Carbohydrate utilization in rat soleus muscle is influenced by carbonic anhydrase III activity

CLAUD E. CÔTE, GUI LAINE PERREAULT, AND JÉ RÔME FRENETTE
Hormonal Bioregulation Research Unit, Laval University Hospital
Research Center, Ste-Foy, Quebec, Canada G1V 4G2

Côté, Claude H., Guyla ine Perreault, and Jérôme Frenette. Carbohydrate utilization in rat soleus muscle is influenced by carbonic anhydrase III activity. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1211–R1218, 1997.—Inhibition of carbonic anhydrase III (CA III; EC 4.2.1.1) activity in type I muscle can influence resistance to fatigue and glycogen utilization. Our aim was to determine if CA III inhibition could influence muscle pH and glycolytic rate. Muscle pH, hexosemonophosphates (HMP), glycolytic intermediates, ATP, and creatine phosphate (CP) were measured at rest and during a fatigue protocol in rat soleus muscles in vitro with or without CA inhibitors (CAI). In resting muscles, CAI resulted in a significant drop in pH (7.11 vs. 7.06, P < 0.05) and in a two-to threefold increase in HMP content compared with control muscles. Measurements of HMP and glycolytic intermediates during the fatigue protocol suggested, however, that the glycolytic flux was not influenced. Globally, muscles incubated with CAI showed larger perturbations of their CP and ATP content than control muscles. The accumulation of HMP induced by the CAI was found to be totally dependent on the combined presence of external glucose and contractile activity, suggesting that inhibiting CA III may augment the responsivity of the contraction-induced glucose uptake process.

muscle fatigue; sulfonamide; glycolysis; hexosemonophosphate; glucose transport; contraction

CARBONIC ANHYDRASE III (CA III; EC 4.2.1.1) is predominantly found in skeletal muscle, liver, and adipose tissue (28). In muscle, CA III is especially abundant in the cytosol of type I slow-twitch fibers where it may count for as much as 15% of the cytosolic protein mass (11, 12). CA III activity is ~5–10 times lower in type IIa fibers, whereas it is basically undetectable in type IIb fibers (12). The membrane-bound CA IV isoform is also present in sarcoplasmic reticulum (SR) (4, 11) and sarcolem mal muscle preparations (16). Finally, ambiguity still exists regarding the presence of the mitochondrial isofor m CA V in rodent mammalian muscle.

Since CA III’s discovery, it has been postulated that its unique function in skeletal muscle was to facilitate the diffusion of CO₂ (2, 15, 17, 31). However, a strong case can be presented that suggests that this may not be its only cellular function. First, CA III has a very low hydratase activity compared with the other cytosolic isozymes CA I and CA II, the activity of CA III being ~1% of that for CA II. Second, there is a virtual absence of correlation between the oxidative capacity of a given muscle fiber type and its CA III activity and content (12), whereas a highly significant negative correlation exists between the level of CA III activity and those of various glycolytic enzymes (1). Third, when rats are made hypo- or hyperthyroid, several changes occur at the energy metabolism level that are coupled with rapid increase and decrease, respectively, in CA III expression. However, even during the transition, a tight coupling persists between the activity of key glycolytic enzymes and CA III activity, whereas no tight relationship can be observed with marker enzymes of the oxidative metabolism (30). In other words, muscles with low glycolytic potentials appear to have a strong need for CA III.

