Metabolic and contractile influence of carbonic anhydrase III in skeletal muscle is age dependent

CLAUDE H. CÔTÉ, FABRISIA AMBROSIO, AND GUYLAINÉ PERREAU LT
Lipid Research Unit, Centre Hospitalier de l'Université Laval Research Center, Ste-Foy, Québec, Canada G1V 4G2

Côté, Claude H., Fabrisia Ambrosio, and Guylaine Perreault. Metabolic and contractile influence of carbonic anhydrase III in skeletal muscle is age dependent. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R559–R565, 1999.—Carbonic anhydrase (CA) III is very abundant in type I skeletal muscle, but its function is still debated. Our aims were to examine CA III expression during growth and determine whether the effects of CA inhibition previously observed in adult muscles could be seen in younger rats in which CA III levels are lower. CA III content and activity were measured in soleus muscles from 10- to 100-day-old rats, and the influence of CA inhibitor on fatigue and hexosemonophosphate content was quantified in vitro. CA III activity and content increased fivefold between 10 and 100 days of age. Data analysis revealed that the influence of CA inhibitor on fatigue was to some extent positively and linearly related to the level of CA III activity. Hexosemonophosphate accumulation with CA inhibition also became more significant with age. In conclusion, CA III level in soleus muscle does not stabilize before 3 mo after birth; data also confirm that the effects of CA inhibitors are due to inhibition of the CA III isom.

fatigue; carbohydrate; metabolism; sulfonamides

CARBONIC ANHYDRASE (CA) III (EC 4.2.1.1), the predominant CA isoenzyme in mammalian skeletal muscle, is found primarily in the cytosol of type I and IIa muscle fibers (10), liver cells (14), and adipocytes (18). Skeletal muscle also contains the sulfonamide-sensitive CA IV isoenzyme associated with sarcoplasmic reticulum (SR) and sarcolemmal membrane (1, 9, 22). However, this CA isoenzyme is clearly distinct from CA III on the basis of its localization, specific activity, and sensitivity to sulfonamides, a family of specific inhibitors.

Since its discovery, it has been assumed that the physiological function of CA III was to facilitate CO2 diffusion across the sarcolemma membrane, and experimental data supporting this claim can be found (12). However, a detailed analysis of the distribution of CA III in the three main types of skeletal muscle fibers reveals no clear relationship between the capacity of a muscle to produce CO2 and its CA III activity. On the other hand, a very significant negative correlation has been reported between CA III activity and activity of key glycolytic enzyme markers (10, 11). This observation, among other factors, led us to investigate the influence of CA inhibition on type I skeletal muscle contractility and energy metabolism.

We reported that CA III inhibition in rat soleus (Sol) muscles incubated in vitro led to an increased resistance to fatigue compared with control muscles during standardized fatigue protocols (6, 11). We went on to demonstrate that, during sustained contractile activity, CA III inhibition could increase the rate of glycogen utilization in rat Sol muscles (5); with testing under resting conditions, we found an increase in hexosemonophosphate (HMP) concentration when CA III was inhibited (8). Globally, these results suggested that CA III can influence the rate of utilization of carbohydrates in type I muscles and is possibly an element of the so-called Randle or glucose-free fatty acid (FFA) cycle. During the course of these experiments, it was found that the effects of the CA inhibitor methazolamide (Meth) were often quite variable and that this lack of consistency seemed to be related to the age of the animals. Riley et al. (19) briefly looked at the level of expression of CA III in rats during development and found that it increased progressively and quite drastically with age in the Sol muscle to reach a plateau at ~75–100 days. Therefore, the aims of the present experiments were 1) to provide further details with regard to the level of CA III expression in type I muscle during development and 2) to determine whether the influence of CA III inhibition on fatigability and HMP accumulation, previously observed with mature adult rats, could be reproduced in younger animals if they possess much lower levels of CA III activity and content. Our working hypothesis is that the influence of CA III inhibition on fatigue and HMP accumulation should be quantitatively related to the level of expression of CA III in the muscle tested.

MATERIALS AND METHODS

Animal care. Sol muscles from 10- to 100-day-old female Wistar rats were used. For most experiments, four age groups were selected: 20–23, 30, 45, and 55 days. For some of the noncontractile measurements, 10-, 70-, and 100-day-old animals were also used. Sample size in all cases ranged from five to seven. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and the Sol muscle was carefully dissected. The use and care of the animals in these studies were approved by and followed the guidelines of the University Hospital Research Center Animal Care and Use Committee. Rats were given food and water ad libitum. At the end of the experiment, animals were killed with an overdose of the anesthetic.

