substrate. In the presence of ouabain (1–10 µM), K removal produced hyperpolarization and K readmission recovery from the hyperpolarization, strongly suggesting that the hyperpolarization following K readmission observed in the absence of ouabain is due to activation of the electrogenic Na-pump. The failure to demonstrate electrogenic pump activity in the absence of substrate may indicate that the pump activity depends highly on the energy supply.

In various vascular smooth muscles, it is known that the production of lactic acid is very high, derived from at least 90% of the glucose utilized, even under fully oxygenated conditions[23,24]. When the Na-pump is inhibited by ouabain or removal of either K or Na, the lactate production is reduced, even though the O_2 consumption is increased. Thus, in vascular smooth muscles, aerobic glycolysis seems to be linked with the Na-K transport at the plasma membrane. The rat myometrium, enriched in Na by exposing to K-free Krebs solution for 18–24 h at 4 °C, recovers Na and K concentration gradients on readmission of K at 37 °C[26]. This recovery is reported not to be impaired by removal of glucose provided that sufficient O_2 is supplied. However, since glycogen may not be easily depleted, it is difficult to conclude from this experiment that the Na-pump can be supported by oxidative phosphorylation alone in the rat myometrium.

In the guinea pig taenia coli, it has been shown that the active transport of Na and K can be maintained either in normal Krebs solution bubbled with N_2 or glucose-free Krebs solution gassed with O_2 even in glycogen-depleted preparations[17]. Although a close correlation between the pump activity and lactate production can also be shown, the Na pump is able to operate without glycolysis at least in the taenia coli. This is supported by our recent observation that in the glycogen-depleted preparation, the degree of hyperpolarization on K readmission in the presence of βHB was the same as in the presence of glucose. In the absence of substrate, K readmission did not produce any clear change in membrane potential even under aerobic conditions (fig. 3b). When substrate was applied within 5 min from K readmission, the transient hyperpolarization was not much different from that caused by K readmission in the presence of substrate. However, the degree of hyperpolarization gradually decreased as the substrate readmission was delayed, and the hyperpolarization nearly disappeared when the interval was more than 20 min. This may suggest that intracellular Na accumulated in K-free solution is gradually pumped out following K readmission without addition of substrate, as shown by Casteels and Wuytack[17]. It is likely that a high rate of energy supply is necessary for strong activation of the Na-pump to produce a large hyperpolarization, but that intracellular Na concentration can be slowly reduced without affecting the membrane potential by a slow utilization of an intracellular energy source, which is partially related to the residual O_2 consumption observed in substrate-free medium.

In the taenia coli, about 50% of glucose consumed flows out as lactate in normal Krebs solution and the remaining 50% is utilized through the TCA cycle. Although not as high as in the vascular smooth muscle, this high rate of lactate production may be linked with the Na-pump activity. However, since the Na-pump can be supported sufficiently by oxidative phosphorylation, without a contribution of glycolysis, the rate of ATP supply seems an important factor and glycolysis may not play any particular role in the pump activity under the normal condition.

Mechanism of changes in membrane potential caused by substrate readmission

The hyperpolarization and depolarization with spike activity produce by glucose or βHB application were not significantly affected by ouabain (5 µM), as shown in figure 4. In this figure, the experimental procedure was the same as in figure 3a, but βHB was used as a substrate. After observing the control responses (a) and the effect of ouabain (b), verapamil (0.1 µM), a Ca antagonist, was added (c). This blocked the spike activity and also the

