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I-3 Compartmentation of ATP synthesis and utilization in smooth muscle: roles of aerobic glycolysis and creatine kinase

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Abstract

The phosphocreatine content of smooth muscle is of similar magnitude to ATP. Thus the function of the creatine kinase system in this tissue cannot simply be regarded as an energy buffer. Thus an understanding of its role in smooth muscle behavior can point to CK function in other systems. From our perspective CK function in smooth muscle is one example of a more general phenomenon, that of the co-localization of ATP synthesis and utilization. In an interesting and analogous fashion distinct glycolytic cascades are also localized in regions of the cell with specialized energy requirements. Similar to CK, glycolytic enzymes are known to be localized on thin filaments, sarcoplasmic reticulum and plasma membrane. In this chapter we will describe the relations between glycolysis and smooth muscle function and compare and contrast to that of the CK system. Our goal is to more fully understand the significance of the compartmentation of distinct pathways for ATP synthesis with specific functions in smooth muscle. This organization of metabolism and function seen most clearly in smooth muscle is likely representative of many other cell types. (Mol Cell Biochem **133/134:** 39–50, 1994)

Key words: smooth muscle, creatine kinase, glycolysis, oxidative metabolism, compartmentation

Introduction

As the phosphocreatine content of smooth muscle is of similar magnitude to ATP, the function of the creatine kinase system in this tissue cannot simply be regarded as an energy buffer. Thus understanding its role in smooth muscle behavior can point to CK function in other systems. From our perspective CK function in smooth muscle is related to the co-localization of ATP synthesis and utilization.

In an interesting and analogous fashion distinct glycolytic cascades also appear to be localized in the regions of the cells with specialized energy requirements. Similar to CK, glycolytic enzymes are known to be localized on the thin filaments, sarcoplasmic reticulum and plasma membrane. In this chapter we will describe the relations between glycolysis and smooth muscle function and compare and contrast to that of the CK system. Our goal is to more fully understand the significance of the compartmentation of ATP synthesis and utilization in smooth muscle.

Aerobic glycolysis and smooth muscle function

Vascular smooth muscle is primarily an oxidative tissue, that is, most of its ATP requirements (> 70%) are calculated to be met by oxidative phosphorylation [1]. However even under fully aerobic conditions, a substantial production of lactate is characteristic of smooth muscle metabolism. The rate of this aerobic lactate production in smooth muscle on a molar basis is similar in magnitude to that of oxygen consumption, and thus it would generate about 20% of the ATP. The production of lactate under aerobic conditions may be similar in other cell types, for example in cardiac or skeletal muscle [2]. However the rate of oxidative metabolism in these cell types is considerably higher than in smooth muscle and their aerobic glycolysis is thus a much smaller component of total metabolism and often overlooked.

The role of this anomalous aerobic glycolysis has long been subject of speculation [1]. Obvious explanations, such as lack of mitochondrial capacity or incomplete oxygenation do not appear applicable. Our work in this area began over a decade ago, when we reported a correlation between the rate of aerobic glycolysis and the activity of the Na-K pump in coronary artery [3]. Oxygen consumption, on the other hand, was found to be strongly correlated with the level of active isometric force, presumably reflecting the energy requirements of the actin-myosin ATPase. For example, ouabain, an inhibitor of the Na-K pump, abolished lactate production, while oxidative metabolism and also force were increased [4]. More recently, quantitative studies of this correlation were undertaken [5]. Na-K pump activity was quantitated by measuring K^+-uptake after readmission of K^+ to K^+-depleted carotid arteries. In the physiological range of pump rates, aerobic glycolysis is its primary energy source and lactate production is stoichiometrically linked to the Na-K pump, i.e., 2 K^+ ions per ATP per lactate. Strikingly, the readmission of K^+ was associated first with a stimulation of lactate production, followed by activation of oxidative metabolism only after the aerobic lactate production was saturated. This 'reverse' Pasteur effect is opposite to that expected from standard biochemistry in which glycolysis is activated only after oxidative capacity is exceeded.

The mechanisms underlying this functional compartmentation of aerobic glycolysis and membrane energy requirements would not appear compatible with the usual notion that glycolytic enzymes are uniformly distributed throughout the 'cytosol'. We investigated this coupling by studying the nature of the substrate underlying aerobic glycolysis. Smooth muscle has a substantial glucose uptake [6] as well as a fully competent mechanism for glycogen utilization [7]. Using radiolabelled glucose, we found that over 90% of the label appeared in the lactate pool. Under stimulated conditions, glucose uptake and glycogen catabolism occur at similar rates, suggesting that lactate could arise from either or both substrates. Surprisingly, the specific activity of lactate was identical to that of glucose, indicating that all of the lactate production originated from glucose, despite the entry of glucosyl moieties from glycogen into the glycolytic pathway [8]. Thus even in the absence of identifiable barriers and the small size of vascular smooth muscle cells relative to diffusion, glycolytic intermediates arising from glucose did not mix with the pool of intermediates originating from glycogen. Measurement of the specific activity of glucose-6-phosphate lent further support to this hypothesis [9]. Under anaerobic conditions, a detectable decrease in the specific activity of lactate was observed, indicating that unlabelled lactate arising from glycogen was produced. Recent studies of Hardin et al. [10] provide further support for the separation of glycogenolytic and glycolytic metabolism. In this study, carbon 13 NMR techniques were used to identify the fate of glycogen and glucose carbon. The NMR label in lactate again originated solely from glucose whereas the decrease in labelled glycogen was attributed to its oxidation to CO_2. Thus the functional compartmentation of glycolysis and membrane energetics and oxidative metabolism with force appears to reflect this compartmentation of glycolytic and glycogenolytic metabolism.

