Effects of Ischemia on Tissue Metabolites in Red (Slow) and White (Fast) Skeletal Muscle of the Chicken

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SUMMARY Brief periods of ischemia have been shown to produce marked reactive hyperemia in both red (slow) and white (fast) skeletal muscle. However, evidence is lacking for specific vasodilator metabolites which are rapidly produced in ischemic skeletal muscle. The present study examined the effects of 1 and 3 minutes of ischemia on creatine phosphate (CrP), adenine nucleotide metabolism, and anaerobic glycolysis in red anterior (ALD) and white posterior latissimus dorsi (PLD) muscles of the chicken. Tissue metabolite concentrations were determined from perchloric acid or trichloroacetic acid extracts using enzymatic assay or high pressure liquid chromatography. CrP or adenine nucleotides were not significantly altered in either muscle following 1 or 3 minutes of ischemia. However, adenosine increased by 811% in the ALD at 1 minute. Following 3 minutes of ischemia, adenosine concentrations were elevated by 439% and 201% in the ALD and PLD, respectively. The PLD showed the greatest increases in inosine and IMP. Inorganic phosphate increased by 67% and lactate increased by 142% in the ALD at 3 minutes. The PLD, which is reported to have a high anaerobic glycolytic capacity, showed no increase in lactate. These results support the hypothesis that adenosine may be a mediator of skeletal muscle reactive hyperemia following short periods of ischemia. Circ Res 45: 366-373, 1979

BRIEF periods of flow arrest are thought to result in the accumulation of substances within the tissue that cause vasodilation (for reviews, see Haddy and Scott, 1968; Johnson, 1974). These hypothetical substances are believed to be linked to anaerobic or aerobic pathways of energy metabolism (Berne et al., 1971; Dobson et al., 1971; Johnson, 1974; Klabunde and Johnson, 1977a). Although data are available on skeletal muscle metabolic changes following ischemic periods ranging from several minutes to hours (Deuticke and Gerlach, 1966; Imai et al., 1964; Rubio et al., 1973), metabolic alterations associated with short-term ischemia are not well defined. Furthermore, the metabolic changes brought about by ischemia have not been compared in red (slow) and white (fast) skeletal muscle. This is of interest because enzyme activity studies in vitro suggest that the anaerobic capacities of the two types of muscle are very different. For example, the slow-contracting anterior latissimus dorsi (ALD) muscle of the chicken is reported to have a high oxidative capacity and a low anaerobic glycolytic capacity, whereas, the converse is reported for the fast-contracting posterior latissimus dorsi (PLD) (Pette, 1971). Also, it has been suggested that the potential for adenosine production is greater in red than in white skeletal muscle (Bockman, 1977). Another reason for studying metabolic responses to ischemia in red and white muscle, and specifically the ALD and PLD, is that a previous study (Klabunde and Johnson, 1977a) has shown that reactive hyperemia is similar in these two types of muscle. Therefore, it was the purpose of this study to determine the effects of short-term ischemia (1 and 3 minutes) on specific glycolytic metabolites and on adenine nucleotide metabolism, in the red ALD and white PLD muscles of the chicken, to see if changes in metabolite concentrations would correlate with the postischemic reactive hyperemia previously observed in these muscles.

Methods

Preparation

Experiments were performed on 61 male White Leghorn chickens (Gallus domesticus) which ranged from 6 to 19 weeks in age. The chickens were anesthetized by a combination of sodium pentobarbital (40 mg/kg) and chloral hydrate (170 mg/kg) given as an intramuscular or intraperitoneal injection. The right ALD and PLD muscles were exposed and separated from underlying tissues. They were left attached to their spinal origin and humeral insertion. The blood supplies and neural innervation were left intact. Both surfaces of the muscles were covered with Saran Wrap and then left undisturbed for at least 20 minutes prior to ischemia. The Saran Wrap prevented the muscles from drying out and served as a barrier to oxygen diffusion from air into tissue. The muscle prepara-
tion is shown in Figure 1. Complete ischemia of 1 or 3 minutes duration was achieved by clamping the muscles next to the spine and near the humerus. Muscles were frozen in situ with a spray of dichlorodifluoromethane (Freon 12), excised, and then placed in liquid Freon 12, cooled in liquid nitrogen. The ALD and PLD were separated from each other and stored below —60°C in vials having tight-fitting caps. We estimated that freezing occurred in 1–3 seconds, depending upon muscle thickness. The different freezing times appeared to have no effect on metabolite concentrations, since control muscles from different size birds (0.4–1.8 kg) had similar metabolite concentrations.

