Control of Energy Metabolism During Muscle Contraction

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Skeletal muscle energetics can be studied noninvasively at rest, during exercise, and in recovery using phosphorus nuclear magnetic resonance (^{31}P-NMR). In resting muscle, inorganic phosphate (P_i) and total cellular phosphate concentration are regulated by Na^+-dependent P_i transport. Insulin was shown to stimulate P_i uptake in G-8 muscle cells, in isolated rat soleus muscle, and in human muscle in vivo under conditions of hyperinsulinemic-euglycemic clamp. The relationship between plasma P_i and intracellular muscle P_i was examined in a group of patients with elevated plasma P_i resulting from renal failure. The total creatine content of muscle cells is controlled by an active creatine uptake in which β_2-receptor stimulation and the activity of the Na^+-K^+-ATPase play a significant role. Recovery after exercise is entirely oxidative; the rate of ATP synthesis is largely controlled by ADP, the concentration of which is determined by the creatine kinase equilibrium that includes the concentration of H^+. At the onset of aerobic dynamic exercise, ATP is maintained largely by glycolysis, producing lactic acid, and by phosphocreatine breakdown. After vasodilation, ATP synthesis becomes predominantly oxidative. The above processes can be quantitatively evaluated by ^{31}P-NMR. *Diabetes* 45 (Suppl. 1):S88–S92, 1996

Skeletal muscle is responsible for more than 70% of glucose utilization and storage and is therefore the main organ ensuring glucose homeostasis. Hormonal control, particularly by insulin, is central to determining muscle glycogen synthesis and glucose utilization. At the same time, the large dynamic work range of skeletal muscle requires that energy input can be rapidly altered and that supply and demand are closely regulated. Substrate (fuel) delivery and selection play an important role in the regulation of energetics, and in this respect muscle glycogen content and utilization (as discussed by Shulman et al. [1]) are major components of muscle function.

The basic bioenergetic processes in skeletal muscle are centered around the generation of ATP by oxidative phosphorylation, anaerobic glycogenolysis, and the conversion of phosphocreatine (PCr) catalyzed by creatine kinase (2). The supply of substrates in part determines the fuel used in oxidative phosphorylation. H^+ ions also play an important role in these processes, as part of the creatine kinase reaction, lactic acid (lactate + H^+) production, and H^+-linked mitochondrial functions.

It is now well established that phosphorus nuclear magnetic resonance (^{31}P-NMR) can be used to measure quantitatively and noninvasively the concentrations of some of the major phosphate-containing metabolites (ATP, PCr, inorganic phosphate [P_i], and hexose phosphates) and of H^+ (strictly the cytosolic pH) in skeletal muscle in vivo at rest, during exercise, and in recovery (2). The time resolution of the measurement (depending on the muscle group being examined) is between 1 s and 1 min. The two most commonly studied human muscles are the flexor digitorum superficialis (involved in finger flexion) and the gastrocnemius (stressed by plantar flexion). Well-worked-out protocols are now available to follow time courses of bioenergetic changes in human and animal muscles (2), and sophisticated analytic procedures have been developed to derive quantitative values and kinetic parameters for several of the major reactions (3), e.g., oxidative ATP synthesis, glycolytic flux, and H^+ production and removal (Fig. 1).

The relationship between contractility of muscle and cellular bioenergetics can be considered at three stages.

1. At rest, the cellular homeostasis of bioenergetic components (e.g., cellular P_i, total creatine, adenine nucleotides, and glycogen) is determined by hormonally controlled transport processes. Insulin has a key role not only in the control of glucose transport but also in modulating P_i uptake and possibly creatine transport. The concentrations of key ions (Na^+, K^+, and Ca^{2+}) are also strictly fixed in the resting cell.

2. During exercise, control of blood flow and control of oxidative ATP synthesis are to some extent linked. The regulation of glycogenolysis, when oxidative metabolism is inadequate, and that of fuel selection are also important in the control of energetics during exercise.

3. In the recovery phase, glycogenolysis is shut down and ATP synthesis, at least in normal situations, is entirely oxidative. At the same time, waste products (largely H^+ and lactate) must be cleared from the muscle cells.