Perhaps at an initial glance, a most puzzling aspect of this compartmentation is that it is not absolute. It has long been known that ionic gradients in smooth muscle can be maintained in the absence of glucose [cf. 5]. It would appear that in the absence of glycolytic production of ATP, oxidative sources can be utilized. We would suggest then that rather than an all or none type phenomenon, compartmentation leads to a more efficient coupling between energy supply and transduction. This is more difficult to demonstrate and less well documented than the compartmentation phenomenon itself, but there are a number of studies to this end. Lynch and Balaban [11] showed that the V_{max} of the Na-K pump in MDCK cells was about twice as large in the presence of both oxidative and glycolytic substrates than with substrate for oxidative metabolism alone. We showed that the restoration of K^+ gradient in K^+-depleted carotid ar-

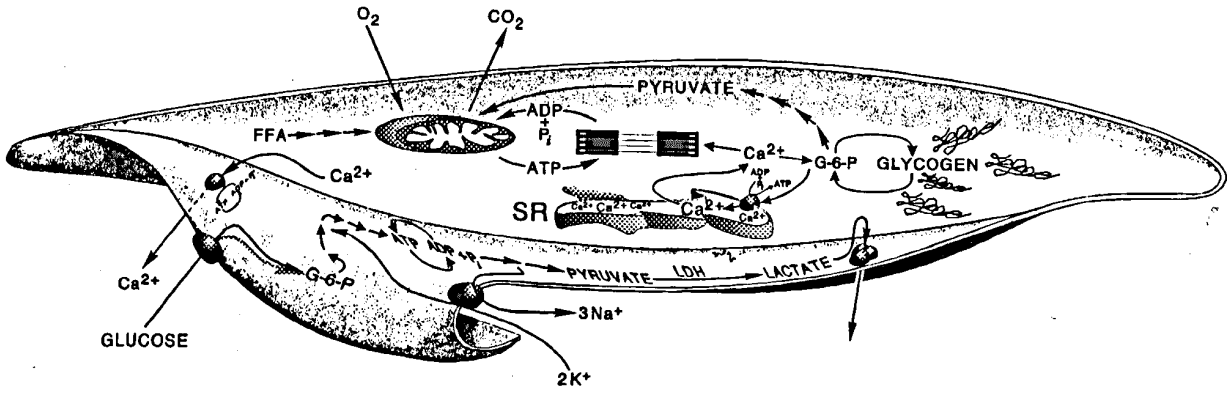

Fig. 1. Model of metabolic compartmentation. Localization of glycolytic enzyme cascades at the plasmalemma and sarcoplasmic reticulum. This schematic of enzyme localization is proposed to underlie the coupling of aerobic glycolysis and membrane energy requirements, whereas oxidative metabolism is more strongly correlated with the level of isometric force.

teries was faster in the presence of glucose, presumably reflecting a similar increase in efficacy of the Na-K pump [5]. In cardiac muscle, ATP from glycolytic sources has been reported to be more effective than that from oxidative sources in closing ATP-dependent K^+-channels [12]. Thus coupling of glycolysis to membrane energetics may be a more general phenomenon not limited solely to smooth muscle.

Our working hypothesis is that these observations are attributable to co-localization of ATP synthesis and utilization systems. We tested this hypothesis using a model system, that of a purified plasma membrane preparation from smooth muscle [13]. Despite purification steps involving 0.6 M KCl, a complete glycolytic enzyme cascade was retained in this membrane preparation. Moreover, with fructose-1,6 diphosphate as substrate, this cascade was functional in terms of production of lactate. Of particular interest was whether this endogenous glycolytic cascade could support membrane energetics, which in this case was measured in terms of Ca^{2+}-pump activity. ATP, as expected, supported Ca^{2+}-uptake. Fructose-1,6 diphosphate also supported uptake, despite the fact that measured bath concentration of ATP produced by this substrate were low (10–30 μM). Moreover, the rate of Ca^{2+}-uptake supported by glycolysis was greater than that supported by ATP infused from an external pump at the same rate as produced by the glycolytic cascade [14]. This suggested that the local ATP concentration produced by the glycolytic cascade in the vicinity of the Ca^{2+}-ATPase might be of greater importance to pump function. We tested the importance of localized ATP production using an immobilized, hexokinase-based ATP trap. Ca^{2+}-uptake was not supported by ATP in the presence of the trap, but significant Ca^{2+}-uptake could be supported by fructose-1,6 diphosphate. Minimally this indicates that ATP produced by the endogenous glycolytic cascade is more accessible to the pump, than to the ATP-trap in the solution. Thus in this model system, co-localization of both the glycolytic enzyme cascade and the Ca^{2+}-pump would appear to optimize function and may underlie the functional compartmentation observed in our studies of intact vascular tissue. A model summarizing our views of smooth muscle metabolic compartmentation based on these studies is shown in Fig. 1.