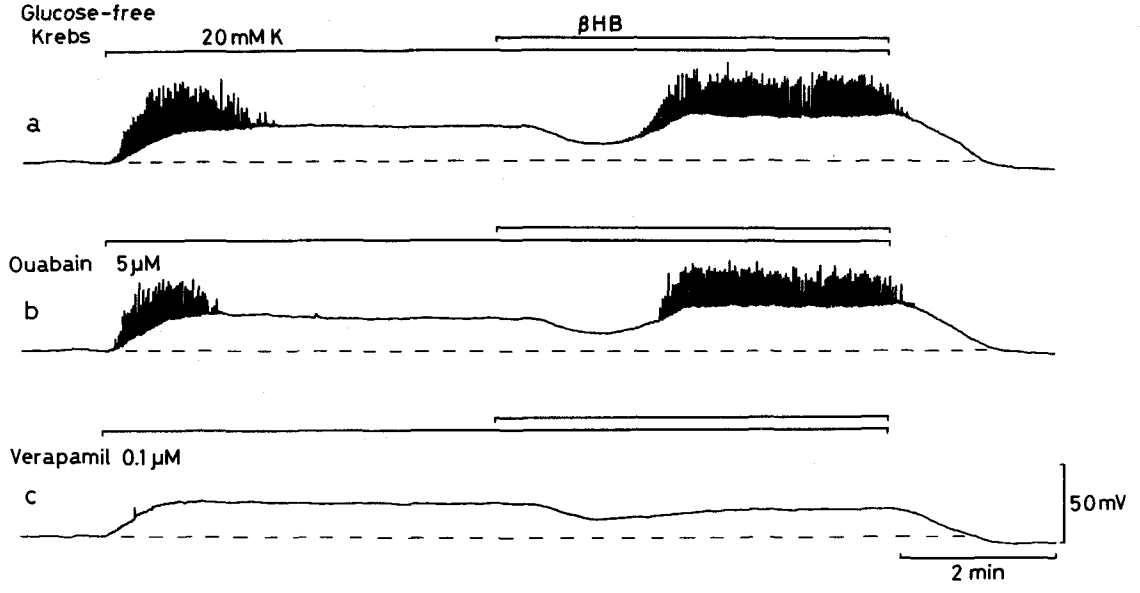

Figure 4. A similar experiment to that shown in figure 3a. Continuous record of membrane potential in glycogen-depleted preparation. K concentration was increased from 5.9 to 20 mM, and 5 min later, βHB (11.8 mM) was applied, as indicated above (a). The procedure was repeated in the presence of ouabain (5 μM) which was applied 10 min before 20 mM K application (b). Similarly, effects of verapamil (0.1 μM) were observed in (c). 10 min after (b), ouabain was removed and verapamil was added. 10 min later, 20 mM K was applied in the continuous presence of verapamil.

delayed depolarization caused by βHB, so that βHB produced only a hyperpolarization which slowly decreased in its presence. When the early part of the hyperpolarization (c) is compared with the transient hyperpolarization (a), it is clear that verapamil does not affect the hyperpolarization caused by βHB. The same results were obtained with another Ca antagonist, nifedipine (0.1 μM). When glucose or βHB was applied to tissues in Ca-containing medium, the presence of verapamil did not inhibit increase in O_2 consumption that occurs, but did inhibit the tension development.

The depolarization with spike activity was also abolished in Ca-free solution containing 0.5 mM EGTA and 12 mM Mg. However, in this solution the hyperpolarization was also slowly suppressed. Thus, it is possible that the depolarization in response to substrate application is a result of an increase in Ca conductance of the membrane, and that the hyperpolarization is not due to an activation of the ouabain-sensitive Na-pump. The idea that an increase in Ca conductance is responsible for the depolarization caused by substrate application is supported by the observation that the depolarization is not affected by complete removal of the external Na (replacing it with choline).

In cardiac muscle, it has been shown that intracellular cyclic AMP increases the Ca conductance of the plasma membrane, giving a basis for β-adrenergic stimulation[14,31]. However, in the taenia coli, dibutyryl cyclic AMP had no effect on the membrane potential in substrate-free medium. Therefore, in this tissue, ATP rather than cyclic AMP produced by substrate application seems to be directly used to increase the Ca conductance. Clear depolarization and accompaning spike activity are observed only when substrate is applied in the presence of depolarizing agents, such as 20 mM K or carbachol. Substrate applied in glucose-free Krebs solution containing normal K (5.9 mM) concentration produces spike activity after a delay of more than 5 min without much depolarization. Thus, ATP may not directly open up Ca channels, but ATP is probably used to prevent inactivation of the channel, or to re-activate some process responsible for the spontaneous spike activity.

The hyperpolarization activated by substrate readmission was also not affected by removal of K or Na from the external medium. Therefore, an increase in K conductance or a decrease in Na conductance is unlikely to be involved in the hyperpolarization. Furthermore, the possibility of a contribution of a Na-Ca exchange process to the hyperpolarization may also be neglected. The hyperpolarization is highly dependent on the temperature compared with the depolarization produced by substrate readmission. When the temperature was lowered below 30 °C, the hyperpolarization nearly disappeared and substrate application produced only depolarization without the preceding hyperpolarization. Since the hyperpolarization is coincident with relaxation and also with an increase in O_2 consumption, it is tempting to speculate that the hyperpolarization is due to activation of an electrogenic Ca-pump. Slow disappearance of the hyperpolarization after exposure to Ca-free solution containing EGTA and high sensitivity to temperature may also be taken as supporting evidence for a Ca-pump.

Correlation of mechanical response and membrane potential in relation to metabolism

Since ATP is necessary for the actomyosin system to produce a mechanical response, a decrease in the intracellular concentration of ATP should result in decrease in tension. However, this may not be the only limiting factor for the reduction of K contracture during exposure to glucose-free solution, since O_2 consumption rate is still 35–40% and the high energy content is more than 25% of

the control even when the mechanical response has disappeared. Furthermore, under these conditions, an increase in the external Ca concentration to 10 mM or an application of carbachol can produce a transient mechanical response[1]. Thus, it is likely that another factor limits the tension development and the evidence suggests that this is reduction of Ca influx. It seems that as intracellular ATP is reduced, Ca channels are inactivated, resulting in decrease in tension. This automatic suppression of energy expenditure would prevent the cell from severe depletion of intracellular ATP. If ATP concentration is lowered to an extremely low level by strong metabolic inhibition, rigor would develop and cellular Ca would be elevated because of membrane leakiness and failure of the Ca-pump[4,5,16,34].

