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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

normal membrane properties in a glucose-free medium supplemented with β-hydroxybutyrate, and that glycogen phosphorylase was not stimulated in the presence of epinephrine[8]. Taken as a whole, these early findings did not allow the development of a generalized model to explain the functional coupling between energy supply and its utilization in smooth muscle. However, these data indicate that glycolytic and oxidative metabolism could independently supply energy for specific exergonic processes. Thus, these early investigations set the basis for the study of metabolic compartmentation in smooth muscle.

2) Recent evidence supporting a functional compartmentation of smooth muscle metabolism

Casteels and Wuytack[11] took advantage of this apparent separability of oxidative and glycolytic metabolism to study the coupling of active Na^+ and K^+ transport to ATP utilization in the smooth muscle of guinea pig taenia coli. They measured lactate production in the absence of molecular oxygen and found a coupling ratio of $3Na^+/2K^+$/ATP, which is not different than the theoretical value. Moreover, the time course of anaerobic lactate production was correlated with the time course of recovery of cell K^+ and extrusion of Na^+ by K^+ depleted tissues, to which external K^+ was administered. This finding indicates that glycolysis can completely support active ion transport. In the presence of oxygen, the recovery of cell K^+ by K^+-depleted taenia coli was again correlated with lactate production, whereas the increase in the rate of oxygen consumption (J_{O_2}) showed a considerable lag time. Moreover, the maximal rate of lactate production J_{lac} was attained within a similar time interval under both aerobic and anaerobic conditions. This evidence is consistent with the hypothesis that glycolysis is important for the maintenance of normal membrane properties. However, the coupling between glycolysis and Na-K transport could not be considered obligatory, since Na^+ transport could be activated in an aerobic, substrate-free medium.

Work done in collaboration with John Peterson and S. Roy Caplan in the early 1970's attempted to characterize the mechanochemical coupling, i.e., ATP utilization associated with mechanical activity, of the vascular smooth muscle of bovine mesenteric vein. Like the guinea pig taenia coli, the high energy phosphagen content (ATP, PCr) of VSM alone would not be sufficient to maintain mechanical activity for more than a few minutes[39]. Thus, intermediary metabolism must also be closely coordinated with contractile events in vascular smooth muscle. Measurements of intermediary metabolism during mechanical activity can be used to estimate tension related ATP utilization. A linear relation was found between active isometric force (P_o) and ATP utilization, as estimated by measuring the steady state rates of oxygen consumption and aerobic lactate production [45–47]. This relation was independent of the stimulus which was used to elicit the mechanical response. Subsequently, similar relations between active isometric force and suprabasal ATP utilization have been found for porcine carotid[44] and coronary[43] arteries, and the rat portal vein[22] and aorta[2].

The level of isometric force in bovine mesenteric vein could be altered either by changing the agonist levels at a fixed tissue length or by varying tissue length at a constant level of stimulus. The rate of ATP utilization (J_{ATP}) per level of active tension, estimated from the slopes of the respective regression analysis, at a fixed length was generally larger than that when tissue length was varied. The difference in ATP utilization may be attributed to tension-independent processes which are activated by the agonist (fig. 1). Additionally, at short lengths where active tension was negligible, a tension independent rate of ATP utilization also could be measured. This component amounted to approximately 10% of the total ATP utilization at L_o or about 20% of the suprabasal ATP utilization rate. We considered this tension-independent component to be primarily ascribable to Ca^{++} and other ion pumping mechanisms.

In these initial studies, the rate of lactate production was characterized by a linear dependence on isometric tension, which was similar to that of J_{O_2} when stimulated with epinephrine, norepinephrine or histamine. Thus, aerobic lactate production under these conditions accounted for a relatively constant proportion of ATP production, which was no greater than 30% of the maximum rate. However, incubation of porcine carotid artery in a medium in which Na^+ was replaced by equimolar K^+ led to the stimulation of oxidative metabolism and mechanical activity without an increase in J_{lac}[18]. Under this condition, J_{O_2} and the estimated J_{ATP} remained correlated with tension[44]. Since incubation in Na^+-free media is known to inhibit active Na-K transport, this finding again raised the possibility that carbohydrate metabolism – in the form of aerobic glycolysis – was specifically coupled to Na-K transport processes in smooth muscle. To test this hypothesis, we used other methods of altering active Na-K transport and measured the effects on isometric tension, J_{O_2} and J_{lac}.