In addition to the preferential support of the Ca^{2+}-pump by fructose-1,6 diphosphate, we also found that phosphoenolpyruvate and importantly phosphocreatine could also support Ca^{2+}-pump activity in the presence of an ATP trap. There was substantial creatine kinase activity in these preparations [14] and its ability to support Ca^{2+}-pump function was similar to that reported for the sarcoplasmic reticulum in striated muscle [15–17]. It is possible that these different ATP producing systems might communicate to the Ca^{2+}-pump via a common, localized ATP pool as suggested by the work of Hoffman and colleagues [18]. Further discussion of the connection or disconnections between the creatine kinase system and glycolytic and oxidative metabolism will be pursued after the ensuing discussion of our studies of smooth muscle creatine kinase.

Compartmentation of creatine kinases in smooth muscle

Smooth muscle is characterized by a remarkably low tension cost, that is, (its rate of ATP utilization per unit isometric force maintained may be up to 1000-fold less than skeletal muscle. Though economical, smooth mus-

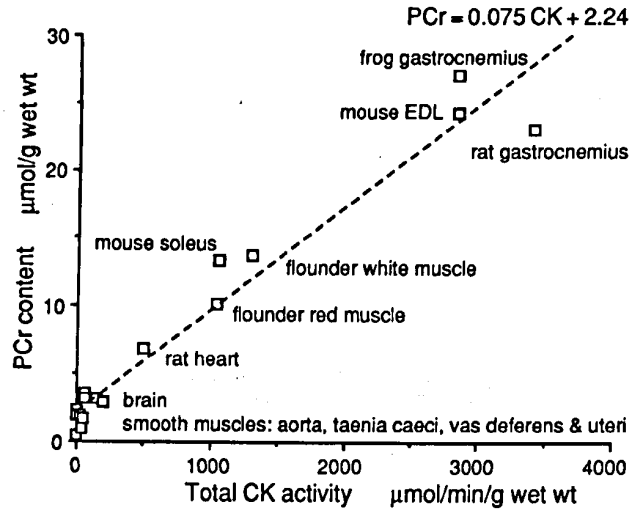

Fig. 2. Relationship between PCr content and total CK activity in various tissues. Values are taken from the review of Iyengar (1984). Our values for guinea pig aorta, taenia caeci and vas deferens are added: the CK activities (Ishida et al., 1991b), PCr for taenia (Ishida and Paul, 1990) and PCr values for aorta and vas deferens (0.5 and 1.9 µmol/g, respectively; Ishida, unpublished). A dashed line represents the linear regression line (r = 0.976).

cle is even more immediately dependent on a continuous supply of metabolic energy for normal function than skeletal muscle. This is primarily due to fact that the preformed pool of high-energy phosphate compounds, phosphocreatine (PCr) and ATP, is an order of magnitude lower in smooth muscle than in striated muscle. In particular, the reported PCr content of various smooth muscle is small, 0.1–4 µmol/g wet weight, and is about 0.5–2 times the content of ATP [1].

Iyengar [19] pointed out that the amount of PCr in a tissue is linearly related to its creatine kinase (CK) activity. Fig. 2 shows this linear relationship between PCr content and CK activity among various tissues including guinea pig smooth muscle. The intestinal smooth muscle of guinea pig taenia caeci contains similar amounts of PCr and CK as that found in rat brain. On the other hand, the vascular smooth muscle of the guinea pig aorta possesses much less. Presumably, the smaller amounts of PCr and CK in the aorta are related to the much smaller ATP utilization upon contraction of tonic, conduit arteries like hog coronary artery [20].