Analytical Procedures

Muscle samples were handled in a cold box at —20°C. After trimming, the muscles were powdered in a stainless steel mortar, precooled in liquid nitrogen. For enzymatic assays, portions of the powder were weighed (8–15 mg) and extracted in 30–50 volumes (based upon muscle weight) of ice cold 0.6 M HClO₄ by use of a Duall homogenizing tube (size 21—Kontes Glass Co.). Following centrifugation, the supernatant solution was neutralized with 3.0 M KHCO₃. The final extract was the equivalent of 20 mg muscle per ml. The concentrations of creatine phosphate (CrP), ATP, inorganic phosphate (Pi), glucose-6-phosphate (G-6-P), lactate, and pyruvate were determined enzymatically based on pyridine nucleotide fluorescence as described by Lowry and Passonneau (1972).

High pressure liquid chromatography (HPLC) was used to measure adenine nucleotides and IMP. ATP and ADP were determined from the same extracts used in the enzymatic assays. AMP and IMP were determined from more concentrated extracts (homogenized in 9 volumes of 0.6 M HClO₄ instead of 30–50 volumes). Labelled AMP[¹³H] and IMP[¹⁴C] were added to the extract to aid in peak identification. The nucleotides were separated on a strong anion exchange column (Partisil 10-SAX, Whatman or Altex) and eluted, using a linear gradient of increasing ionic strength from 0.0075 M to 0.3 M KH₂PO₄ with 0.6 M KCl. Buffer pH was 4.1. Nucleotide absorbance was determined at 254 nm, and adenosine was used as a standard in each sample run. Fractions of the eluant were collected and counted by liquid scintillation for ³H and ¹⁴C. The retention times of the labeled nucleotides were compared to the chromatogram peaks to identify AMP and IMP.

For the measurement of adenosine and inosine, 30 mg of tissue (frozen powder) were extracted in 9 volumes of 7% trichloroacetic acid. Following centrifugation, the supernatant solution was extracted repeatedly with diethyl ether. Nucleotides were removed by treatment with a few milligrams of air-dried Dowex-1-formate beads (strong anion exchanger). The extract (100 µl) then was injected onto a Lichrosorb RP18 (Altex) reverse-phase column for the separation and detection of adenosine and inosine by HPLC. The initial solvent was 4 mM KH₂PO₄ (pH 6.0). The nucleosides were eluted sequentially, using a linear gradient consisting of 80% CH₃OH:20% H₂O against the initial solvent. Solvent flow rate was 1.5 ml/min. Both inosine and adenosine eluted in less than 15 minutes. Absorbance was measured at 260 nm. A sample chromatogram is shown in Figure 2. The nucleosides were quantified by relating the areas under the absorbance peaks to an AMP standard peak of known concentration. Overall recovery was 100% for both inosine and adenosine. Extracts treated with adenosine deaminase (E.C. 3.5.4.4) and nucleoside phosphorylase (E.C.2.4.2.1) prior to injection onto the column were used to verify that no other ultraviolet absorbing
substances were cochromatographing with adenosine and inosine.

**Materials**

The enzymes used in the metabolite assays were obtained from Sigma Chemical Co. or Boehringer Mannheim Corp. Metabolite standards were obtained from Sigma, P-L Biochemicals, Inc., or Mallinckrodt. $^3$H-AMP and $^3$C-IMP were purchased from New England Nuclear and Schwarz/Mann, respectively. Analytical grade reagents were used in all assays. Solvents for HPLC were high purity reagents: KH$_2$PO$_4$ (Fisher, primary standard), KCl (BDH Chemicals, Analar), methanol (Fisher, HPLC grade).