It was possible that the tonicity of the Na^+-free saline used (Na^+ replacement by K^+ produces a hypotonic solution) was a primary factor involved in the inhibition of J_{lac}. Therefore, isotonic Na-free media (Na^+ replacement by K^+ plus 50 mM sucrose or K_2SO_4) were also used to inhibit Na-K transport, eliminating any osmotic effects. Similar to the initial finding, J_{lac} was significantly reduced, whereas J_{O_2} and P_o were stimulated[40]. Ouabain also caused J_{lac} to decrease concomitant with stimulation of J_{O_2} and P_o. On the other hand, stimulation of active Na-K transport by the addition of KCl (5mM) to K^+ depleted tissues lead to a significant increase in J_{lac}, and a decrease in J_{O_2} associated with the relaxation of isometric force. The mechanism underlying these metabolic alterations is inherently different from that responsible for the Pasteur effect (increases in J_{lac} due to limitation of J_{O_2}). This is best exemplified when media K^+ is further increased by 80 mM, evoking dramatic increases in P_o, J_{O_2} and J_{lac}, indicating that J_{O_2} is not limiting.

These observations are consistent with the hypothesis that aerobic glycolysis is specifically coupled to active Na-K transport, and that oxidative and glycolytic metabolism are independently regulated in VSM. The independence of oxidative and glycolytic metabolism suggested that substrate utilization may also be independently regulated.

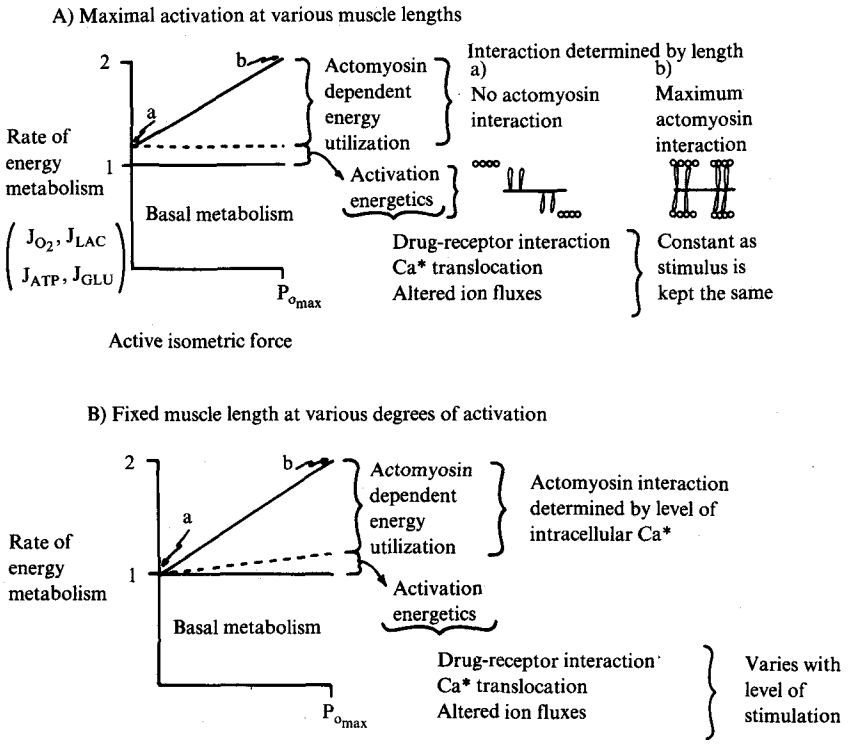

Figure 1. Determinants of vascular smooth muscle energy metabolism under isometric conditions. *A* Top line represents total metabolic rate as a function of active isometric force, measured under conditions of maximal stimulation at various muscle lengths. Metabolic rates are given in terms of ATP utilization in arbitrary units. The ordinate, however, could be expressed in terms of the rates of oxygen consumption, as these metabolic parameters show a similar dependence on isometric force. Changes in isometric force are assumed to reflect change in number of available actomyosin interaction sites. Solid horizontal line represents basal metabolism measured in the absence of stimulation. Distance between solid and broken horizontal line represents requirements of activation processes. *B* Top line represents total metabolic rate as a function of active isometric force measured under conditions of fixed muscle length with graded contractions produced at various levels of activation. Difference in slopes of the relation between metabolism and force under the different conditions (a, b) can be attributed to different levels of activation at the same force. Broken line represents metabolism associated with activation processes. From Paul[39] (1980), American Physiological Society (with permission).