Lohmann equilibrium and high-energy phosphate compounds in smooth muscle

Muscle contraction requires ATP. Direct evidence for ATP utilization in the intact muscle was first shown by experiments using 1-fluoro-2,4-dinitrobenzene (FDNB), a CK inhibitor [21]. Unless muscle metabolism is adequately suppressed, changes in ATP content directly associated with muscle contraction are not observed. Similarly, ATP and PCr levels of many smooth muscles are scarcely altered by the contractile stimulus in aerobic medium with substrate at 37° C [22, 23]. For example, ATP and PCr levels of guinea pig taenia caeci and porcine vascular smooth muscles (coronary and carotid arteries) stimulated for 30 min in high potassium were not significantly different from control [24–26]. Measurements of pyridine nucleotide, flavoprotein and fura-2-Ca^{2+} fluorescence suggest that the stimulation-induced elevation of the cytoplasmic Ca^{2+} concentration could independently activate glycogenolysis, oxidative phosphorylation and actomyosin ATPase; activation of the metabolic pathways preceded activation of the contractile proteins [27]. Therefore, a physiological temperature in tonic smooth muscle, the rate of aerobic ATP synthesis matches the increased ATP breakdown upon stimulation, resulting in virtually no change in ATP + PCr content of the muscle.

Rat portal vein, which is characterized by rapid phasic contractions and a relatively high ATPase activity, appears to be an exception in that PCr breakdown during contraction can be measured at 37° C [28]. At 23° C, at which resynthesis may be impeded, a loss of PCr, but not ATP, was reported using NMR techniques in the rabbit bladder as well as taenia coli [29]. These reported preferential losses of PCr in smooth muscle are well predicted by the Lohmann equilibrium. In contrast, at 18° C, after electrical stimulation, an initial ATP reduction followed by a decline in PCr was reported in the rabbit taenia coli and the initial phase of changes in ATP and PCr apparently deviated from the Lohmann equilibrium [30]. Whether this deviation directly correlates to the compartmentation of metabolites and enzymes is ambiguous, for at low temperatures the ATP utilization by the contractile proteins may start earlier than the onset of CK reaction.

Changes in ATP and PCr levels of smooth muscle were much more clearly observed when the muscle was exposed to poisons or metabolic inhibiting conditions such as hypoxia and glucose-depletion. Administration of 0.8 mM DNFB elicited a gradual decline in ATP, but not PCr, in electrically stimulated bovine carotid artery [31, 32]. Also, the presence of 0.4 mM DNFB for 60 min elicited a marked loss of ATP of 95% of control but did not alter the PCr level in non-stimulated guinea pig taenia caeci (Ishida and Paul, unpublished data). These re-

sults indicate that CK is capable of catalyzing the transfer of high energy phosphate between creatine and adenylate in non-DNFB treated smooth muscle.

Exposure of various smooth muscles to conditions inhibiting oxidative phosphorylation, such as hypoxia (N_2 bubbling instead of O_2), cyanide, etc., has been reported to elicit a loss of PCr [25, 29, 33–38]. In guinea pig taenia caeci with or without contractile stimulation, imposition of hypoxia elicited a significant ATP loss only after PCr was reduced to approximately 25% of control [34, 25]. This early loss in PCr followed by an ATP loss with a lag time is consistent with conversion of PCr to ATP and the equilibrium constant of Lohmann reaction.

In guinea pig taenia caeci exposed to hypoxia, raising the glucose concentration of the medium from 5.5 to 55 mM dose-dependently elevated the ATP content from ~ 50 to ~ 80% of the level under normoxia, and increased lactate production [39]. Whereas, under hypoxia, the PCr content of the taenia was not significantly elevated by high glucose concentrations [25, 37]. This preferential ATP production observed after increasing the glucose concentration under hypoxia seemed to follow the Lohmann equilibrium during the recovery process.

On the other hand, when oxygen was readmitted after hypoxia in the guinea pig taenia caeci, PCr increased rapidly and overshot the original level in 5 min after reoxygenation [37]. The ATP content also gradually increased and attained 75% of the original level. The relation between PCr and ATP in the taenia during reoxygenation, however, differed substantially from those after imposition of hypoxia and after increasing glucose under hypoxia. This rapid increase in PCr could not be predicted by the Lohmann equilibrium. Although the incomplete recovery ATP and the overshoot of PCr could be explained by the hypoxia-induced reduction of the total adenylate content of the muscle to 70% of control, the observed difference in the relations between PCr and ATP after imposition of hypoxia, or increasing glucose under hypoxia, and that after reoxygenation could not completely be defined by the effects of pH, loss of creatine and insufficient creatine kinase, as discussed previously [37].

When substrate, glucose in most experiments *in vitro,* was removed from the medium bubbled with oxygen, the PCr and ATP content of guinea pig taenia caeci smooth muscle also decreased. Under glucose-depleted conditions in the presence of oxygen, when the PCr and ATP of the muscle were partially reduced, a subsequent imposition of hypoxia was reported to elicit a further rapid decrease in PCr and reoxygenation elicited a rapid recovery of the PCr content of guinea pig taenia caeci [40]. In our recent experiments, 60 min after exposure of taenia caeci to aerobic, glucose-depleted conditions in the presence of high potassium, the PCr content was still about 2.5 times greater, but the ATP content was smaller, than that in the muscle exposed to hypoxia for 15 min (Ishida, unpublished data). Similar to hypoxia, a gradual decrease in PCr of the taenia caeci was reported after glucose-depletion using NMR techniques [35]. These results also suggest that the relations between PCr and ATP after exposure of the muscle to substrate depletion are apparently different from that seen after imposition of hypoxia. Since an appreciable amount of oxygen consumption still persisted in the taenia in glucose-free medium [41], the rapid loss of PCr after hypoxia and its gradual loss after glucose depletion further suggest that the PCr production of the taenia is highly dependent on oxidative metabolism.