**Statistical Methods**

Statistical differences among control and ischemic groups were determined in most cases by one-way analysis of variance (anova). The nonparametric Kruskal-Wallis test (Sokal and Rohlf, 1969) was used when the homogeneity of variances criterion of the anova was not met rigidly.

**Results**

**Nucleotides and Inorganic Phosphate**

Under control conditions, the content of CrP and ATP was about 2.5 times greater in the fast-contracting PLD than in the slow-contracting ALD muscle (Fig. 3). Although the absolute concentrations of CrP and ATP differed in the two muscles, the CrP/ATP ratios were the same (ALD = 3.45 ± 0.18 (SE); PLD = 3.55 ± 0.14). ATP and CrP were measured in the same enzymatic assay system for determinations of this ratio. The ATP concentrations in Figure 3 were determined by HPLC and were not significantly different from values obtained by enzymatic assay. ADP concentrations were higher in the control PLD muscle than in the ALD (Fig. 4). The AMP and IMP concentrations were very low in both muscles (Fig. 5). The AMP concentration was 20.4 ± 3.1 nmol/g wet weight in the ALD and 23.3 ± 1.3 in the PLD. IMP concentrations were 12.7 ± 7.5 and 4.14 ± 0.88 nmol/g in the control ALD and PLD muscles, respectively. The high concentrations of ATP and ADP, coupled with low concentrations of AMP, resulted in a high adenylate energy charge in both muscles. This value, calculated from the equation, energy charge = [(ATP) - 0.5 (ADP)]/[(ATP) + (ADP) + (AMP)], was 0.911 ± 0.002 and 0.942 ± 0.003 in the ALD and PLD, respectively (Table 1). ATP concentrations, as well as ADP and AMP, were determined by HPLC for these calculations. The control phosphorylation potential, (ATP)/[(ADP) X (Pi)], was greater in the PLD principally due to its much greater ATP content (Table 1). Inorganic phosphate concentration was slightly higher in the PLD (Fig. 4).

Following 1 and 3 minutes of ischemia, CrP and ATP did not significantly decline in either muscle (Fig. 3). However, there was a tendency for the mean concentrations of CrP to fall in the ALD during ischemia by about 2 nmol/g. ADP concentrations were unaltered by ischemia in both muscles (Fig. 4). Therefore, the ATP/ADP ratio was unchanged by ischemia in both muscles (Table 1).

Figure 5 shows the effects of ischemia on AMP and IMP concentrations. Ischemia caused no change in the AMP content of either muscle. Therefore, ischemia had no significant effect on the total tissue content of adenine nucleotides (Table 1). The apparent rise in AMP ($P > 0.05$) at 3 minutes in the PLD largely reflected an unusually high concentration (123 nmol/g) in one of the eight muscles assayed. IMP concentrations increased more than 6-fold after 3 minutes of ischemia in the PLD. The ischemic ALD showed no significant increase ($P > 0.05$) in IMP.

The adenylate energy charge was found to have very little intergroup variation, as indicated by a standard error of about 0.3% of the mean (Table 1). The energy charge did show a small significant decline following 3 minutes of ischemia in the ALD.

The Pi content of the ALD was found to increase by about 3.5 µmol/g wet weight following 3 minutes of ischemia (Fig. 4). The source of this increase was
Effects of ischemia (1 and 3 minutes) on ADP and Pi content of PLD (unfilled bars) and ALD (stippled bars) muscles. Tissue concentrations (ordinate) are in μmol/g wet wt. Number of muscles (n) is given within each bar. Vertical bars represent the se. * indicates a significant difference from control group (C) tested at the 0.05 level of statistical significance.

not evident. CrP and ATP hydrolysis during ischemia did not account for the net increase in Pi. Separate experiments failed to demonstrate any appreciable hydrolysis of CrP during perchloric acid extraction. Furthermore, Pi was assayed in the same extract from which CrP and ATP were determined. The increase in Pi, whatever its origin, caused a fall in the calculated phosphorylation potential in the ALD (Table 1).