We have previously demonstrated that soleus (Sol) muscle incubated with CA inhibitors (CAIs) can show an increased resistance to fatigue compared with control (Ctr) muscles and that this effect is attributable to the loss of cytosolic CA III activity (9, 13). An analysis of the kinetics of the effect on fatigue showed that the largest difference between Ctr and methazolamide (Meth) muscles was observed in the early minutes of the fatigue protocol, a period when creatine phosphate (CP) hydrolysis and glycolysis are the predominant energy metabolic pathways (13). Geers and Gros (15), using in vitro muscles, also found that CAI could influence muscle contractility and CP concentrations. We subsequently proposed, on the basis of studies that examined glucose and glycogen metabolism under conditions of CA inhibition, that CA III may have a direct or indirect downregulatory effect on the glycogenolytic and/or glycolytic rate (9). Indeed, it was found that Sol muscles deprived of their CA III activity were using their glycogen at a higher rate than the Ctr muscles when measured over a 45-min in vitro fatigue protocol. According to this hypothesis, the inhibition of the CA III activity should allow a greater rate of carbohydrate utilization, which can translate into a lower fatigability, depending on the exercise duration. Because the handling of CO₂ by CA III may be involved in maintaining the cytosolic pH and because the key enzymes of glycolysis and glycogenolysis are both sensitive to pH fluctuations, it is therefore possible that inhibition of the CA III activity may influence one or both of these metabolic pathways. The objective of this study was, therefore, to determine if inhibition of CA III activity can influence 1) muscle pH at rest and during sustained muscle activity, 2) the accumulation of various glycolytic and glycogenolytic metabolites, and 3) the glycolytic rate.

MATERIALS AND METHODS

Measurement of contractile properties. For all experiments presented, isometric contractile properties were measured before initiation of the fatigue protocols as described previously (13). Female Wistar rats weighing 140–160 g were anesthetized with pentobarbital sodium (50 mg/kg ip), and whole Sol muscles were carefully dissected. Rats were killed

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by means of pneumothorax performed under anesthesia. Contractile properties were measured in vitro in a buffered physiological solution (Kreb's-Ringer bicarbonate) supplemented with curare (20 mg/ml) and glucose (2 mg/ml), unless otherwise specified, and maintained at 25°C. One tendon was attached to a rigid support at the bottom of the bath, and the other end was connected to an isometric force transducer (Grass FT-03) through a stainless steel hook. Muscles were stimulated with 25-V square pulses of 0.2 ms duration delivered through platinum field electrodes. The bathing solution of the experimental group contained a CAI in the form of 1 mM Meth. This concentration represents the highest amount of this compound that can be solubilized in the solution used for these experiments. In a small number of experiments, 0.1 mM ethoxyzolamide (Ethox) was also used as a CAI, and although it theoretically inhibits roughly 80% of the Sol muscle CA III activity compared with 100% inhibition for Meth, no difference was found between the two CAIs. Therefore, all metabolic data were pooled. These CAIs were selected mostly for their characteristics of high permeability through the cellular membrane (18). Previous studies in our laboratory indicate that the time course for the effect of Meth on fatigability is ~10 min (13).

For all protocols, an initial period of 30 min was allowed for muscles to equilibrate with the incubation medium (with or without CAI). During this period, muscles were adjusted to their optimum length, defined as the length at which maximal isometric twitch tension was produced. This process usually required 10–12 twitch contractions. At the end of the equilibration period, one single twitch contraction was elicited, and the following measurements were obtained: maximum twitch tension, contraction time (time-to-peak tension), and one-half relaxation time (RT½). After measurement of the twitch parameters, muscles were stimulated for 1 s at frequencies of 35, 50, 80, 100, and 120 Hz to determine maximum tetanic tension (Pₒ). Each tetanic contraction was separated by a 60-s resting period. Ten minutes after the last tetanic contraction, muscles were submitted to a fatigue protocol that consisted of eliciting one 0.5-s tetanic contraction at 10 Hz every 5 s for 30 min. This stimulation frequency produces a partially fused tetanic contraction, with tension reaching ~50% of Pₒ at the beginning of the test. This is the same rest used in previous experiments (9, 13); it was designed to induce a significant amount of tension loss over a relatively long period to allow recruitment of the aerobic metabolism and therefore increased CO₂ production.