3) Smooth muscle substrate utilization patterns

VSM relies on the oxidation of endogenous substrat stores and exogenous substrates to support basal energy utilizing processes[12]. Under unstimulated conditions various substrates can be oxidized, however, the preferred substrate for oxidation appears to be free fatty acids. The oxidation of exogenously supplied glucose can account for only 5–12% of the resting oxygen consumption rate. Increasing bathing media glucose, paradoxically, decreases the respiratory rate rather than increasing absolute glucose oxidation[36]. In addition, exogenous glycogen levels remain constant in unstimulated VSM, indicating a low total carbohydrate oxidation. Recently, Chace and Odessy[12] demonstrated that addition of various substrates to a glucose supplemented media significantly decreases the rate of glucose oxidation by rabbit aorta. Moreover, they reported that oxidation of endogenous substrates other than glycogen accounted for at least 30% of the basal J_{O_2}[38]. These findings agree with data acquired using radiolabeled glucose to follow carbohydrate metabolism. Hashimoto and Dayton[21] demonstrated that free fatty acids depressed the conversion of glucose carbon to CO_2, however, octonoate had no significant effect on glucose uptake (J_{glu}) by rat aorta. Free fatty acid utilization also has no apparent effect on the rate of lactate production by rabbit aorta[12]. Morrison et al.[36] have found that as little as 1% of ^{14}C-glucose uptake is oxidized to $^{14}CO_2$ and H_2O by swine, rabbit and monkey aorta. The major fraction of glucose is simply catabolized to lactate (80–86%) with approximately 5–10% incorporated into glycogen, 1% into lipid and 5–8% into DNA-RNA-protein fractions.

The apparent coupling between Na-K transport and aerobic glycolysis indicates that the carbohydrate supply is coordinated with alterations in active ion transport. The source of carbohydrate moieties is thought to be derived primarily from glucose uptake[36]. Although glucose uptake is substantial, the intracellular concentration of glucose generally has been shown to be negligible[3]. This observation implies that glucose transport is rate-limiting for its utilization, and that glucose transport must be regulated to match increases in metabolic demand. Alternatively, glycogen could act as a source of substrate during increased activity.

4) The role of glycogen phosphorylase

We studied the effects of alterations in ion transport on glycogen phosphorylase activity and glucose transport to determine if either of these processes could act as the rate-limiting step for aerobic glycolysis. Glycogen phosphorylase is converted from its inactive *b* to active *a* form

upon phosphorylation by phosphorylase kinase. Phosphorylase kinase is also found in an active and inactive form. Phosphorylase kinase is activated by Ca^{++} in concentrations which stimulate mechanical activity, therefore phosphorylase activity generally is correlated with contractile activity[37]. The utilization of glycogen by skeletal and cardiac muscle during contractile activity is well documented[16,20], however, its role as an energy source in smooth muscle is not known with certainty. Although glycogenolysis is generally associated with mechanical activity, contraction can be elicited without concomitant glycogen utilization in rabbit gut and bovine mesenteric artery and tracheal muscle[28]. This dissociation between glycogenolysis and contraction may be attributable to the differential activation of glycogen phosphorylase by the various agents used to stimulate mechanical activity. Elevation of the medium K^+ concentration by 80 mM stimulates mechanical activity, J_{O_2} and J_{lac}, in a variety of VSM[39]. There is also evidence that active Na-K transport is elevated under this condition[1]. In porcine carotid artery and rabbit aorta, glycogen phosphorylase acitivity also was substantially elevated[41]. Ouabain also elicited an increase in phosphorylase activity which was parallel to its stimulatory effect on P_o and J_{O_2}, however as previously stated, aerobic glycolysis was significantly depressed. Similar observations were made for porcine carotid artery[29]. This evidence demonstrates that phosphorylase does not act in limiting the rate of aerobic glycolysis.