Measurement of contractile properties and fatigability. Contractile and fatigue data were obtained from all groups of animals except the 10-day-old rats. Contractile properties were measured in vitro in Krebs-Ringer bicarbonate buffer maintained at 25°C and supplemented with curare (20 mg/
metabolism and, therefore, an increased CO2 production. Relatively long period to allow recruitment of the aerobic

Table 1. Contractile properties of soleus muscles

<table>
<thead>
<tr>
<th>Age</th>
<th>Muscle Mass, mg</th>
<th>TPT, ms</th>
<th>RT½, ms</th>
<th>P0, g</th>
<th>N/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18.4 ± 0.8</td>
<td>53.2 ± 6.6</td>
<td>51.0 ± 3.9</td>
<td>5.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Meth</td>
<td>20.0 ± 0.7</td>
<td>53.1 ± 6.9</td>
<td>50.8 ± 3.9</td>
<td>5.2 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>30 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>27.2 ± 4.9</td>
<td>57.7 ± 2.7</td>
<td>58.5 ± 2.5</td>
<td>9.9 ± 2.2</td>
<td>48.8 ± 5.1</td>
</tr>
<tr>
<td>Meth</td>
<td>30.7 ± 1.1</td>
<td>58.4 ± 1.1</td>
<td>67.5 ± 3.1*</td>
<td>10.3 ± 1.9</td>
<td>51.8 ± 4.9</td>
</tr>
<tr>
<td>45 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>60.2 ± 5.6</td>
<td>64.5 ± 5</td>
<td>82.2 ± 5</td>
<td>14.8 ± 0.9</td>
<td>79.2 ± 4.8</td>
</tr>
<tr>
<td>Meth</td>
<td>60.4 ± 5.6</td>
<td>62.3 ± 2.9</td>
<td>85.3 ± 2.9</td>
<td>13.9 ± 0.8</td>
<td>74.6 ± 3.7</td>
</tr>
<tr>
<td>55 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>67.9 ± 4.4</td>
<td>68.2 ± 2.5</td>
<td>76.6 ± 4.9</td>
<td>18.6 ± 1.8</td>
<td>95.1 ± 5.2</td>
</tr>
<tr>
<td>Meth</td>
<td>70.1 ± 2.7</td>
<td>72.2 ± 3.1</td>
<td>80.1 ± 5.3</td>
<td>21.8 ± 1</td>
<td>99.1 ± 4.2</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5-6 determinations in all groups. TPT, time to peak tension; RT½, half relaxation time; P0, maximum twitch tension; Po, maximum tetanic tension; Meth, methazolamide. *Significantly different from age-matched control group, P < 0.05.

RESULTS

Table 1 presents data for muscle mass and contractile properties of Sol muscles dissected from rats of different age groups. As expected, muscle mass increased with age. Age also had a significant effect on
contractile properties. Twitch contractile speed became slower, as shown by increases in TPT and RT½ values. Values of TPT for the 23-day-old animals were significantly lower than those for the 45- and 55-day-old animals, whereas values for the 30-day-old animals were statistically different from those for the oldest group only. The same general trend could be observed for RT½ values as stabilization occurred by 45 days. However, this was the only contractile variable for which a significant effect of Meth could be observed; indeed, a significant increase was noted at 30 days, whereas only tendencies could be seen with the two older groups. Absolute twitch and tetanic force production increased constantly over the time span covered in our experiment, whereas normalized P0 stabilized at 30 days. All values for force production were not influenced by the presence of Meth in the bathing medium.

Values for Sol muscle CA III specific activity are shown in Fig. 1. CA III activity increases almost constantly from birth to adulthood, with values increasing roughly fivefold between 10 and 100 days of age. The slope of the increase in CA III activity is very high between 10 and 25 days; the increase is slower between 25 and 100 days. A parallel increase in CA III content is also clearly visible on the immunoblot shown in Fig. 2. In contrast to CA III, the level of sensitive CA activity, which represents CA isoforms in cytosolic skeletal muscle extract other than CA III, stabilized quite rapidly after a significant increase between 20 and 40 days of age to remain quite constant until 100 days of age (data not shown).

The influence of age alone on Sol muscle resistance to fatigue can be observed in Fig. 3. The stimulation parameters were kept the same for the three groups of muscles tested, even though contractile speed was changing over this age period. Muscles from the 23-day-old group produced significantly more tension than muscles from the other groups, except at the first time point. At 30 days a significantly higher resistance to fatigue between 10 and 30 min was observed in 30- than in 45-day-old animals. Values are means ± SE; n = 5–7 muscles for each group.