It is an attractive idea that glycolytic and oxidative pathways may have different functions. For example, the glycolytic process may be more accessible to the Na-pump located in the plasma membrane[23,24]. The mechanism responsible for lowering cytoplasmic Ca concentration is considered to be supplied better by ATP produced from aerobic than anaerobic metabolism[21]. However, as far as electrophysiological observations are concerned, no clear difference was found between the responses caused by glucose and βHB application. More careful quantitative experiments are necessary to discriminate possible functional difference between metabolic pathways.

When substrate is applied to a glycogen-depleted preparation in the presence of Ca, the first response observed is a transient hyperpolarization and relaxation. It may be that maintenance of intracellular Ca concentration low enough for the resting state is one of the most important functions for the cell to be activated by substrate readmission, and that cellular metabolism provides energy preferentially for this function, i.e. Ca-pump activity. Then, inactivation of Ca channels is removed, allowing Ca influx. When the intracellular Ca concentration is increased beyond the capacity of Ca-pump activity, the tension starts to develop and simultaneously extra energy production will be initiated to maintain the Ca influx and to meet with the consumption by the contractile machinery.

Summary

In the guinea pig taenia coli, when glycogen is depleted by repeating Ca-induced contracture in excess K solution containing no glucose, the tension cannot be maintained. The decrease in tension is accompanied by reduction of high energy phosphate compounds and oxygen consumption. When substrate is readmitted to the glycogen-depleted preparation in the presence of 2.4 mM Ca and 20 mM K, the first response is hyperpolarization of the membrane and relaxation, and this is followed by depolarization and development of contracture. The latter response is blocked by verapamil, suggesting that energy supply increases the Ca conductance of the plasma membrane. The early response is considered to be due to activation of electrogenic Ca pump, since this is not affected by ouabain as well as removal of Na and K. ATP produced by substrate readmission is probably preferentially utilized for Ca pump activation to reduce the intracellular Ca. The recovery of tension is likely to be brought about by ATP supply not only to the contractile machinery but also to the plasma membrane to remove inactivation of Ca conductance. It is postulated that as the energy source is depleted, energy consumption is automatically limited by suppressing Ca influx, as a self-defence mechanism. Since βHB is as effective as glucose in the recovery of these processes, and also in the activation of electrogenic Na pump, the matabolic pathway of oxidative phosphorylation alone can support these functions without a contribution of the glycolytic pathway.

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Energy metabolism and transduction in smooth muscle

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1) Historical perspective

The comprehensive and pioneering work of Bülbring and her school in the field of smooth muscle electrophysiology can sometimes overshadow their early and important contributions toward the understanding of the metabolism and energetics of smooth muscle. In the process of investigating the mechanism of adrenergic relaxation of guinea pig taenia coli, the hypothesis was advanced that epinephrine elicited relaxation of smooth muscle tone by increasing the supply of energy to the plasmalemma, thereby activating ion transport[10]. Work performed in the 1950's and early 1960's was undertaken to characterize the coupling of specific energy sources with contractile activity and active ion transport in taenia coli.

As early as 1953, Bülbring[9] demonstrated that oxygen consumption increased in proportion to the degree of tension development, during a variety of interventions which altered the resting tone of taenia coli[9]. Subsequently, Born and Bülbring[7] and Born[6] showed that this relation was dependent on the maintenance of the tissue content of high energy phosphagen (ATP, PCr). Since the level of high energy phosphagen in taenia coli is low, this finding indicated that tension maintenance was dependent on intermediary metabolism. On the other hand, Axelsson and Bülbring[4] investigated the relation between carbohydrate metabolism and the electrical activity of the plasmalemma. They found that cooling or removal of glucose from the perfusion medium decreased the electrical stability of the cell membrane of taenia coli. Rewarming or addition of glucose would restore the normal membrane stability. However, treatment with iodoacetate eliminated these stabilizing effects. Iodoacetate also had been shown to abolish the increased rate of Na^+ extrusion seen in the presence epinephrine[10]. Additionally, replacement of external Na^+ with Li^+ also inhibited the recovery of normal membrane stability indicating that active Na-K transport could be responsible for at least a portion of the membrane effect. Axelsson et al.[5] later concluded that the maintenance of the membrane potential of taenia coli is dependent on carbohydrate metabolism and, this relation was qualitatively or quantitatively different from that of the contractile mechanism. Lundholm's group reached a similar conclusion for VSM, after finding a dissociation between lactic acid production and mechanical activity in bovine mesenteric arteries, under a variety of conditions[28].

Upon further characterization of the adrenergic effects on the electrical activity of the taenia coli plasmalemma, Bülbring concluded that there was no obligatory requirement for carbohydrate metabolism for the epinephrine induced relaxation[8]. This conclusion was based on the finding that glycogen depleted taenia coli could maintain