β-Adrenergic stimulation with isoproterenol causes a relaxation of existing tone of porcine coronary arteries and rabbit aorta, as well as taenia coli. Isoproterenol alone does not significantly alter phosphorylase activity[41]. Addition of isoproterenol to a porcine coronary artery ring which is mechanically stimulated with elevated K^+ leads to a relaxation of isometric force in parallel to a decrease in J_{O_2}, whereas both phosphorylase activity and J_{lac}remain elevated. These findings support the hypothesis that Ca^{++} is the primary regulator of phosophorylase activity, since the intracellular concentration of Ca^{++} is likely to be elevated during membrane depolarization with elevated K^+ even when mechanical activity is decreased during β-adrenergic stimulation[34,35,41].

Thus, phosphorylase did not appear to act as an important regulatory step for aerobic glycolysis. However, these data are consistent with a coordination of phosphorylase activity and increased oxidative metabolism in association with muscle contraction.

Since J_{lac} demonstrates a consistent coordination with Na-K transport, and lactate production can be altered independently of oxidative metabolism and glycogen phosphorylase activity, we postulated that carbohydrate metabolism is compartmentalized in VSM. To test this hypothesis, we measured glucose uptake and glycogen breakdown and determined the contribution that each could make to the observed rate of lactate production.

5) Compartmentation of glycolysis and glycogenolysis in VSM

Tissue glycogen content was measured to determine if the amount of substrate which was derived from glycogen, during treatment with elevated KCl, could supply the substrate necessary for the observed increased in intermediary metabolism. Stimulation of mechanical activity and intermediary metabolism in the presence of elevated KCl was associated with a rapid glycogenolytic response which was complete within approximately 30 min (fig. 2). The breakdown of glycogen could account for the total increase in the rate of lactate production or the additional substrate necessary for the observed increase in oxidative metabolism. Under the same condition, glucose uptake was stimulated and could also account for the total amount of lactate produced[31]. We then studied the incorporation of radiolabel into lactate from glucose (U-[^{14}C]-glucose) to determine the relative contribution of each substrate source to lactate production. Input into the lactate pool can be derived from various sources (glucose, glycogen, amino acids. Therefore, the lactate specific activity (μCi/mmole) depends on the isotopic input from glucose and the relatively unlabeled input from the other possible sources. If all lactate were derived from medium glucose, a specific activity of 0.5 relative to medium glucose (lactate specific activity/glucose specific activity = lactate specific activity ratio) theoretically would be measured, since two moles of lactate are produced from one mole of glucose via glycolysis. Data in figure 3 show that the lactic acid pool reaches a steady state with the radiolabel ([^{14}C]) at a specific activity ratio of 0.5, within 120 min of incubation. These data demonstrate that the sole source for J_{lac} is glucose which is taken up from the bathing medium, under unstimulated control conditions. This finding is not surprising, since glycogen turnover is not substantial in resting VSM[38]. Approximately 92% of the radiolabel from glucose was incorporated into lactate, which is consistent with the literature. This evidence is also in agreement with the chemically measured lactate production: glucose uptake ratio[31], which indicates that lactate production can account for 93% of glucose uptake. Approximately 6% of the radiolabel was recovered

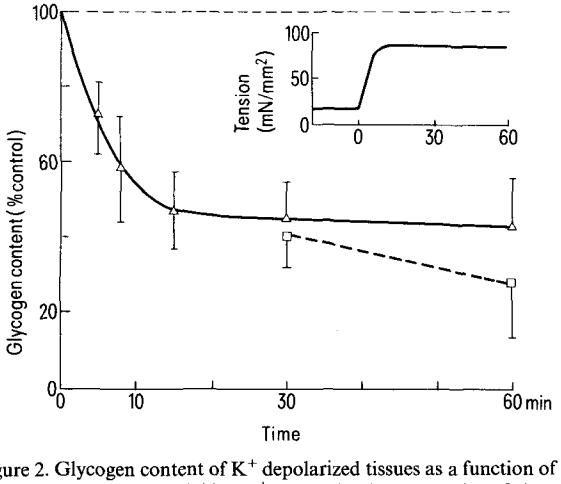

Figure 2. Glycogen content of K^+ depolarized tissues as a function of the duration of treatment with high K^+ (80 mM). The mean value of glycogen content of all control artery segments, based on glucosyl equivalents, (n = 67) was 2.82 ± 0.20 μmoles · g^{-1} (± SEM). The glycogen content of arteries incubated in aerobic (95% O_2) high K^+ (△) and anaerobic (95% N_2) high K^+ (□) media is expressed as a percentage of the glycogen content of their respective experimental control artery segments. The inset diagram displays the time course of a typical K^+ induced isometric contraction of a segment of porcine carotid artery over the same time period during which glycogen content was recorded (from Lynch and Paul[31], with permission).