Nucleosides

Although no measureable changes occurred in adenine nucleotide concentrations, the tissue content of adenosine and inosine showed significant changes with ischemia. The adenosine concentrations in the ALD were about half as great as the PLD concentrations in control muscles (Fig. 6). Following 1 minute of ischemia, adenosine concentration increased by 61% (6 nmol/g) in the ALD. No change occurred in the PLD at 1 minute; however, at 3 minutes, the adenosine increased by 201% (3.9 nmol/g) in the PLD. This compared to a 439% (4.3 nmol/g) increase in the ALD at 3 minutes. Therefore, both red and white muscles showed large increases in adenosine at 3 minutes of ischemia, and only the red muscle showed an increase at 1 minute. Inosine concentrations significantly increased to 456% (17 nmol/g) in the PLD following 3 minutes of ischemia (Fig. 6). The 110% increase (6.8 nmol/g) in the ALD was significant (P < 0.05). No significant increases (P > 0.05) were observed at 1 minute.

Glycolytic Intermediates

The absence of any significant changes in ATP suggested that anaerobic production of ATP may be stimulated during ischemia. Therefore, concentrations of lactate, pyruvate, and G-6-P were measured. Lactate and pyruvate will accumulate under anaerobic and ischemic conditions. The rate of accumulation of lactate should reflect the rate of glycolysis. G-6-P accumulation will reflect glycogen degradation and the activation state of phosphofructokinase (PFK). Lactate did not increase significantly in either muscle following 1 minute of ischemia (Fig. 7), indicating a very low rate of lactate production and accumulation under control conditions and after 1 minute of ischemia. Three minutes of ischemia resulted in a 2.5-fold increase in lactate in the ALD, but no change occurred in the PLD, which has been reported to have the higher glycolytic capacity of these two muscles. Pyruvate concentrations were not found to change significantly from control values of 0.25 ± 0.05 (se) and 0.14 ± 0.06 μmol/g wet weight in the ALD and PLD, respectively. Similarly, G-6-P concentrations...
TABLE 1  Effects of Ischemia on Total Adenine Nucleotide Content (AXP), ATP/ADP Ratio, Adenylate Energy Charge, and Phosphorylation Potential

<table>
<thead>
<tr>
<th></th>
<th>AXP</th>
<th>ATP/ADP</th>
<th>Energy charge</th>
<th>Phosphorylation potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>ALD</td>
<td>3.37 ± 0.17 (9)</td>
<td>5.08 ± 0.14 (19)</td>
<td>0.911 ± 0.002 (9)</td>
</tr>
<tr>
<td></td>
<td>PLD</td>
<td>7.78 ± 0.27 (9)</td>
<td>8.44 ± 0.27 (21)</td>
<td>0.942 ± 0.003 (9)</td>
</tr>
<tr>
<td>1-min ischemia</td>
<td>ALD</td>
<td>3.51 ± 0.19 (7)</td>
<td>5.05 ± 0.15 (17)</td>
<td>0.906 ± 0.003 (7)</td>
</tr>
<tr>
<td></td>
<td>PLD</td>
<td>7.03 ± 0.30 (7)</td>
<td>8.23 ± 0.30 (17)</td>
<td>0.938 ± 0.003 (7)</td>
</tr>
<tr>
<td>3-min ischemia</td>
<td>ALD</td>
<td>3.19 ± 0.18 (8)</td>
<td>4.67 ± 0.16 (16)</td>
<td>0.899 ± 0.002 (8)</td>
</tr>
<tr>
<td></td>
<td>PLD</td>
<td>7.57 ± 0.28 (8)</td>
<td>8.60 ± 0.32 (15)</td>
<td>0.938 ± 0.003 (8)</td>
</tr>
</tbody>
</table>

Values given are the mean ± SE; numbers in parentheses = number of muscles.
Energy charge = [(ATP) + 0.5 (ADP)]/[(ATP) + (ADP) + (AMP)].
Phosphorylation potential = (ATP)/[(ADP) - (Pi)].
* Indicates a significant difference from control group tested at the 0.05 level.