Similarly in vascular smooth muscle, the change in PCr content measured using NMR techniques was reported to be more resistant to substrate depletion compared to hypoxic conditions in hog carotid artery [36, 42] and in the sheep aorta [10]. Biochemical determination of PCr and ATP in the guinea pig aorta also showed similar results as seen in NMR studies (Ishida, unpublished data). A marked loss of PCr after hypoxia was also observed in the rabbit aorta [38]. In the sheep aorta, oxygen consumption was reported to increase after glucose removal [10]. In the hog coronary artery, a substantial amount of oxygen consumption was maintained even in the glucose-free medium plus 2-deoxyglucose, an inhibitor of glycolysis [24]. These results obtained in vascular and intestinal smooth muscles suggest that, under glucose-depleted conditions, the remaining oxidative metabolism effectively supplies PCr. These observations demonstrating a strong association between PCr production and oxidative phosphorylation led us to investigate the presence and localization of CK isoenzymes in smooth muscle.

Identification and localization of CK isoenzymes in smooth muscle

Since the early demonstration of CK in chicken gizzard smooth muscle [43], the isoforms of BB-, MB- and MM-CK have also been detected in smooth muscles [19, 44–49]. BB-CK is the predominant isoform in all smooth muscles investigated. In smooth muscle, some MB- or