in glycogen after 120 min of incubation. The average glycogen specific activity was approximately $^1/_{10}$ that of the medium glucose. Certain predictions can be made from the observed pattern of radiolabel incorporation. Since the glycogen specific activity is low compared to that of medium glucose, a substantial amount of unlabeled glucosyl moieties are available for utilization during KCl stimulated glycogenolysis. In this situation, the specific activity of the products of carbohydrate metabolism should be decreased substantially. The specific activity of lactate was measured, after incubation with elevated KCl, to determine the contribution of glycogenolysis to aerobic glycolysis. Artery rings were equilibrated with [^{14}C]-glucose for 120 min prior to treatment, therefore, subsequent alteration of the lactate specific activity ratio would be indicative of changes in the pattern of substrate utilization.

In order to evaluate the precision of these experiments in detecting alterations in the lactate specific activity, equilibrated artery rings were incubated in an anaerobic medium with elevated KCl (80 mM). Under this condition, all glucosyl moieties derived from glycogen must be released as lactate, since oxidative phosphorylation is inhibited by the absence of molecular oxygen. Within 30 min of treatment, the lactate specific activity was decreased to a value of 0.42 ± 0.02 (n = 4) (fig. 3), demonstrating a substantial incorporation of glucosyl units from glycogen into lactate.

Under aerobic conditions, elevation of the medium K^+ concentration by 80 mM was found to have no significant effect on the steady state lactate specific activity ratio (fig. 3), indicating that glucose remained the sole source of substrate for aerobic glycolysis. The constancy of the specific activity ratio indicates that glucosyl moieties

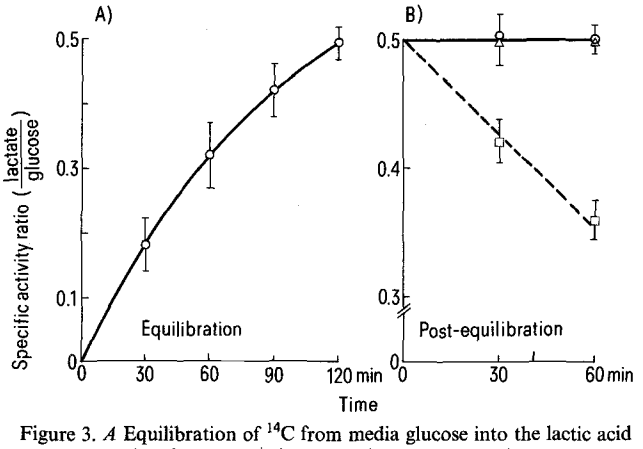

Figure 3. *A* Equilibration of ^{14}C from media glucose into the lactic acid pool and *B* the effect of K^+-depolarization on the steady state lactate specific activity ratio under aerobic and anaerobic conditions. The data are presented as a ratio of lactate to glucose specific activities (lactate sp. act./glucose sp. act.). Two moles of lactate are produced from one mole of glucose via glycolysis; therefore a lactate specific activity ratio of 0.50 indicates that all lactate is derived from glucose, assuming the specific activities of all other possible sources of lactate are substantially lower than that of media glucose. The data for the equilibration period (A) are shown only through 120 min by which time a steady state had been reached. Therefore, all experimental tissues (B) were preincubated in PSS containing U-^{14}C-glucose for at least 120 min prior to treatment, to insure that the label had reached a steady state. Mean values ± SEM are given for the lactate specific activity ratio measured from unstimulated control (○), and K^+ depolarized artery rings under aerobic (△) and anaerobic (□) conditions (from Lynch and Paul[31], with permission).

derived from glycogen degradation are not utilized for lactate production, and therefore do not enter the same pool of glycolytic intermediates as do moieties derived from glucose uptake. This conclusion is valid whether or not preferential degradation of radiolabeled glycogen occurs during the initial minutes of stimulation[30]. Separate enzymatic pathways for glycogenolysis and aerobic glycolysis must be evoked to explain this observation.