The first series of experiments, including the determination of muscle pH and measurement of glucose 6-phosphate (G-6-P), fructose 6-phosphate (F-6-P), fructose 1,6-phosphate (F-1,6-P), glyceraldehyde 3-phosphate (G-3-P), pyruvate (Pyr), lactate (Lact), CP, and ATP was performed with Sol muscles incubated in the presence of exogenous glucose in the bathing solution as described previously. Other series of experiments were performed thereafter. In one of these, G-6-P concentration was also measured in Sol muscles submitted to the same experimental protocol described previously (with or without CAI) but with an incubation medium containing no glucose and one containing 25 µM cytochalasin B (Cyto) to abolish glucose uptake by the muscle. Finally, to verify the specificity of the inhibitor, the extensor digitorum longus (EDL) muscle, a type II muscle known to be devoid of CA III activity (26), was submitted to the same experimental protocol used for Sol muscle in the presence or in absence of CAI and G-6-P concentrations were determined.

Determination of muscle pH. The homogenate method was used to investigate the influence of CA III inhibition on muscle pH variation. All muscle samples obtained in situ at rest and at various times during the in vitro fatigue protocol (0, 1, 3, 5, 15, and 30 min) were frozen with metal tongs precooled in liquid nitrogen ~3 s after interruption of the fatigue test. The muscle samples were weighed frozen, dissected free of tendons and other nonmuscular elements, and then homogenized at 0°C in 5 vol. of 145 mM KCl, 10 mM NaCl, and 5 mM imidazole. The pH of the homogenate was measured at 38°C according to the method described by Sahlin (27).

Measurement of glycolytic intermediates. Muscle samples were frozen at rest and at various times during the fatigue protocol as described previously. Samples were homogenized at 0°C in 5 vol. (wt/vol) of 0.6 M perchloric acid and centrifuged at 5,000 g for 10 min. The supernatant was recovered and neutralized with 2 M KHCO₃. Measurements of G-6-P, F-6-P, F-1,6-P, G-3-P, Pyr, Lact, CP, and ATP concentrations were obtained fluorometrically or spectrophotometrically as described by Lowry and Passonneau (21) and Bergmeyer (3).

Statistical analysis. All results for metabolic concentrations are expressed in millimoles per gram wet muscle, unless specified otherwise, and are presented as means ± SE. Data for muscle metabolic concentrations and muscle pH were analyzed by a two-way analysis of variance for repeated measures followed by Fisher’s protected least significant differences test when a significant F ratio was obtained. Data on muscle contractile properties were analyzed by a one-way analysis of variance. In all cases, the level of significance was set at P < 0.05.

RESULTS

Data for isometric contractile properties of Sol muscles from Ctr and experimental groups are shown in Table 1. All measurements were unaffected by CAI except for RT½, which was significantly prolonged, an effect previously shown to be related to the inhibition of the SR-CA IV isoform (15). As shown in Fig. 1, when incubated in presence of CAI, muscles showed an increased resistance to fatigue compared with Ctr muscles. The difference in tension production could be observed as soon as 1 min after initiation of the fatigue protocol.

Mean pH value for muscles incubated with CAI at time 0 of the fatigue protocol was significantly different from the value obtained with Ctr muscles (Table 2). Fluctuations in muscle pH during contraction, although quite small, were more pronounced in the Ctr group than in the CAI muscles. Indeed, significant decreases were observed in Ctr after 5, 15, and 30 min

| Table 1. Contractile properties of control and experimental soleus muscle |
|-----------------|-----------------|-----------------|
|                 | Control (n = 36)| Ethox (0.1 mM) (n = 30) | Meth (1 mM) (n = 30) |
| TPT, ms         | 68.2 ± 0.2      | 71.1 ± 0.2*       | 67.7 ± 1.2         |
| RT½, ms         | 88.2 ± 4.2      | 95.7 ± 2.5*       | 97.6 ± 3.9*       |
| Rₑ, ms          | 21.5 ± 1.0      | 20.1 ± 0.1        | 20.5 ± 0.4        |
| Pₒ, g           | 89.7 ± 3.8      | 90 ± 0.5          | 95 ± 1            |

Values are means ± SE; n = no. of muscles. Muscles were incubated at 25°C for 30 min before measurements of contractile properties. Ethox, ethoxyzolamide; Meth, methazolamide; TPT, time to peak tension; RT½, one-half relaxation time; Pₒ, maximum tetanic tension. *Significantly different from control, P < 0.05.
of stimulation compared with the value at time 0, whereas the difference was almost significant already at 3 min \( (P = 0.06) \). In the CAI group, no acidification took place during the first 3 min of the fatigue protocol, a period when CP utilization is important, and significant changes appeared only after 15 min.