REGULATION OF RESTING CONCENTRATIONS

Intracellular phosphates. P_i is derived from the blood and is a determining factor in setting the total phosphorus level in the cell. P_i transport is Na^+ dependent (4), with two Na^+ ions taken up for one P_i. The steady-state intracellular P_i concentrations are determined by the rate of Na^+/P_i entry, counteracted by passive P_i efflux. P_i entry is under hormonal control

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IGF-I, insulin-like growth factor I; NMR, nuclear magnetic resonance; PCr, phosphocreatine; P_i, inorganic phosphate.

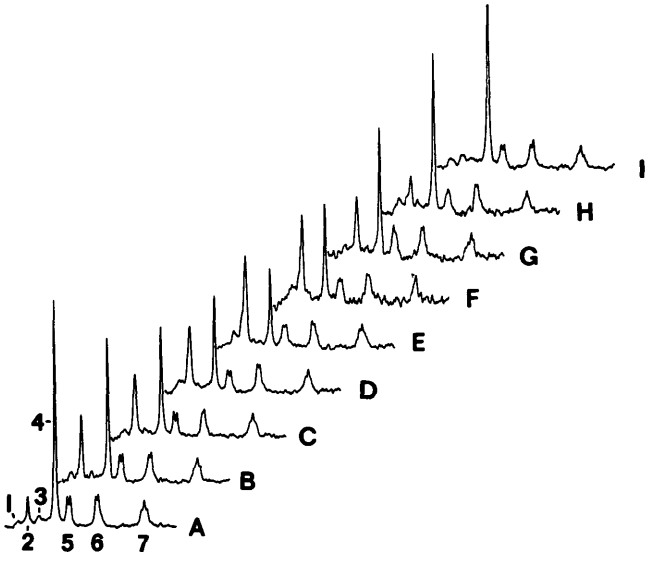

FIG. 1. ^{31}P-spectra from human gastrocnemius muscle in vivo. Data were collected using a 6-cm diameter surface coil with an 80-μs pulse length and a 2-s interpulse delay. On the *x*-axis is the chemical shift in parts per million and on the *y*-axis the signal intensity. Peak assignments: 1, phosphomonoesters; 2, P_i; 3, phosphodiesters; 4, PCr; 5, γ-phosphate of ATP; 6, α-ATP + NADH and NAD^+; 7, β-ATP. The spectra show muscle at rest (A), during aerobic dynamic exercise (B–E), and in recovery from exercise (F–I). The number of accumulations for each spectrum was 64, 32, 8, and 16 for A, B–E and I, F and G, and H, respectively. The intracellular pH at rest (A) was 7.03, decreasing to 6.74 at the end of exercise (E).

(5). For example, insulin and insulin-like growth factor I (IGF-I) stimulate the rate of P_i uptake in G-8 cells (Fig. 2). In isolated rat soleus muscle, insulin at 10,000 μmol/ml stimulated P_i influx by 55%, and IGF-I at 72–720 nmol/l stimulated P_i influx by 48–129% (5). Neither had any effect on the Na-independent component of P_i influx. The mechanisms of the enhancement of P_i uptake by the two substances are different in that IGF-I stimulation is delayed compared with insulin activation and is inhibited by dicyclohexylcarbodiimide. This suggests that the effect of IGF-I requires new protein synthesis, unlike the insulin effect.

Because skeletal muscle is one of the major sites of insulin action, the potential to influence metabolism as well as ionic fluxes is substantial. Administration of this hormone leads to an increase in the K^+ content of muscle, whereas Na^+ efflux is increased because of changes in both Na^+-K^+-ATPase activity and Na^+-H^+ exchange (6). P_i flux across the muscle cell membrane may also be increased, because P_i uptake by muscle is thought to be important in the hypophosphatemia associated with insulin infusion (6,7). It has been argued that insulin-induced changes in ion fluxes, particularly in Na^+-H^+ exchange, stimulate phosphofructokinase activity by increasing intracellular pH, thereby accounting for the stimulation of glycolysis (8).