6) The coordination of substrate supply with energy transduction

The demonstration of separate enzymatic pathways for the utilization of glucose and glycogen is of itself interesting, since the enzymes and substrates of metabolism historically have been thought to interact in a homogenous cytoplasm[13]. The ramifications of this observation in the realm of the regulation of energy transduction and metabolism are quite pervasive. A schematic figure of this proposed compartmentation is shown in figure 4.

A) Modulation of glucose uptake by Na-K transport

The coordination of lactate production with Na-K transport processes has been documented in VSM as well as various other cell types[19,27,48]. The coupling of this membrane localized active transport mechanism with glycolysis appears to be expressed as a distinct metabolic compartment under basal and KCl stimulated conditions in porcine carotid artery. The primary control point for aerobic glycolysis in VSM is the membrane transport of glucose[3,31], since glucose uptake is rate limiting for glycolysis under these conditions. Therefore, [Na^+] pump function may be interrelated with the regulation of glucose transport. We measured the net rate of uptake of the glucose analog 3-0-methyl D-glucose, as well as lactate production and the intracellular concentration of glucose, to determine if sugar transport were altered under conditions which are known to alter active Na-K transport. Stimulation of active Na-K transport by elevating the K^+ concentration of the bathing medium was found to be associated with a concomitant increase in the rate of 3-0-methyl D-glucose uptake and lactate production, however, glucose transport remained rate limiting for its utilization[31]. Since we had previously demonstrated that glucose is the sole source of substrate for aerobic glycolysis, the observation that lactate production is elevated under this condition independently confirms that glucose transport is regulated in VSM. The nature of the intracellular signal which is responsible for modulating glucose transport is unknown. The turnover of ATP and PCr has been suggested as a regulatory factor in striated muscle[33]. In accordance with this hypothesis, increased energy utilization during activation of the Na-K transport mechanism could regulate glucose uptake. Stimulation of glucose transport in the presence of elevated KCl is consistent with this hypothesis. However glucose transport would be expected to decrease when active ion transport is inhibited, but neither ouabain or incubation in a Na^+ free medium were found to alter glucose uptake. Thus, this hypothesis needs further examination. It also is not likely that the increase in glucose transport is dependent on an increase in the intracellular concentration of Ca^{++} subsequent to membrane depolarization in the presence