Values for metabolite concentrations of in vivo muscles at rest and for muscles submitted to the in vitro fatigue protocol when glucose was present in the bathing solution are presented in Tables 3 and 4. Very significant differences between both groups in terms of hexosemonophosphate (HMP) content are already present before the start of the fatigue protocol. Although it is clear that the dissection, preparation, and incubation procedure did not affect Ctr muscles, as shown by the fact that values for HMP at rest in vivo and at time 0 of the in vitro protocol are similar, large increases in G-6-P and F-6-P levels are found in the muscles incubated with CAI. On the contrary, values for all glycolytic intermediates located at steps after the phosphofructokinase (PFK) reaction did not show such an increase.

Variations in HMP content during the stimulation protocol were also quantitatively and qualitatively

![Graph](http://ajpregu.physiology.org/)

Fig. 1. Effects of inhibition of carbonic anhydrase III (CA III) activity with carbonic anhydrase inhibitors (CAIs) on fatigue. Rat soleus (Sol) muscles were incubated in the presence of CAI (0.1 mM ethoxyzolamide or 1 mM methazolamide; data pooled) or without inhibitor [control (Ctr)] for \( \sim 45 \) min before a 30-min stimulation protocol. Stimulation consisted of 1 500-ms train at 10 Hz every 5 s for 30 min \( (n = 30–65 \) muscles in each group). A significant difference was found for all time points between 1 and 30 min (analysis of variance, \( P < 0.05 \)).

Table 2. Values for pH obtained during fatigue protocol for soleus muscles incubated with or without carbonic anhydrase inhibitor

<table>
<thead>
<tr>
<th>Group</th>
<th>Time, min</th>
<th>0</th>
<th>3</th>
<th>5</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr</td>
<td>7.11 ± 0.01</td>
<td>7.06 ± 0.01</td>
<td>7.03 ± 0.01*</td>
<td>7.01 ± 0.02*</td>
<td>7.00 ± 0.01*</td>
<td></td>
</tr>
<tr>
<td>CAI</td>
<td>7.06 ± 0.01†</td>
<td>7.07 ± 0.01</td>
<td>7.03 ± 0.01</td>
<td>7.00 ± 0.01*</td>
<td>6.99 ± 0.01*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6 \) muscles. Muscles were stimulated at 10 Hz for 500 ms once every 5 s during the fatigue protocol. Time 0 is the beginning of the stimulation protocol. Muscles had been incubated for \( \sim 45 \) min before initiation of the fatigue protocol. CAI, carbonic anhydrase inhibitor. *Significantly different from value at time 0; \( P < 0.05 \); †Significantly different from corresponding control value; \( P < 0.05 \).

Table 3. Concentrations of various hexosemonophosphates at rest and during fatigue protocol in soleus muscles from control and CAI groups

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Control, min</th>
<th>CAI, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-6-P</td>
<td>0.141 ± 0.011</td>
<td>0.198 ± 0.020</td>
<td>0.372 ± 0.060†</td>
</tr>
<tr>
<td>F-6-P</td>
<td>0.033 ± 0.007</td>
<td>0.028 ± 0.010</td>
<td>0.064 ± 0.01†</td>
</tr>
<tr>
<td>F-1,6-P</td>
<td>0.023 ± 0.002</td>
<td>0.028 ± 0.01</td>
<td>0.021 ± 0.004</td>
</tr>
<tr>
<td>F-6-P/F-1,6-P</td>
<td>1.43 ± 0.21</td>
<td>1.07 ± 0.010</td>
<td>3.04 ± 0.53†</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 5–9 \) muscles. Values at rest are from muscle sample taken in situ in anesthetized rats. G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; F-1,6-P, fructose 1,6-phosphate. *Significantly different from value at time 0; \( P < 0.05 \). †Significantly different from corresponding control value; \( P < 0.05 \).