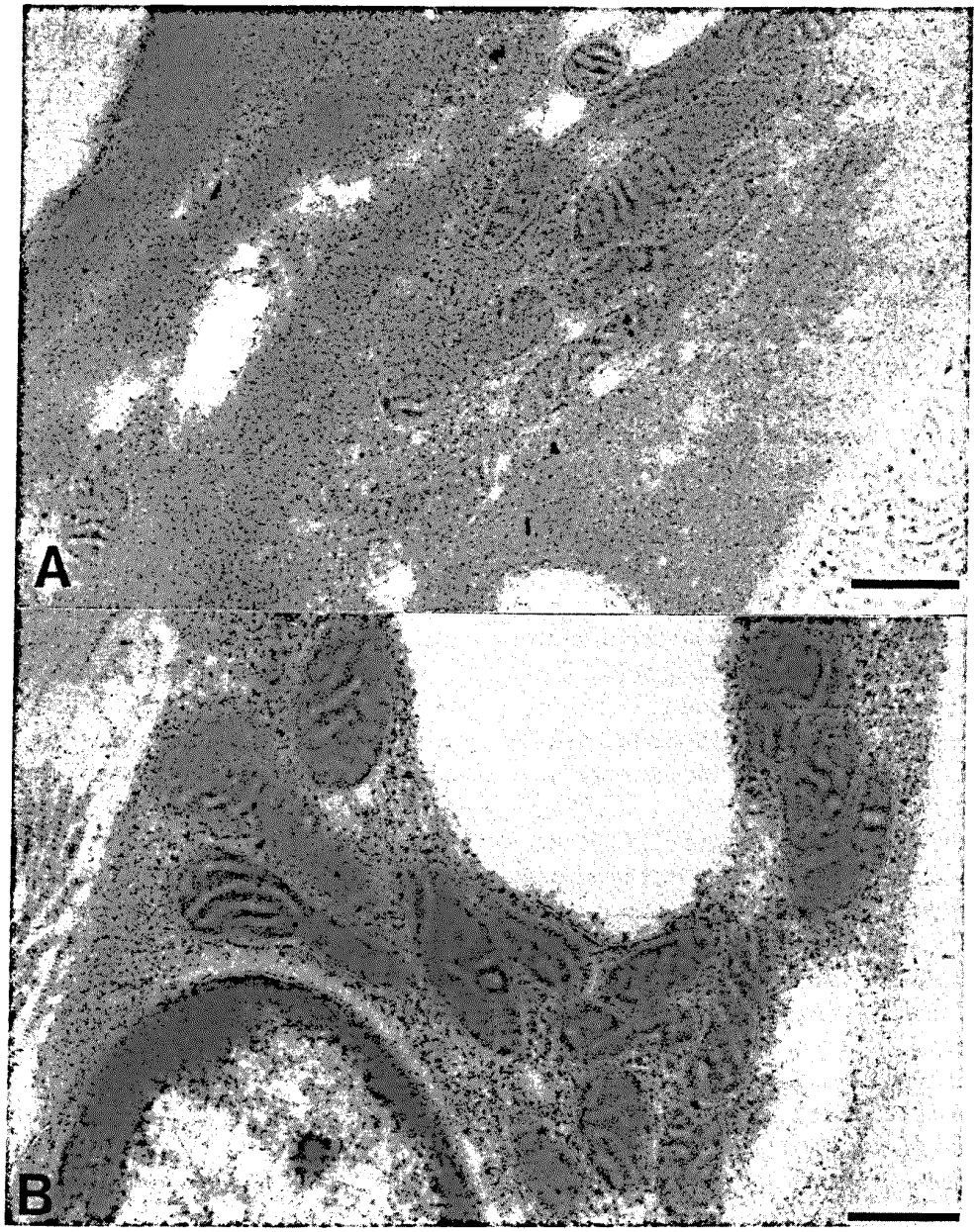

Fig. 3. Immunolocalization of mitochondrial creatine kinase (Mi-CK) in guinea pig aorta. Ultrathin cryosections were incubated with anti-chicken cardiac Mi-CK antibodies followed by goat anti-rabbit IgG coupled with 10 nm gold particles. Gold particles are specifically associated only with mitochondria. Bars indicate 0.5 μm in A and 0.3 μm in B.

even MM-CK could be found but when identified, they are of low abundance.

Another important CK isoform in terms of energetics is mitochondrial CK (Mi-CK). An appreciable amount of CK activity was detected in the enriched mitochondrial fractions of some smooth muscles [37, 50, 51]. Recently, using cellulose polyacetate electrophoresis, Mi-CK predominantly in the octameric form was identified in mitochondrial fractions prepared from guinea pig aorta, taenia caeci and vas deferens [48]. In the guinea pig, smooth muscle Mi-CK migrated more slowly in cathodic direction than cardiac muscle Mi-CK, and it was relatively resistant to dimerizing conditions, suggesting that smooth muscle Mi-CK possesses properties more similar to the brain isoform than to cardiac Mi-CK.

Smooth muscle tissue usually contains a substantial number of nerve cells. Thus, we tried to directly show the localization of Mi-CK in smooth muscle mitochondria using immunoelectron microscopy [52]. Fig. 3 shows that immunogold labeling of cryosectioned gui-

nea pig aorta, using an anti-chicken cardiac Mi-CK antibody. The immunogold specifically labeled the aortic mitochondria. Similarly, specific immunogold labeling of mitochondria was also observed in guinea pig taenia caeci and vas deferens. Fig. 3 further shows that a substantial number of gold particles seemed to be located in areas where the inner and outer mitochondrial membranes are in close proximity. These results are consistent with recent reports indicating that octameric Mi-CK may accumulate within the contact sites [53–56].

On the other hand, when cryosections of guinea pig taenia caeci were stained with anti-chicken BB-CK antibodies, immunogold labeling was observed in the cytosol. Although substantial amounts of BB-CK in the taenia caeci were rather soluble, thus cytosolic, the myofibrils of this smooth muscle were also clearly labeled, with a substantial amount of immunogold particles closely associated with the thick filaments of the taenia caeci. This is corroborated by a recent report which showed that after Triton X-100 skinning, only BB-CK, but not MB- or MM-CK, was left in guinea pig taenia caeci [49]. Thus, it will be interesting to test whether some of the BB-CK present in smooth muscle is indeed an integral protein of the contractile apparatus. This may be analogous to the MM-CK in skeletal muscle which is a genuine structural element of the M-line, i.e., representing the M4 m-bridges [57–59]. In addition, on these cryosections significant immuno-reactivity was also observed on mitochondria, presumably due to cross-reactivity of the anti-chicken BB-CK antibody with Mi-CK, a phenomenon observed previously (Riesinger, unpublished data).

The data show an isoenzyme-specific compartmentation of CK isoenzymes in smooth muscle and thus provide the cytological evidence for the phosphocreatine circuit model [60] in smooth muscle, a scheme for phosphagen transfer from mitochondria to the cytoplasm via Mi-CK at mitochondrial sites and cytosolic CK (predominantly BB-CK) at myofibrillar sites.

Physiological significance of CK compartmentation in smooth muscle

Compared with skeletal muscle, smooth muscle contractions are much longer in duration. Tonic smooth muscle contractions can last for hours and are accompanied by sustained increases in lactate production and oxygen consumption, suggesting a relatively tight coupling between contractile response and metabolism in smooth muscle. In these tonic muscles, PCr may not be simply metabolized as a dead-end product in reactions of phosphorylation and dephosphorylation via ATP, although its concentration in smooth muscle cell is small. Interestingly, chicken gizzard smooth muscle is characterized with short lasting (< 1 min) contractile activity [61, 62]. It does not possess Mi-CK, it cannot be detected even in the mitochondrial fraction, but only BB-CK [52]. Presumably, the presence of Mi-CK in mitochondria and BB-CK in the cytoplasm of smooth muscle presumably provides evidence for an active role of PCr as a shuttle compound between the mitochondrial ATP producing site and the cytoplasmic energy transducing sites. PCr produced by mitochondria moves to the cytoplasm and recycles back to mitochondria as creatine after its phosphate is transferred to adenylate in the cytoplasm. This may facilitate the maintenance of tension in smooth muscle over long periods without fatigue.