Discussion

A previous study (Klabunde and Johnson, 1977a) on reactive hyperemia in ALD and PLD muscles demonstrated that the microvascular responses to ischemia of 3.5-180 seconds in duration were similar in two muscle types, which are known to differ in resting oxygen consumption (Hudlicka, 1969) and anaerobic glycolytic capacity (Pette, 1971). It was postulated that a common vasodilator metabolite produced during ischemia could account for the similarities in reactive hyperemia. However, there did not change during ischemia in either muscle (Fig. 7).

**FIGURE 6** Effects of ischemia (1 and 3 minutes) on adenosine and inosine concentrations in PLD (unfilled bars) and ALD (stippled bars) muscles. Tissue concentrations (ordinate) are in nmol/g wet wt. Legend is same as Figure 4.

**FIGURE 7** Effects of ischemia (1 and 3 minutes) on lactate (LAC) and G-6-P content of PLD (unfilled bars) and ALD (stippled bars) muscles. Tissue concentrations (ordinate) are in µmol/g wet wt. Legend is same as Figure 4.
there are problems with this hypothesis. First, biochemical studies in vitro have suggested that the metabolic responses to ischemia should be different in the ALD and PLD (Pette, 1971). This would decrease the probability of a common vasoactive substance mediating vasodilation. Second, to account for the rapid onset of vasodilation during ischemia, the metabolite must accumulate very rapidly within the tissue spaces surrounding arterioles. In this regard, there are very few data in the literature on changes in tissue metabolites during brief periods of ischemia in skeletal muscle. Therefore, it was the purpose of this study to (1) compare the effects of short-term ischemia on adenine nucleotide metabolism and anaerobic glycolysis in ALD and PLD muscles, and (2) determine if changes in certain metabolite concentrations correlate in time with reactive hyperemic responses previously observed in these muscles.

Initial experiments measured CrP and adenine nucleotides in control and ischemic muscles. CrP is not thought to be an important regulator of blood flow; however, it is hydrolyzed more rapidly than ATP during ischemic hypoxia in skeletal muscle and heart (Imai et al., 1964) and, therefore, viewed as a sensitive indicator of hypoxia. Adenine nucleotides were of interest because of their vasoactive properties (Haddy and Scott, 1968; Forrester and Lind, 1969), as well as their role in cellular energetics (Atkinson, 1968; Ramaiah, 1974; Shen et al., 1968). Our results showed that neither CrP nor ATP was significantly hydrolyzed during 1 or 3 minutes of ischemia in ALD or PLD muscles. No measurable changes occurred in the adenylate pools; however, some loss of adenine nucleotides probably occurred, as indicated by increases in adenosine and inosine during ischemia in both muscles.

The concentrations of AMP and IMP measured in this study are considerably lower than those reported in other studies (Berne et al., 1971; Deuticke and Gerlach, 1966; Imai et al., 1964; Rubio et al., 1973). These differences probably are species related. The recoveries for AMP and IMP on the high pressure liquid chromatograph were 102 ± 3 (SE) and 86 ± 1.5%, respectively, and therefore cannot account for the low tissue values.

Prolonged ischemia and hypoxia are known to stimulate anaerobic glycolytic production of ATP (Ramaiah, 1974). There is evidence (Atkinson, 1968) suggesting that a fall in adenylate energy charge may stimulate glycolysis through activation of PFK. Our data showed that a fall in the energy charge occurs in the ALD only after 3 minutes of ischemia. The fall was very small and would have only a slight effect on PFK activity according to studies relating energy charge to PFK activity (Shen et al., 1968). The phosphorylation potential also may regulate PFK activity (Ramaiah, 1974). Our results showed that the phosphorylation potential decreased considerably in the ischemic ALD. Therefore, there was a potential stimulus to increase glycolytic activity in the ischemic (3-minute) ALD.