Fig. 1. Changes in soleus muscle carbonic anhydrase (CA) III specific activity as a function of age. CA III specific activity was measured with cytosolic extracts prepared from soleus muscles dissected from rats of different ages. CA III specific activity was calculated by subtracting value obtained for sensitive CA activity [with 5 µM methazolamide (Meth)] from value for total CA activity (no Meth). Values are means ± SE; n = 5–7 determinations for each point.

Fig. 2. Changes in CA III content of soleus muscle as a function of age. CA III content was quantified by Western blotting. Soleus muscle cytosolic extracts were electrophoretically separated and transferred on nitrocellulose membranes for immunodetection with a CA III-specific antibody. CA III content increased steadily over time during growth. Bottom: typical blots, with molecular weight markers on left. Values are means ± SE. Optical density values represent mean of 4 different experiments performed in duplicate.

Fig. 3. Resistance to fatigue of control (Ctr) soleus muscle from rats at different ages. Fatigue curves were obtained with control soleus muscles from 23- to 45-day-old rats. Muscles were stimulated as described in MATERIALS AND METHODS. Significantly more tension was produced by muscles from 23-day-old animals than by muscles from 2 other groups, except at 1st time point. At 30 days a significantly higher resistance to fatigue between 10 and 30 min was observed in 30- than in 45-day-old animals. Values are means ± SE; n = 5–7 muscles for each group.
old animals developed significantly higher relative tension than the two other groups starting from 1 min until the end of the protocol. The 30- and 45-day-old animals were more closely matched, inasmuch as significant differences could be observed only between 10 and 25 min. Muscles from 55-day-old animals were also tested, despite their large size, but are not shown in Fig. 3, whereas muscles from 10-day-old animals were too small for manipulation in our set-up. In Fig. 4 the impact of Meth on muscle fatigue is presented for 23-, 30-, and 45-day-old rats. As previously observed with 45-day-old animals, tension production was clearly different between Meth-treated and control muscles, the muscles incubated with Meth showing significantly higher values for tension production than the control muscles between 1 min and the end of the fatigue test. The influence of Meth was very similar in muscles from 55- and 45-day-old animals (data not shown). No statistically significant difference could be observed in the 23-day-old animals, even though there was an obvious tendency for the Meth-treated muscles to perform better than the control muscles, especially in the middle of the protocol. At 30 days of age the Meth-treated muscles showed an increased resistance to fatigue compared with control muscles very similar to that documented for Sol muscles from 45- to 55-day-old animals, but some time points did not reach statistical significance.

To better analyze the influence of CA III content and activity on resistance to fatigue, the average difference in tension production between control and Meth-treated muscles over the 30-min duration of the fatigue test (tension-time integral) was calculated and plotted against the muscle CA III specific activity (Fig. 5). The data analysis revealed a significant relationship between Sol muscle CA III activity and the difference in the integrated value for tension production between control and Meth-treated muscles. The relationship was especially solid for activity levels between 25 and 35 U/mg, whereas a plateau was observed thereafter with increasing levels of activity. Not surprisingly, this plateau would suggest that, in adult animals, a factor(s) other than CA III activity obviously modulates resistance to fatigue. Overall, the relationship was best
type I fibers are ideally suited for postural work, their involvement in postural work, the fact that the Sol muscle is involved in postural work, the oxidative and glycolytic capacities of each fiber type undergo changes over this period as the animal reaches sexual maturity. Along with changes in fiber types, the oxidative and glycolytic capacities of each fiber type undergo changes over this period, as does capillary density (21). Although all

**DISCUSSION**

Contractile properties. Data obtained for isometric contractile measurements are in good agreement with published values for rat Sol muscles (4). Few studies have examined the influence of age on Sol muscle contractility; however, the general trend from a few weeks after birth to adulthood is a slowing of isometric and isotonic contractile speed and an increase in capacity for tension production (3, 15). Although the increased tension production is basically the result of an augmented cross-sectional area with age, the diminished speed of contraction is mainly associated with the maturation of the myosin heavy chain population of the Sol muscle. As documented previously, the proportion of type I fiber in the Sol muscle seems to be constantly increased with age, but slowly increasing with age. At 1 wk after birth, rat Sol muscles are composed of 55% type I and 45% type IIc fibers; at 9 wk, type I fibers account for 66%, IIa for 30%, and IIb for 4% (21). Thereafter, the proportion of type I fibers continues to increase to reach values as high as 95% in 24-mo-old animals (15). This evolution of the type I fiber population with age may be related to the fact that the Sol muscle is involved in postural functions, where load is a major determinant. Because type I fibers are ideally suited for postural work, their increasing presence with animal growth appears logical.