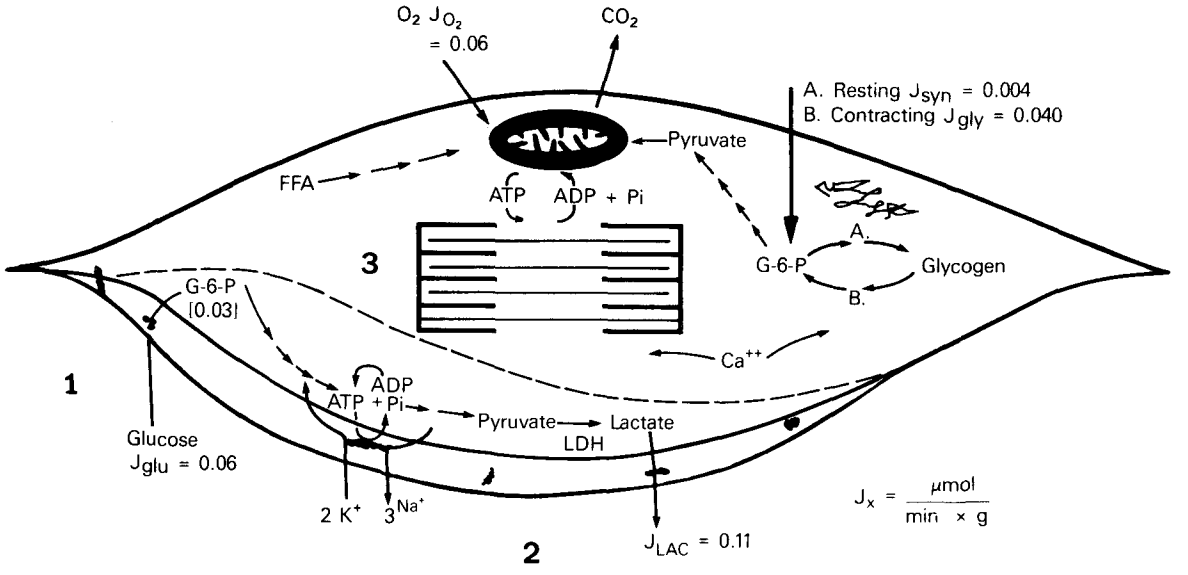

Figure 4. Diagrammatic representation of carbohydrate metabolism by vascular smooth muscle. *1.* Glucose is the sole substrate source for aerobic glycolysis in unstimulated and mechanically activated porcine carotid arteries. Since glucose transport is rate limiting for its utilization, transport must be regulated under conditions were carbohydrate utilization is increased. *2.* Aerobic glycolysis can account for approximately 95% of glucose uptake, and is apparently coupled to Na-K transport at the sarcolemma. The coordination between glucose supply and its utilization, at the membrane level, indicates a sensitive and efficient means of regulating carbohydrate metabolism. *3.* On the other hand, glycogen utilization is coordinated with the increase in the rate of oxidative metabolism which is associated with the stimulation of mechanic activity. Glycogen breakdown is rapid and substantial during the initial minutes of mechanical activity, which may be particularly important for energy production during the presteady state of tension generation. The functional independence of the oxidative and glycolytic components of intermediary metabolism is expressed as a physical compartmentation of substrate utilization during increases in mechanical activity (represented by the dotted line). Although the rate of glycogenolysis is comparable in magnitude to the rate of glucose uptake under this condition, glucose remains the sole source of substrate for aerobic glycolysis. Rates are approximate values, expressed as μmol/min · g. The stimulated rate of glycogenolysis (J_{gly}) is time averaged over the initial 30 min of treatment.

of elevated [K^+], since neither ouabain nor incubation in [Na^+]-free media were found to alter glucose transport although both treatments stimulate mechanical activity. On the other hand, incubation with ouabain or in [Na^+]-free media lead to the accumulation of glucose in the intracellular space, subsequent to the inhibition of Na-K transport[31]. These data indicate that the inhibition of active Na-K transport directly decreases glucose utilization by shifting the rate limiting step for glycolysis from glucose transport to a step in the glycolytic pathway. This is consistent with the hypothesis that aerobic glycolysis is regulated by alterations in Na-K transport.

The compartmentation of glycolysis with Na-K transport offers a sensitive and efficient means of coordinating energy transduction with production. If direct enzyme coupling at the plasmalemma is responsible for this coordination, as found for the human RBC[32], then the ability of cellular metabolism to quickly and efficiently respond to changes in active ion transport could be maximized. Since VSM tone is dependent on its resting membrane potential[24], this distinct mode of coupling metabolism to ion gradients is a particularly sensitive control point for excitation-contraction coupling. This may be of significance to the understanding of certain vascular myopathies, such as hypertension and atherosclerosis, in which alterations in both ion transport[17,23] and carbohydrate metabolism[14,36] have been reported.

B) Control of substrate utilization during isometric contraction

The linear relation between the rate of oxygen consumption and isometric force in VSM has been recognized for about a decade[39]. However, the substrate utilized for the maintenance of mechanical activity has yet to be defined adequately. Oxidation of glucose, which is taken up from the bathing medium, can account for only a small amount of the resting or activated ATP production. Moreover, glucose utilization decreases whereas oxygen consumption and isometric tension increase during inhibition of Na-K transport[29,31]. These observations indicate that glucose is not directly utilized as a substrate for oxidative phosphorylation. However, measurement of glycogen turnover indicates that this substrate pool could be important during the initial minutes of an isometric contraction. Activation of glycogen phosphorylase and glycogenolysis concomitant with the stimulation of mechanical activity was consistently observed for porcine carotid artery[29]. Moreover, glycogen degradation is rapid and substantial during KCl stimulated mechanical activity, yet little if any of this substrate is utilized for the production of lactate. Since all lactate is derived from media glucose and glycogen degradation is substantial, glycogen is most likely oxidized. The activation of phosphorylase is likely to be communicated by the increase in the intracellular Ca^{++} concentration concomitant with the activation of actin-myosin interaction. This mechanism assures the availability of substrate for the increase in oxidative metabolism. The coordination of this substantial glycogenolytic response with the increase in oxidative metabolism during the initial minutes of tension generation may be especially important in relation to the energetic properties of actin-myosin interaction in the presteady state.