Changes in the concentration of lactate may serve as an index of the rate of anaerobic glycolysis, since its removal (washout or metabolic reutilization) is impaired during ischemia and accompanying hypoxia. The PLD has a high glycolytic capacity (Pette, 1971) but showed no significant increase in lactate content after 3 minutes of ischemia. In contrast, a 2.5-fold increase in lactate occurred after 3 minutes of ischemia in the ALD. The rate of lactate production averaged over the 1st minute of ischemia was 0.84 μmol/g-min. This rate increased to 1.1 μmol/g-min between 1 and 3 minutes of ischemia, suggesting a slight increase in anaerobic glycolysis. It is possible that the fall in the phosphorylation potential stimulated anaerobic glycolysis by activating PFK. Additional experiments involving measurement of fructose-6-phosphate and fructose-1,6-diphosphate would be necessary to determine if PFK is activated.

G-6-P concentrations showed no detectable changes during ischemia. These do not provide conclusive evidence concerning glycogen breakdown, since the concentration of G-6-P also is determined by the activity of PFK. However, when the G-6-P data are coupled with the lactate data, which indicate no significant increase in anaerobic glycolysis in the PLD, and very little, if any, in the ALD, then the failure to observe an increase in G-6-P suggests that a large breakdown of glycogen did not occur during ischemia in either muscle. It would be interesting to see if activation of phosphorylase occurred in these muscles, since the conversion of phosphorylase b to a occurs quite rapidly in ischemic and hypoxic cardiac tissue (Dobson and Mayer, 1973).

Therefore, this study has shown that ATP and CrP concentrations are maintained in the ischemic ALD and PLD without large increases in anaerobic glycolysis during short periods of ischemia. Mitochondrial oxidative phosphorylation probably accounts for little, if any, ATP production, since the ALD is virtually anoxic (tissue PO2 < 0.01 mm Hg) at 1 minute of ischemia (extrapolated from published data, Klabunde and Johnson, 1977b). With a resting oxygen consumption of 3 ml/min-100 g (Hudlicka, 1969), the ALD under aerobic conditions produces about 8 μmol of ATP/min-g by oxidative phosphorylation (assuming 6 mol ATP formed/mol O2). If oxidative phosphorylation is abolished by anoxia (Jöbsis, 1972; Starlinger and Lübers, 1973), then the ALD should experience a deficit of approximately 8 μmol of ATP/min-g. However, no significant decline in ATP or CrP occurred. One explanation is that ATP utilizing processes within the tissue (e.g., anabolic pathways, active transport mechanisms, heat production), slow down to conserve ATP. Alternatively, published values for oxygen consumption (Hudlicka, 1969) may overesti-
nate true resting oxygen consumption. If the oxygen consumption in the ALD is severalfold less than its reported value, then CrP and ATP would not be expected to fall significantly during the ischemic period. This argument gains support from a recent investigation (E. L. Bockman, personal communication) that shows that the soleus (red) and gracilis (white) muscles of the cat have oxygen consumptions of 0.28 ± 0.03 (SE) and 0.24 ± 0.03 ml/min-100 g, respectively. These values are severalfold lower than those reported by other investigators (Hudlicka, 1969, 1975).

The finding that adenosine accumulates in ischemic red and white muscle is very significant, not only because adenosine appears to be a sensitive indicator of ischemia, but also because adenosine is a very potent vasodilator (Berne et al., 1971). Previous investigations have shown that adenosine accumulates in muscles subjected to more than 20 minutes of ischemia (Rubio et al., 1973) and in muscles undergoing ischemic contractions for 5 minutes or more (Berne et al., 1971; Dobson et al., 1971). The present study demonstrated, for the first time, that adenosine accumulated in noncontracting red and white muscle following 3 minutes of ischemia. Furthermore, the increase was similar in both types of muscle (4.3 nmol/g in the ALD; 3.9 nmol/g in the PLD). This similarity is of interest because enzyme activity studies in vitro suggest that 5'-nucleotidase activity (dephosphorylation of AMP to adenosine) is higher in red skeletal muscle than in white (Bockman, 1977). If differences in the capacity for adenosine production do exist in red and white muscle, then these differences are not reflected in vivo following 3 minutes of ischemia. After 1 minute of ischemia, the red ALD showed a large increase in adenosine. This may reflect a more rapid onset of hypoxia and subsequent degradation of adenine nucleotides. The failure to detect a fall in ATP when adenosine increased can be explained by the relative concentrations of these two substances. A 5 nmol/g increase in adenosine represents less than 1% of the tissue content of ATP. In addition, the increase in adenosine at 1 minute may be explained by a more rapid activation or disinhibition of 5'-nucleotidase by allosteric effectors in the ALD than in the PLD.