Table 4. Concentrations of various 3-C glycolytic intermediates at rest and during fatigue protocol in soleus muscles from control and CAI groups

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Control, min</th>
<th>CAI, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-3-P, nmol/g wet wt</td>
<td>23.1 ± 3.0</td>
<td>19.3 ± 2.9</td>
<td>20.2 ± 2.7</td>
</tr>
<tr>
<td>Pyr, nmol/g wet wt</td>
<td>37.4 ± 4.4</td>
<td>21.9 ± 2.1</td>
<td>24.1 ± 1.6</td>
</tr>
<tr>
<td>Lact, µmol/g wet wt</td>
<td>1.71 ± 0.14</td>
<td>0.72 ± 0.07</td>
<td>5.76 ± 0.74*</td>
</tr>
<tr>
<td>Lact/Pyr</td>
<td>45.7 ± 4.6</td>
<td>239.0 ± 22.5*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 5–9 \) muscles. Values at rest are from muscle sample taken in situ in anesthetized rats. Pyr, pyruvate; Lact, lactate; G-3-P, glyceraldehyde 3-phosphate. *Significantly different from value at time 0; \( P < 0.05 \).
different between both groups (Table 3). Significant increases in the levels of G-6-P during the fatigue protocol only occurred in the Ctr group, with values for G-6-P content in the CAI group even showing a significant decrease after 1 min compared with the value at time 0. G-6-P content was approximately two- to four-fold greater in the CAI group than in the Ctr group for all time points selected. Levels of F-6-P in Ctr muscle did increase gradually during the fatigue test in parallel with the values for G-6-P. However, F-6-P levels in the CAI muscles had a tendency to show changes in a direction opposite to the one for G-6-P, with a significant accumulation already present after 1 min of stimulation compared with the value at time 0. Concentrations of F-6-P became similar in both groups at 20 min. At all time points selected, no significant difference was found for F-1,6-P between Ctr and CAI. The ratio F-6-P/F-1,6-P, which can be used to determine if an accumulation of metabolite occurred at a critical point of the glycolytic flux, was three- to fivefold higher in the CAI group for all time points (Table 3). Muscle content of G-3-P, Pyr, and Lact during the fatigue protocol is shown in Table 4. Only Lact levels increased very significantly over time during the fatigue test, but, as is the case for G-3-P and Pyr, no difference could be found between groups. Overall, one is left with the conclusion that the glycolytic flux was similar in both Ctr and muscles deprived of CA activity.

To investigate the origin of this accumulation of HMPs in the muscles incubated with CAI, G-6-P measurements were made in both Ctr and CAI Sol muscles incubated in a bathing solution with (+) and without (−) glucose; also, for each condition (+ or − glucose), G-6-P levels were obtained on muscles submitted to the usual contractile activity preceding the fatigue test and in muscles that were installed under passive tension in the bath but not submitted to any contractile activity at all for the 45 min period. Because hexokinase (HK) (type II, EC 2.7.1.1) cannot provide G-6-P to the muscle cell in the absence of external glucose, glycogenolysis becomes the only possible pathway to produce G-6-P. In the absence of external glucose, CAIs do not have an effect on G-6-P accumulation, regardless of whether muscles undergo contractile activity during the 45-min incubation (Fig. 2). Interestingly, the accumulation of G-6-P previously described in the presence of external glucose cannot be demonstrated if muscles are not submitted to contractile activity during the incubation. To further confirm these data, experiments were conducted with muscles incubated in the presence of external glucose and undergoing contractile activities but with the bathing solution containing Cyto (25 µM), an inhibitor of glucose uptake. Under such conditions, CAI could not induce any increase in G-6-P content compared with Ctr muscles (Fig. 2). When similar protocols were carried out with the EDL muscle, which is basically devoid of CA III activity, no influence of CAI on HMP content was observed. Values of 0.143 ± 0.050 and 0.130 ± 0.030 were obtained for Ctr and CAI groups, respectively, suggesting that the accumulation of HMPs observed with the Sol muscles is a specific effect related to the inhibition of CA III.