Generally, measurements of J_{O_2}, phosphorylase activity, J_{lac}, and P_o are made under steady state conditions. These data supply information on the mechanisms which are

responsible for the maintenance of the existing state of VSM, i.e., either resting or mechanically activated. However, there are transient responses in which these parameters reach maximal values during the initial minutes of stimulation prior to the attainment of the steady state[42]. The unloaded shortening velocity (V_{us}) of VSM, which is an intrinsic measure of the myosin ATPase activity, also reaches a maximum within the first minute of stimulation and then decreases to a steady state value prior to the attainment of maximal isometric tension[15,26]. J_{O_2} exhibits a similar transient behavior decreasing to the steady state within 5–10 min of activation[26]. If the J_{O_2} measurements are corrected for the temporal delays introduced by diffusion and the response of the oxygen electrode, then the measured time to peak values of J_{O_2} and V_{us} differ by only 60 s. This lag time may be due to inherent differences in the methods of measurement. The important observation for this discussion is that ATP utilization by the myosin ATPase and ATP production via oxidative phosphorylation rapidly increase to maximal levels which then decline 2–3-fold to the steady state. This sudden and substantial increase in oxidative metabolism is apparently coordinated with the rapid rate of glycogen degradation, although the temporal relation exhibits a significant lag time. However, a portion of the lag time is likely to be related to agonist diffusion and inherent differences in these measurements. Thus, it is highly likely that glycogen is utilized as an oxidative substrate in coordination with muscle activation and early steady state tension maintenance.

7) Summary

Early investigations into the nature of the coupling between energy transduction and metabolism in smooth muscle, particularly from the laboratories of Bülbring and Lundholm, suggested that specific metabolic pathways could independently supply energy for ion transport and actin-myosin interactions. Subsequent work has solidified the concept that oxidative phosphorylation is specifically coupled to tension generation and maintenance, whereas, aerobic glycolysis is not only a vital characteristic of smooth muscle metabolism, but also is likely to be independently coupled to Na-K transport at the plasmalemma. The independence of oxidative and glycolytic metabolism is reflected as a compartmentation of carbohydrate metabolism in the porcine carotid artery. The coupling of these independent metabolic pathways with specific energy utilizing processes, indicates a means by which energy production and transduction can be closely and efficiently regulated. The coupling of glycogenolysis to mitochondrial respiration may have evolved as a direct response to the energetic needs of VSM. That is, the large glycogenolytic response in the initial minutes of stimulation may be necessary to maximize the cellular production of ATP during the presteady state.

Likewise, the coupling between aerobic glycolysis and Na-K transport indicates a sensitive and efficient means of coordinating energy metabolism with ion transport at the membrane level. Additionally, the regulation of substrate supply, i.e. glucose transport, also may be closely coordinated with changes in ion transport. One may speculate that alterations in the microenvironment of each compartment can independently regulate intermediary metabolism and therefore allow the cell to quickly and efficiently respond to localized stimuli. Thus, stimulation of Na-K transport could effectively regulate energy production at the membrane level without mobilizing or competing with the energy transduction of other cellular processes. This compartmentation of energy utilization may be highly advantageous, since oxidative metabolism is closely coordinated with mechanical activity and therefore regulation of blood flow.

Future investigations will attempt to elucidate which intracellular signals which are responsible for the regulation of these functionally independent compartments of energy metabolism and transduction in VSM. In more general terms, our findings provide a basis from which future questions concerning the regulation of cellular metabolism must be directed. The cellular cytoplasm can no longer be envisioned as a homogenous compartment, but rather a complex array of functional subcompartments which may be individually regulated. What are the advantages of compartmentation in terms of metabolic efficiency and the response of energy metabolism to localized stimuli? Do compartments confer individualistic responses to physiological regulators, and what are the factors which are involved in the evolution of specialized cellular compartments for a given cell type? These questions need to be approached at the level of cellular organization and function for which VSM appears well suited.

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