We have used ^{31}P-NMR and the hyperinsulinemic-euglycemic clamp simultaneously to assess the effect of insulin on intracellular pH and the major phosphorus-containing metabolites of normal human skeletal muscle in vivo in four normal subjects (9). Insulin and glucose were infused for 120 min. Plasma insulin increased ~10-fold over preclamp levels (5.6 ± 0.9 and 54 ± 5 mU/l for preclamp and over the last hour of infusion, respectively; mean ± SE, n = 4). Plasma glucose concentration did not change significantly (5.4 ± 0.2 and 5.5 ± 0.1 mmol/l for preclamp and over the last hour of infusion, respectively).

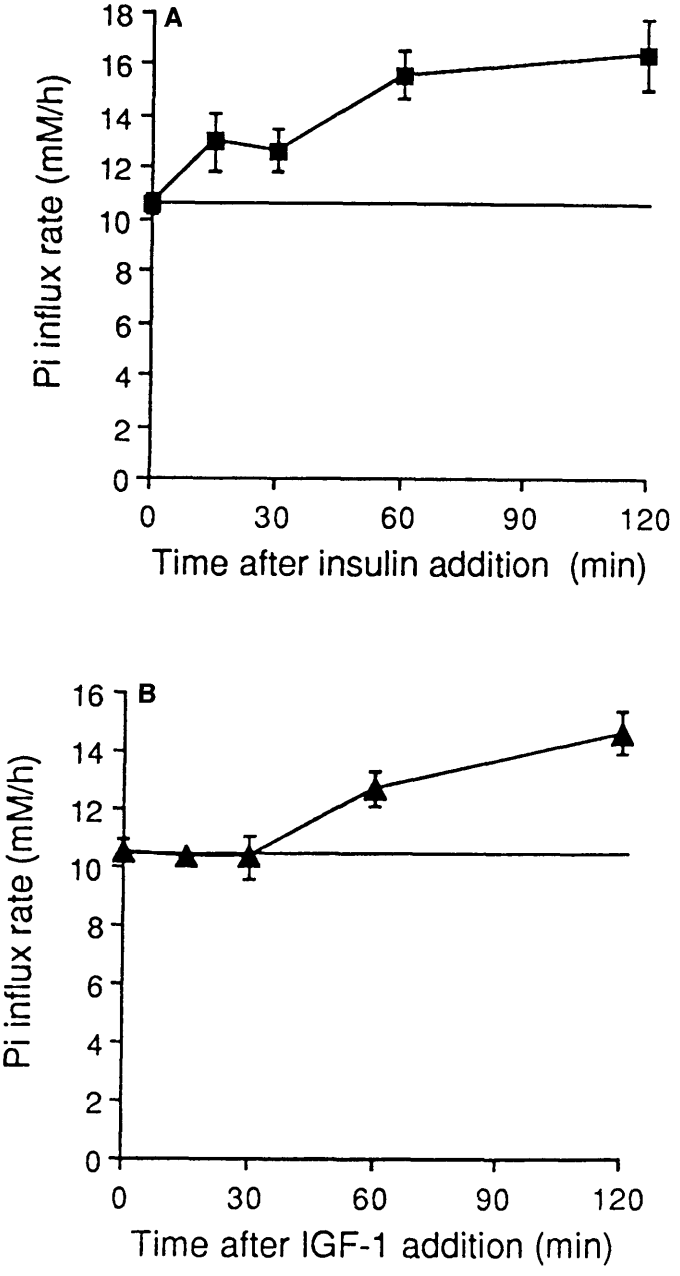

FIG. 2. Time course of the effects of insulin and IGF-I on P_i influx in G-8 cells. The figure shows the onset of stimulation by 10,000 μU/ml insulin (*A*) and 720 nmol/l IGF-I (*B*) as a function of exposure time. Horizontal lines show control values at time 0. Incubations were performed in 120 mmol/l sodium (all n = 6). Adapted from Polgreen et al. (5).