Results obtained for ATP and CP contents in Ctr and CAI groups just before and during the fatigue test are shown in Figs. 3 and 4. For both ATP and CP levels, values at time 0 did not differ from those obtained on Sol muscles from resting (in vivo) anesthetized rats (3.03 and 13.3 µmol/g, respectively). Values for ATP levels in Ctr muscles did show a small but significant increase at 5 min but did not differ significantly from the value at time 0 thereafter during the protocol, while significant drops in ATP content did occur at 10 and 30 min in the CAI group. Muscle CP levels decreased significantly in both groups by roughly 50% after the 30-min period. The absolute value for CP content in the CAI group was significantly lower than the Ctr value only at time 5 min in the protocol. This kinetic of CP disappearance may appear slow compared with many other contractile protocols and has to be related to the fact that 1) the contractile protocol used was of a fairly moderate intensity with a high rest-to-work ratio and 2) type I muscle fibers have a much lower rate of CP hydrolysis than the mixed or fast-twitch muscles used in most published experiments. Using mean values for CP content, one can estimate that the rate of CP utilization in the first 5 min of the protocol was higher in muscles with the presence of CAI than in the Ctr muscles (Fig. 5).
Muscle contractile properties. The data presented for contractile properties are in agreement with previously published reports using rat Sol muscle (7, 8). The results obtained for Po and other isometric contractile properties in this study confirmed the integrity of the isolated muscles used. Aside from RT‰, which is significantly prolonged in the presence of CAI, the inhibitors used do not interfere with the cellular reactions involved in the contractile process. Such a prolongation of RT‰ in rat Sol muscles incubated in vitro in the presence of CAI has been confirmed by others (15), and thorough investigations have revealed that the prolongation is linked to inhibition of the SR-CA IV isoform, which can influence calcium release and reuptake by the SR. Possible mechanisms underlying this effect have been discussed in detail in previous papers (9, 10, 13).

Muscle pH. The pH values obtained for skeletal muscle with the homogenate technique are, in general, significantly higher, by roughly 0.1 unit, than the values obtained with pH microelectrodes (23), and this difference is likely related to the presence of noncytosolic fluids. Although absolute values must be looked at lightly, this technique may be useful in the comparison of relative changes of two different groups submitted to a similar protocol. The homogenate pH reported here, 7.11 ± 0.06, is similar to the value of 7.09 ± 0.06 reported by Troup et al. (31) and the value of 7.16 reported by Geers and Gros (15), who used the 5,5-dimethyl-2,4-oxazolidinedione method. The lower muscle pH in the Meth group before the beginning of the fatigue protocol (7.11 vs. 7.06) is consistent with the results presented by Geers and Gros (15), who observed a 0.1-unit drop in pH in Sol muscle incubated for 6 h (compared with ~45 min in the present study) with a CAI. They attributed this effect to the suppression of facilitated CO₂ diffusion and to a reduction in CO₂ diffusivity. Under these conditions, CO₂ level should increase inside the muscle cell and the pH should drop because of the low uncatalyzed hydration activity that occurs naturally. No significant decrease in pH was noticed during the first 5 min of the fatigue protocol in the CAI group, whereas a significant decrease of pH occurred in the Ctr group during this same period. CP
hydrolysis and glycolysis are two energy metabolic pathways used by the muscle during this early period. Hydrolysis of CP is a proton-consuming reaction, whereas glycolysis leads to H⁺ production. The fact that no visible decrease in pH occurred in muscles deprived of CA activity during the early portion of the fatigue test is consistent with the higher rate of CP utilization observed in this group compared with Ctr muscle and suggests that CA III has a significant impact on proton accumulation. Globally, even though the pH data must be interpreted with care, we nevertheless believe that 1) the fact that the pH value measured in both groups of muscles was identical for the last 25 min of the stimulation protocol, at a time when oxidative metabolism is active, does not support the idea that CA III is there only to facilitate the diffusion of CO₂ and 2) it is very unlikely that the increase in resistance to fatigue induced by CAI is the direct result of changes in cytosolic pH.