Adenosine increased in both muscles without a concomitant increase in AMP. In other studies, using ischemic or hypoxic heart and skeletal muscle preparations (Berne et al., 1971; Deuticke and Gerlach, 1966; Rubio et al., 1973), it has been observed that AMP increased when adenosine was elevated. The present study differs from these studies in that very short periods of ischemia were employed. This observation poses some interesting questions regarding the 5'-nucleotidase reaction (AMP → adenosine + Pi). Is this a non-equilibrium reaction in which the enzyme activity is regulated by allosteric activators or inhibitors? That implies that adenosine production is limited by the activity of 5'-nucleotidase under normal conditions, rather than by substrate (AMP) availability. In support of this concept, diphosphate and triphosphate nucleotides are known to be effective competitive inhibitors with Ks in the μM range (Baer et al., 1966; Fox and Marchant, 1977). An alternative explanation is that AMP and adenosine are compartmentalized with respect to the enzyme, such that 5'-nucleotidase catalyzes an equilibrium reaction with AMP availability, or adenosine removal, limiting the reaction rate. While no doubt there are other possible explanations, additional discussion would go beyond the scope of the present study.

Inosine also accumulated in both muscles after 3 minutes of ischemia. The major pathways of inosine formation are by deamination of adenosine (adenosine deaminase) and dephosphorylation of IMP (5'-nucleotidase). IMP is formed mainly by deamination of AMP (AMP-deaminase). The much larger increase in IMP and inosine in the white muscle, compared to the red, suggests that there is a higher activity of AMP-deaminase in the white muscle. This is consistent with studies in vitro demonstrating that AMP-deaminase activity is greater in white skeletal muscle (Bockman, 1977; Raggi et al., 1975).

The results of this study are most consistent with the notion that adenosine could be the mediator of reactive hyperemia in these muscles. Following 3 minutes of ischemia, adenosine concentrations increased by 4.3 and 3.9 nmol/g in the ALD and PLD, respectively. A previous study (Klabunde and Johnson, 1977a) demonstrated that there was pronounced and similar reactive hyperemia in these two muscles following 3 minutes of ischemia. At 1 minute of ischemia, adenosine increased only in the ALD, although both muscles demonstrated reactive hyperemia following 1-minute blood flow occlusions (Klabunde and Johnson, 1977a). This suggests that some other metabolite or nonmetabolic factor (e.g., myogenic) may be responsible for reactive hyperemia in the PLD following 1 minute of ischemia. In the case of the PLD, the increase in adenosine at 3 minutes may contribute to the increase in peak reactive hyperemia flow and duration as the occlusion length is increased from 1 to 3 minutes. These conclusions are not meant to imply that adenosine is the sole metabolic mediator of reactive hyperemia. Adenosine may act in concert with other vasoconstrictor substances.

If not explicitly stated, then it often is implied that arresting blood flow to skeletal muscle causes substantial metabolic alterations, resulting in a fall in high energy phosphate stores and a stimulation of anaerobic metabolism (Johnson, 1974; Haddy and Scott, 1968). This has led many investigators to hypothesize that specific vasoactive substances accumulate during ischemia and cause vasodilation. Although this hypothesis is attractive and has received considerable attention, our results fail to support the underlying premise that substantial metabolic changes occur during ischemia. Brief pe-
nods of ischemia do not result in large changes in CrP, adenine nucleotide content, or in anaerobic metabolism in resting red and white skeletal muscle. A major finding of this study is that adenosine rapidly accumulated in ischemic red and white muscle before measurable changes occurred in CrP, adenine nucleotides, and lactate. These results support the hypothesis that adenosine may play an active role in red and white muscle vasodilation during short periods of ischemia.

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