Insulin and glucose infusion resulted in a decline in the intracellular pH of forearm muscle of 0.027 ± 0.007 U/h ($P <$ 0.01), whereas in control studies of the same subjects, pH rose by 0.046 ± 0.005 U/h ($P < 0.001$). In the clamp studies, intracellular P_i concentration rose by 18%/h, whereas ATP and phosphocreatine and phosphomonoester concentrations did not change. In plasma, P_i concentration was 1.16 ± 0.05 mmol/l before infusion, and this decreased by a mean rate of 0.14 mmol · h^{-1} · l^{-1}. No change was observed in any of these intracellular metabolites in the control studies.

The results showed that under physiological conditions, insulin does not raise intracellular pH in human muscle and thus cannot influence muscle metabolism by this mecha-

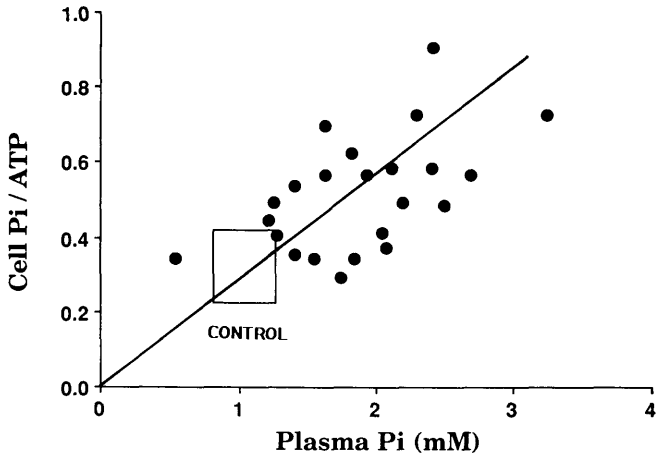

FIG. 3. The effect of alterations in plasma P_i concentration on cell P_i/ATP in uremic patients not undergoing dialysis. A significant linear correlation was observed ($r = 0.5$, $P < 0.01$). The mean ± SE cell P_i/ATP for 10 age-matched control subjects is indicated in the boxed area.

nism. We also found that the insulin-induced decrease in the plasma P_i concentration was accompanied by an increase in the P_i concentration in muscle. It is widely believed that P_i-shift hypophosphatemia of the kind we observed is secondary to increased incorporation of P_i into organic phosphate compounds (10). This requires a fall in the cytosolic P_i concentration, and the greater the permeability of the cell membrane to net P_i influx, the smaller this fall need be. Steady-state exchange fluxes are readily measured by isotopic techniques, but this important net flux permeability has rarely been studied. For human skeletal muscle, the near constancy of the sum of the concentration of P_i and PCr during exercise (2), despite the manyfold rise in the cytosolic P_i concentration, suggests that the effective net flux permeability for P_i is low. If the observed fall in the plasma P_i concentration were due solely to muscle P_i uptake, it would mean that muscle uptake was ~0.1 mmol · h^{-1} · l^{-1}. This would require the cytosolic P_i concentration to fall by at least 25%, which would be detectable by NMR spectroscopy. In contrast, the significant rise in the muscle P_i concentration observed suggests a primary increase in P_i influx, perhaps due to an increase in Na^+-linked P_i uptake (4). Also, no phosphate sink in the form of an NMR-visible organic phosphate pool was seen.

The relationship between extracellular (blood) P_i and in-tracellular muscle P_i has been studied in a group of patients in renal failure who have high plasma phosphate concentrations (11). In control subjects, extra- and intracellular P_i is maintained within narrow limits. In the patient group, intracellular P_i increases linearly with blood P_i (Fig. 3). Im-portantly, despite the increase in intracellular P_i, the phosphorylation potential remains constant. The reciprocal phosphorylation potential is 4.5 ± 0.7 and 4.9 ± 0.1 mol/l × 10^{-6} in control subjects and patients, respectively. Creatine kinase plays a role in ensuring constancy in the phosphorylation potential, because an increase in cellular P_i is accompanied by an increase in PCr and a decrease in ADP.

The human studies are entirely in accordance with our observations on muscle cells and on isolated rat muscle (see above).