Guinea pig taenia caeci exposed to high potassium contract and can maintain maximum levels of force for more than 60 min. With maximal stimulation, oxygen consumption of this muscle increased from 0.25 to 0.45 μmol/min/g wet wt at 37° C [25], and the maximum calculated ATP turnover rate was approximately 2.7 μmol/min/g wet wt. The CK activity of a mitochondrial fraction isolated from taenia caeci was approximately 1 μmol/min/mg mitochondrial protein at 37° C [37, 48]. Assuming a mitochondrial content of 5 mg/gwet wt, as estimated in the canine tracheal smooth muscle [63], the Mi-CK activity seems to well match the oxygen consumption rate in the taenia caeci.

In contrast, the total CK activity, ~ 150 μmol/min/g wet wt at 25° C in guinea pig taenia caeci, which is predominately cytosolic BB-CK activity, is much higher than the maximally activated ATP turnover rate (~ 5 μmol/min/g wet wt at 37° C) calculated from rates of oxygen consumption and lactate production [25, 48]. Recently, Clark et al. [49] reported that after Triton X-100 skinning, only BB-CK was left in the taenia caeci. Although the relevance of this high activity of BB-CK is not known at present, it will be interesting to investigate whether in smooth muscle a fraction of the cytosolic BB-CK is also compartmentalized and bound to subcellular locations with high energy turnover, taking a dual role in enzymatic reactions and structural formation of myofilaments and organelles [60, 64].

Clarifying a more direct role of CK isoenzymes for contractile function of smooth muscle is an ultimate goal of our studies. Earlier, Born [34] reported that the hypoxia-induced reduction in PCr content, but not ATP,

was linearly correlated with spontaneously developed tension, in guinea pig taenia caeci. We also [25, 37] reported that after imposition of hypoxia, PCr loss was associated with reduction of active tension induced by high potassium. Based on several lines of evidence we concluded that the reduction in force with hypoxia was a straightforward consequence of energy limitation. Clark et al. [49] reported that substantial BB-CK remained after Triton X-100 skinning of the taenia caeci. This remaining BB-CK was sufficient to support a near maximal contraction when the skinned taenia caeci was bathed in a medium containing PCr and ADP. Moreover, BB-CK was reported to have a low Km for PCr compared with the MM-CK isoform [65, 66]. These results suggest that BB-CK could take a more direct role for contraction in taenia caeci, producing ATP though rephosphorylation of ADP by PCr in a localized contractile protein compartment. Since the contractile response of taenia caeci requires relatively more energy than vascular smooth muscles [20, 67], the production of PCr via Mi-CK may limit the BB-CK reaction at the contractile protein site, thus eliciting a relaxation of the taenia caeci.

Interestingly, the effects of hypoxia are dependent on the type of stimulation. For example, vasoconstrictions induced by high potassium, in contrast to agonists such as norepinephrine, show a different sensitivity to oxygen. In the rabbit aorta, hypoxia and cyanide preferentially inhibited norepinephrine-induced contraction but elicited only a small inhibition of a potassium-induced contraction [68]. This suggests that an energy limitation at the contractile protein level is not directly associated with the vasorelaxation induced by the inhibition of oxidative phosphorylation.

Covalent modification via phosphorylation [66] may also play a role in defining the function of BB-CK in smooth muscle. Recently, BB-CK has been shown to be phosphorylated by protein kinase C and this phosphorylation lowered the Km for PCr [69]. During a norepinephrine contraction in rabbit aorta, hypoxia inhibited the formation of inositol phosphate and myosin light chain phosphorylation in addition to reducing its PCr content [38, 70]. Therefore, in vascular smooth muscle inhibition of oxidative phosphorylation may cooperatively suppress inositol phosphate formation shortly after receptor stimulation and reduce the affinity of BB-CK for PCr in the vicinity of the contractile proteins. This could result in a more effective vasorelaxation in the presence of agonist than in the presence of high potassium. However, the exact role of CK isoenzymes in vascular smooth muscle remains speculative.

In contrast to the sharp loss of force and PCr induced by hypoxia, the level of ATP did not decline drastically and would appear to remain above that required for many cellular process, including in particular, contractile protein function in taenia caeci [25, 34, 37] and rabbit aorta [38]. ATP has been reported to be present in a bound form in the cell [71, 72]. Therefore, some ATP may exist in a form or functional compartment not readily accessible to the contractile proteins.