CA III and carbohydrate metabolism. The values reported for resting content of HMPs compare well with the data reported from other laboratories (29). Surprisingly, differences in HMP concentrations were already present before initiation of the fatigue test. Because muscles underwent 10–12 twitch contractions followed by 5–6 600-ms tetanic contractions during this ~45-min period, it is obvious that the metabolic rate does not have to be very high to see the metabolic influence of CAI. Three main metabolic components control the concentration of HMPs: two generating pathways and one consuming chain of reactions. G-6-P can be generated through glycogenolysis and through phosphorylation of exogenous glucose by HK, whereas degradation of HMPs is totally dependent on the glycolytic flux, which is considered to be mostly under the influence of PFK and possibly pyruvate dehydrogenase (PDH, EC 1.2.4.1) also. At a first look, data obtained for HMPs and PFK and possibly pyruvate dehydrogenase (PDH, EC 1.2.4.1) also. At a first look, data obtained for HMPs and PFK was obviously blocking the breakdown of HMP, the concentration of which kept increasing despite the well-known negative feedbacks that should have been exerted on HK and glycogenolysis. PFK is an allosteric enzyme that could be influenced by CA III activity whose modulation at rest and during exercise is quite complex (20, 24). Whether the small decrease in pH measured before the fatigue test could have induced PFK blockade and the subsequent HMP accumulation deserves further examination with more accurate methods.

The most significant finding of the present series of experiments is that the accumulation of HMP observed is dependent on both the presence of external glucose and contractile activity. Although the amount of contractile activity performed during the equilibration period was not enough to increase the G-6-P level in Ctr muscle, G-6-P content was increased by 100% in muscles incubated with CAI. If one assumes the noninvolvement of HK activity in this scheme, this suggests that inhibiting the CA activity of the Sol muscle does in some way enhance the responsivity of the contraction-induced glucose uptake process. However, a mechanistic explanation for this influence would clearly be speculative at this point in the investigation. Skeletal muscle is a very important tissue for glucose disposal and contains two facilitative glucose transporters, GLUT-4 and GLUT-1. GLUT-4 is specially abundant in oxidative muscles like the Sol muscle (25) and is regulated by both insulin and contraction through two different intracellular processing schemes possibly involving the contribution of two different pools of GLUT-4 transporters (22) where phosphorylation-dephosphorylation reactions are numerous. Although the possible influence of CAI on insulin-induced glucose uptake needs to be investigated to get a more global picture of the impact of CA activity on muscle glucose uptake at rest and during exercise, the fact remains that this first demonstration of the influence of CA activity on the uptake of exogenous energy substrate strengthens the notion that CA III is possibly a key modulator of energy metabolism.

The influence of CAI on carbohydrate metabolism during the fatigue test seems to be, however, quite different from what was noticed during the pretest period. Once the stimulation protocol was initiated, there was a gradual increase in HMP concentrations in the Ctr Sol muscles, with the first significant difference observed after 5 min of stimulation for G-6-P; after 20 min of stimulation, the ratio F-6-P/F-1,6-P in Ctr muscles was twice as high as the value at time 0, indicating that PFK is somehow limiting the flux. On the contrary, HMPs did not increase over time during the fatigue test in the CAI group. If one assumes that the flux through PFK is the same in both groups during the fatigue test, as the values for 3-C compounds would suggest, it is then implied that HMP production in the CAI group is smaller than in the Ctr muscles. However, this does not agree with the previous demonstration of an increased glycogen consumption during the same fatigue test with CAI. Thus a strict logical approach would suggest that, during the fatigue protocol, a time when the cellular environment is obviously very different from the situation at rest, CAI can probably impact on some key downstream reactions, like the one catalyzed by PDH, making increases in 3-C compounds invisible. Such an hypothesis would agree with the work of Geers et al. (14), who found that CAI could increase oxygen consumption of rat Sol muscle incubated in vitro during very long periods by ~25%. PDH is a multienzyme complex that bridges glycolysis and the tricarboxylic acid cycle and plays a pivotal role in the regulation of carbohydrate metabolism as a whole. Its modulation is quite complex and involves, among other things, a phosphorylation-dephosphorylation cascade.