Total creatine. Relatively little is known about the regulation of the cellular content of total creatine (PCr + creatine), which is a fundamental bioenergetic parameter. A sodium-creatine cotransporter responsible for creatine uptake has been described in several cell lines (12). We investigated creatine transport and accumulation in a cultured G-8 myoblast line (13). Creatine uptake is rapid, saturable, and sodium dependent. The total cellular creatine concentration is relatively independent of extracellular creatine levels. This is consistent with a high-affinity active uptake balanced by slow passive efflux. The kinetic characteristics of the transporter in the G-8 cells are similar to those in human muscle, suggesting that creatine homeostasis in muscle is likely to be modulated by factors similar to those we demonstrated in the G-8 cell. Among these, we found that net creatine uptake is stimulated by isoproterenol, 3,3',5-triiodothyronine, or amylin, all factors that stimulate the Na^+-K^+-ATPase (14). In further studies on G-8 mouse myoblasts, we provided evidence that there is a β-adrenergic receptor–mediated pathway that regulates intracellular creatine and hence net creatine uptake. Creatine uptake was stimulated not only by isoproterenol but also by norepinephrine at physiological concentrations (0.1–1 μmol/l). The cAMP analogue N^6,2-*O*-di-butyryladenosine 3:5'-cyclic monophosphate also enhanced creatine uptake. The effect of norepinephrine in enhancing creatine uptake was inhibited by the nonspecific adrenergic antagonist labetalol but not by the α_1-adrenergic receptor agonist methoxamine. The effect of norepinephrine was inhibited by propranolol (10 μmol/l), whereas the α_2-antagonist phentolamine (10 μmol/l) failed to inhibit. The β_2-antagonist butoxamine (0.1–10 μmol/l) was more effective than the β_1-antagonist atenolol (0.1–10 μmol/l) in inhibiting the effect of norepinephrine on creatine uptake. Clenbuterol (100 μmol/l), a β_2-agonist, enhanced creatine uptake, where-as ouabain (2 mmol/l) decreased it without affecting cell viability (Tables 1 and 2).

TABLE 1
Effects of isoproterenol, norepinephrine, methoxamine, dibutyryl cAMP, and clenbuterol on creatine uptake

	Creatinine content (mmol/kg)
Isoproterenol	
0 nmol/l	60 ± 5
0.5 nmol/l	83 ± 10*
100 nmol/l	98 ± 6†
10,000 nmol/l	94 ± 11*
Norepinephrine	
0 nmol/l	27 ± 3
10 nmol/l	41 ± 10
100 nmol/l	48 ± 5*
10,000 nmol/l	42 ± 3‡
Methoxamine	
0 μmol/l	110 ± 7
0.2 μmol/l	73 ± 9*
2 μmol/l	67 ± 14
20 μmol/l	63 ± 6*
Dibutyryl cAMP	
0 μmol/l	53 ± 15
100 μmol/l	77 ± 12
150 μmol/l	88 ± 6
200 μmol/l	120 ± 13‡
Clenbuterol	
0 μmol/l	140 ± 12
100 μmol/l	180 ± 9*

Data are means ± SE. The cells were of passage numbers 8–12 and plating density 250,000 ($n = 6$). The creatine concentration in the extracellular medium was 0.5 mmol/l. *$P < 0.05$, †$P < 0.001$, and ‡$P < 0.01$ compared with control with no addition.

TABLE 2
Effect of antagonists on norepinephrine mediated creatine uptake

	Creatine content (mmol/kg)
Labetalol	
0 μmol/l	100 ± 4
0.1 μmol/l	95 ± 4
1 μmol/l	85 ± 6*
10 μmol/l	92 ± 8
Propranolol	
0 μmol/l	125 ± 5
10 μmol/l	81 ± 4*
Phentolamine	
0 μmol/l	33 ± 2
100 μmol/l	35 ± 3
Atenolol	
0 μmol/l	120 ± 5
0.1 μmol/l	127 ± 6
1 μmol/l	84 ± 13*
10 μmol/l	94 ± 9*
Butoxamine	
0 μmol/l	119 ± 5
0.1 μmol/l	98 ± 4†
1 μmol/l	62 ± 7†
10 μmol/l	72 ± 11†