On the roles of aerobic glycolysis and creatine kinase in smooth muscle energetics

ATP originating from oxidative or glycolytic metabolism does not appear to be simply interchangeable in smooth muscle. Oxidative metabolism is more strongly associated with the ATP requirements of the contractile apparatus, where aerobic glycolysis is identified with membrane-related energy-dependent processes. One of the more striking examples of the differences between these two ATP sources can be found by comparing the effects of oxygen to those of high exogenous glucose in restoring phosphagen content after hypoxia. In either case, ATP levels are restored to about 70–80% of control. However, while added glucose under hypoxia restores ATP, PCr is little affected and remains depressed at about 20% of control. In contrast oxygen elicits a strong recovery of PCr to 120% of control and a similar recovery of ATP as glucose. What then is the significance of this observation? Is mitochondrial ATP preferred or essential for PCr formation? Perhaps, the constraints posed by hypoxia alter the reaction-diffusion dynamics in the cell such that the ATP produced by glycolysis is not as effectively converted to PCr. We have argued that a membrane-associated glycolytic cascade is located near ATP utilizing enzymes. In the absence of mitochondrial PCr, perhaps competition for glycolytic ATP in such a membrane compartment would favor the ion transport ATPases, rather than conversion to PCr by creatine kinase. This would be somewhat different than the PCr-circuit model [60] in which glycolysis is considered to be a second producing site of the PCr-circuit.

There is substantial evidence for coupling of glycolysis to the PCr-circuit. During anoxia, the patterns of phosphocreatine utilization and tissue acidification are

similar in fish skeletal muscle [73]. In skeletal muscle, creatine kinase is co-localized with glycolytic enzymes in the I-band [74]. Interestingly, there is evidence that creatine kinase can couple to pyruvate kinase to form a diazyme complex [75]. Characteristic of these systems, however is a large pool of phosphocreatine and large changes in energy demand that far exceed the metabolic capacity for ATP synthesis. Another example is the coupling of the Na-K pump to the PC-circuit in the electric fish, Torpedo [76, 77]. In this case again, limited aerobic and anaerobic metabolic capacity in the face of large step changes in energy demand appear to have evolved a strategy whereby phosphocreatine pools are utilized to meet the transient demands followed by slower rates of recovery.

The role of the creatine kinase system in smooth muscle is not yet known with certainty. The differences in energy utilization and replenishment strategy are quite different from skeletal muscle. When generating maximal isometric force, the total ATP utilization of smooth muscle is often less than twice the basal rate [1]. The energy utilization during contraction in skeletal muscle may exceed basal rates by orders of magnitude. As noted above, in the steady state of contraction in smooth muscle, there often is no change in either ATP or PCr from prestimulus levels. Thus the term 'functional coupling' used to describe systems in which PCr provides the immediate source of ATP for skeletal muscle contraction or the Torpedo Na-K pump has a somewhat different meaning when used to describe the coupling between aerobic glycolysis and membrane function in smooth muscle. We have used the term 'preferential coupling' to describe this phenomenon [14]. Under normal physiological conditions, both oxidative and glycolytic metabolism function in parallel to provide for smooth muscle energy needs. However, glycolytic ATP appears to be the preferred source for membrane energy requirements. It is not known whether PCr plays an intermediate role in these energy transductions in smooth muscle.

Why then is there substantial creatine kinase in smooth muscle? As in skeletal muscle, CK and glycolytic enzymes in smooth muscle tend to show a similar localization pattern [14, 78]. The relatively low ATP requirements for contractility coupled with the fact that the PCr content is of similar magnitude to ATP would suggest that a role as an ATP buffer is limited. CK has also been ascribed a 'spatial' buffering function, whereby smaller gradients of ATP and ADP can maintain a given diffusive flux of high-energy phosphate [79]. However, in small diameter cells, as most smooth muscles, this also would not be anticipated to be a major factor. Perhaps this type of facilitated diffusion might be important in axial distribution of energy in smooth muscle.

Experimental tests of CK function in smooth muscle are limited. Ekmehag and Hellstrand [80] used β-guanidino propionic acid treatment to reduce PCr to about 1/10 control values. In rat portal vein, no changes in function or metabolism were detected. It can be argued, however, that even at these reduced PCr levels, a transport function for the CK system would be largely preserved. Likewise for glycolysis, smooth muscle cells can maintain normal function in the absence of glucose, provided other substrate is available. If as we have argued that a particular energy-dependent function might not be eliminated by inhibition of CK or glycolysis, but simply not optimized, one might only clearly define the importance of co-localization of energy transduction and utilization under conditions of stress.

A role for these systems in local regulation might perhaps be of more significance to smooth muscle [81]. Many energy-dependent cellular functions are differentially regulated. For example, relaxation of smooth muscle would involve a decrease in the actin-myosin ATPase but by an increase in Ca^{2+}-pump ATPase activity. Often these pathways involve common second messengers. For example, ADP is a common activator or inhibitor of many processes including metabolism, and localization of CK or glycolysis would restrict the effects of ADP to the region where a specific function was co-localized. The other side of this coin is that high-energy phosphagen produced in the local region would be at a higher concentration and perhaps more effective in a kinetic sense, if located in proximity to the utilization site. However both this regulatory and efficacy arguments are not unique to smooth muscle and remain speculative until more definitive experiments are devised.

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