Globally, the evidence is quite convincing that 1) the energy demand was higher in the group deprived of CA
activity than in the Ctr group, as suggested by data for CP, ATP, and HMP and 2) in the presence of CAI, the Sol muscle has an increased reliance on carbohydrate for ATP production during contractile activity. The overall impression is that inhibition of CA leads to some loss of homeostatic control as it specifically relates to energy metabolism at large. The higher rate of CP disappearance noted during the first 5 min could be one of the metabolic mechanisms that allows muscles with CAI to sustain higher tension production during the early part of the fatigue test. However, there seems to be a price to pay for this consistently higher level of muscle performance recorded during the 30-min test as, for one, ATP level decreased to unexpectedly low levels after 30 min of contractile activity in the presence of CAI. On the basis of this observation, one would predict that the positive influence of CAI on fatigue should diminish gradually thereafter, assuming that the difference in ATP levels between Ctr and CAI muscle keeps increasing.

More specifically, two key observations deserve more in-depth analysis. First, it is clear that the action of CAI on HMP is restricted only to muscles undergoing some contractile activity; second, evidence shows that the influence of CAI (and consequently of CAIII) is not the same during rest and sustained contractile activity. Such a contrast in enzymatic behavior could possibly be related to the fact that CAIII is the only member of the CA family of isozymes to possess both a hydratase and a phosphatase activity. The possible physiological or biological significance of the phosphatase activity of CAIII has remained elusive until quite recently (19). Indeed, recent results suggest that CAIII, under conditions leading to increased oxidative stress (like exercise) and, consequently, to CAIII glutathiolation (5, 6), could possibly act as a protein phosphatase with tyrosine, serine, and threonine as preferred substrates. This opens a very exciting and interesting avenue for the biology of CAIII in skeletal muscle because both carbohydrate and lipid metabolism in this tissue contain cellular cascades that involve protein phosphatases. It is therefore quite possible that the influence of CAIII under resting and contracting conditions may be very different, because CAIII could act as a hydratase and/or phosphatase, respectively, under such conditions.

In conclusion, we have shown that inhibition of the CA activity of the Sol muscle can slightly influence muscle pH. The metabolic correlates obtained during these same experiments suggest that the increased resistance to fatigue induced by the CA inhibitor may be partially related to an accumulation of glycolytic substrates that occurred during the period of incubation before the fatigue protocol. Inhibition of CA activity in an oxidative type I muscle is undoubtedly linked to a loss of regulatory metabolic processes. More specific experiments will have to be conducted to identify more precisely the underlying regulatory mechanism(s) through which inhibition of CAI can influence muscle metabolism.

Perspectives

The present results can be added to the data obtained previously for glycogen utilization that show that it is increased when CA activity is abolished. Globally, it is becoming evident that an active CAIII in a type I muscle contributes to the sparing of the glycogen store and could therefore be part of the complex and still-debated glucose free fatty acid (FFA) cycle. Glycogen sparing is amazingly highly efficient in type I muscle fibers, where the highest CAIII contents and activities are found. The present data that show that CAIII may influence glucose uptake during contractile activity are provocative and very interesting. Whether CAIII has a direct effect on carbohydrate utilization or an indirect action linked to the preferential use of FFA as oxidative substrate also remains to be investigated. Type I fibers do possess an important pool of triglycerides, and it has been estimated that ~50% of the fat oxidized during exercise of moderate intensity comes from this pool. Because skeletal muscle hormone-sensitive lipase is sensitive to pH fluctuations and is activated through a cascade of phosphatase reactions, CAIII could also potentially participate in the modulation of FFA metabolism. The fact that CAIII is found in very high concentrations in the three specific tissues controlling energy metabolism (fat cells, skeletal muscle, and liver) makes it a good candidate for a potential role as a metabolic modulator.

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Address for reprint requests: C. Côté, Laval Univ. Hospital Center, Research Center, 9500, 2705 Blvd. Laurier, Ste-Foy, Québec, Canada G1V 4G2.

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