Data are means ± SE. The cells were of passage numbers 8–12 and plating density 250,000 (n = 6). The creatine concentration in the extracellular media was 0.5 mmol/l; 0.1 μmol/l norepinephrine was present in the extracellular media. Preincubation with 2 mmol/l ouabain for 1 h resulted in a final creatine content of 76 ± 12 mmol/kg of protein after 48 h in a 0.5 mmol/l creatine–containing medium, compared with the non–ouabain-exposed cells' content of 106 ± 8 mmol/kg protein ($P < 0.05$); the cells incubated in a creatine-free medium (Dulbecco's modified Eagle's medium) had final creatine content of 79 ± 4.3 mmol/kg protein. The passage number was 9 (n = 12). *$P < 0.05$ and †$P < 0.01$ compared with control with no additions.

These results show the importance of the β_2-receptor in the control of active creatine uptake and the role of the Na^+-K^+-ATPase according to the model shown in Fig. 4, which explains the relationship between creatine influx, efflux, and the Na^+-K^+-ATPase.

There is considerable interest in the possible benefits to skeletal muscle function of dietary creatine supplementation, which increases both muscle creatine content and maximal voluntary muscle contraction (15). Our results suggest that creatine supplementation should be particularly beneficial when coupled to a training regimen.

METABOLIC RECOVERY AFTER EXERCISE

The quantitative analysis of recovery data, unlike that during exercise, is simplified because muscle mass and the nature and severity of exercise do not influence the metabolic changes directly. At the end of exercise, glycogenolysis is switched off, so the rate of PCr recovery provides a measure for mitochondrial ATP synthesis. The other major processes are involved in the removal of the products of exercise (H^+ and lactate). The kinetic analysis of PCr and pH recovery is therefore of major importance for our understanding of the mechanisms of recovery in normal and disease states. The initial rate of PCr recovery after exercise has a hyperbolic relationship to the end-exercise cytosolic ADP concentration (16), as does the rate of PCr synthesis throughout recovery to the ADP concentrations during the recovery period. The near-equilibrium process catalyzed by creatine kinase ensures that PCr, ADP, and intracellular pH are related by the expression $[ADP] = \{([\text{total Cr}]/[PCr]) - 1\}\,[ATP]/K[H^+]$, where K is the equilibrium constant (1.66×10^9 l/mol) for the creatine kinase reaction and brackets indicate concentration. The relationship between the rate of mitochondrial ATP synthesis (Q, measured in millimoles per minute) and cytosolic ADP, $Q = Q_{max}/(1 + K_m/[ADP])$, can be used to calculate Q at half-maximal ADP concentration, giving K_m (30 μmol/l cell water) and the maximum value (Q_{max}; 40 $mmol \cdot l\ cell\ water^{-1} \cdot min^{-1}$). The pH variations are thus allowed for in the calculation of free cytosolic ADP, although low pH may also have a direct effect on the mitochondrial ATP synthase. Thus ADP can be taken as the error signal for ATP control.

It is important to reemphasize that H^+ ions play an important role in determining this error signal through the creatine kinase equilibrium. Thus the rate of pH recovery is an essential element in regulating ADP throughout the recovery phase. pH change is approximately linear during the recovery phase, and the proton efflux rate varies linearly with intracellular pH, increasing from ~0.5 mmol/min at pH 6.8 to 10 mmol/min at pH 6.3 (3). We examined pH control in rat skeletal muscle in more detail and have shown that, during recovery from exercise, protons were removed by a pH-dependent extrusion from the cell, similar to that in human muscle (17). The pH-dependent efflux of protons was partially inhibited by amiloride or 4,4′-diisothiocyanostilbene-2,2′-disulfonate, implicating both sodium proton and bicarbonate chloride exchange as approximately equal contributors to H^+ removal. In early recovery, up to 30% of proton efflux was mediated by lactate-proton cotransport.

BIOENERGETIC CHANGES DURING EXERCISE

The quantitative evaluation of the bioenergetic parameters during exercise is somewhat more complex because the increased demand for ATP hydrolysis is met by three processes: glycogenolytic (anaerobic) ATP synthesis, oxidative ATP synthesis, and the conversion of PCr to ATP. The last is directly measured, whereas the contributions of the former two (and their rates) can be estimated provided the rate of lactate production can be measured. This is done by measuring cytosolic pH, from which lactate production can be calculated if we know the passive buffering capacity of the

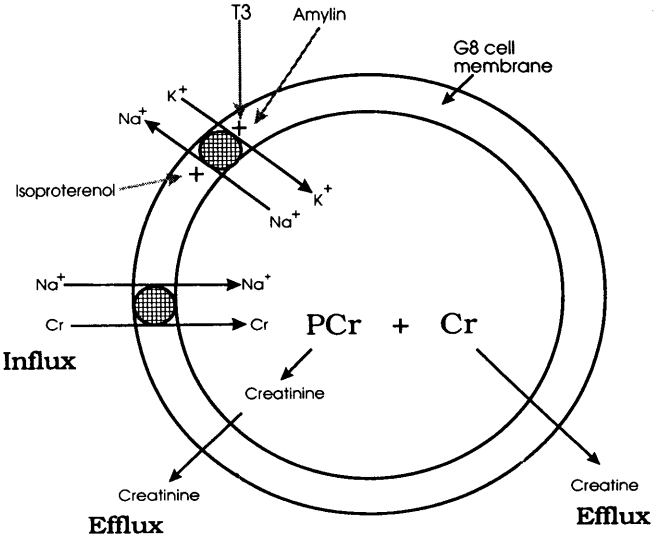

FIG. 4. A proposed model for creatine transport.

cell and the rate of proton removal. This last parameter can be measured during recovery. From this, H^+ removal during exercise can be calculated if we assume that the same pH dependence for H^+ efflux applies during exercise as has been demonstrated during the recovery phase (3).

We can analyze the various contributions to ATP synthesis during exercise by comparing the changes observed under ischemic and aerobic conditions at the same muscle performance. We have carried out such a study on the flexor digitorum superficialis in adult men during dynamic ischemic and aerobic exercise at three different work rates and during recovery from aerobic exercise (18). During exercise, changes in pH and PCr were larger at higher power, but in aerobic exercise neither the end-exercise ADP concentration nor the initial postexercise PCr resynthesis rate altered with power. In ischemic exercise, we estimated total ATP resynthesis from the rates of PCr depletion and glycogenolysis inferred using an analysis of proton buffering. The estimate of total ATP turnover remained constant with time and was shown to be proportional to work rate. As PCr decreased, the relative contribution from glycogenolysis to ATP synthesis increased in parallel so as to maintain the total ATP synthesis rate. Comparison of the calculated ATP turnover in ischemic and aerobic exercise suggested that oxidative ATP synthesis was small during the first minute of aerobic exercise and increased with a half-time of ~0.5–1 min. This suggests that oxidative ATP synthesis is negligible during the first 30 s of aerobic exercise at mechanical power output levels of 0.5, 0.67, and 1 W. Using the values of proton efflux derived from studying pH recovery after exercise, we could estimate ATP synthesis by glycogenolysis during aerobic exercise from the measured pH changes. The P_i concentration has a significant effect on the rate of glycogenolysis; this may be of regulatory significance, because P_i is a substrate for glycogen phosphorylase and an activator of phosphofructokinase.

We can measure hemoglobin desaturation during aerobic and ischemic exercise by infrared spectroscopy. At 0.5 W, hemoglobin desaturation was higher at the end of the 1st min of aerobic exercise (in fact as high as during ischemic exer-cise) than at the end of exercise. This mirrors the calculated rate of oxidative ATP synthesis. A possible explanation is a delayed increase in blood flow so that oxidative ATP synthesis is limited first by the rate of arterial oxygen supply and then, at later stages, by the cytosolic ADP concentration when oxygen supply is adequate (18).

CONCLUSIONS

^{31}P-NMR provides a quantitative method for measuring the concentrations of key phosphate-containing metabolites and of cytosolic H^+. By following the time course of changes in these components during exercise and recovery, we can derive quantitative information about glycogenolytic, mitochondrial, and ionic fluxes. Thus the regulation of these events can be investigated in normal and diseased skeletal muscle.

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