In parallel with the decrease in contractile speed, the rate of relaxation also slows significantly with age. This change is mainly related to qualitative and quantitative modifications at the level of the SR. The SR Ca\(^{2+}\)-ATPase activity will indeed determine the rate of Ca\(^{2+}\) reuptake and, therefore, the duration of the active state and the rate of relaxation. We and others have demonstrated previously that 1 mM Meth can have a significant effect on twitch relaxation time (6, 23). This effect was subsequently attributed to the presence in the SR fraction of the membrane-bound CA IV isoform (22). Unlike CA III, this isoform is present in all types of muscle fibers (9). It is postulated that it could be involved in the exchange of ions within the SR during contraction, inasmuch as its inhibition is known to influence the Ca\(^{2+}\) transient (23). Our observation of a prolongation of twitch relaxation time in the presence of Meth in 30-day-old rats suggests that CA IV activity in the SR fraction of the Sol muscle does not reach a functional level before that age. However, to the best of our knowledge, no study of the evolution of CA IV activity over time in the SR fraction of skeletal muscle is available.

Influence of age on fatigability. The stimulation parameters used for the fatigue protocol have been selected on the assumption that the level of muscle contraction would be of moderate intensity, allowing us to conduct a long-duration test where oxidative metabolism would be recruited and CO\(_2\) would be produced. This is the same test we have used in previous experiments, and it allows a clear distinction between Sol muscles incubated with and without CA inhibitor (5, 6, 8). The first striking observation with regard to the fatigue tests is that young and small Sol muscles tolerate this protocol much better than older and larger muscles. Several factors can possibly explain the difference in the fatigue curves of control Sol muscles from 23-, 30-, and 45-day-old animals. First and most importantly, the stimulation frequency (10 Hz) was kept constant for all age groups; however, the force-frequency curves for these groups are slightly different because of the difference in speed of contraction, which leads to a leftward shift of the curve for old muscles compared with young muscles. A consequence of such a choice is that the level of tension the muscles were asked to develop at the onset of the fatigue test was higher in the older and slower muscles, expressed in relative (percent maximum tetanic tension) or absolute values. Muscles from 23-day-old animals were producing 36.5 ± 3.3% of P\(_0\) at 10 Hz, whereas Sol muscles from 30- and 45-day-old rats reached values of 45.4 ± 2.0 and 47 ± 2.1%, respectively. In other words, the workload was lower for the young than for the older muscles. Second, the metabolic machinery of the Sol muscle is undergoing significant changes over this period as the animal reaches sexual maturity. Along with changes in fiber types, the oxidative and glycolytic capacities of each fiber type undergo changes over this period, as does capillary density (21). Although all

---

**Influence of age on fatigability.** The stimulation parameters used for the fatigue protocol have been selected on the assumption that the level of muscle contraction would be of moderate intensity, allowing us to conduct a long-duration test where oxidative metabolism would be recruited and CO\(_2\) would be produced. This is the same test we have used in previous experiments, and it allows a clear distinction between Sol muscles incubated with and without CA inhibitor (5, 6, 8). The first striking observation with regard to the fatigue tests is that young and small Sol muscles tolerate this protocol much better than older and larger muscles. Several factors can possibly explain the difference in the fatigue curves of control Sol muscles from 23-, 30-, and 45-day-old animals. First and most importantly, the stimulation frequency (10 Hz) was kept constant for all age groups; however, the force-frequency curves for these groups are slightly different because of the difference in speed of contraction, which leads to a leftward shift of the curve for old muscles compared with young muscles. A consequence of such a choice is that the level of tension the muscles were asked to develop at the onset of the fatigue test was higher in the older and slower muscles, expressed in relative (percent maximum tetanic tension) or absolute values. Muscles from 23-day-old animals were producing 36.5 ± 3.3% of P\(_0\) at 10 Hz, whereas Sol muscles from 30- and 45-day-old rats reached values of 45.4 ± 2.0 and 47 ± 2.1%, respectively. In other words, the workload was lower for the young than for the older muscles. Second, the metabolic machinery of the Sol muscle is undergoing significant changes over this period as the animal reaches sexual maturity. Along with changes in fiber types, the oxidative and glycolytic capacities of each fiber type undergo changes over this period, as does capillary density (21). Although all
these age-related changes will not be specifically discussed, it should be understood that changes at these levels can impact fatigue resistance. Finally, one could also isolate muscle size as one factor governing the muscle's capacity to maintain tension over time, inasmuch as O₂ diffusion in vitro is basically a function of muscle thickness. Although the evidence is clear that the Sol muscles from 45-day-old animals can maintain their functional integrity for up to 4 h in such an in vitro set-up at 25°C, diffusion of O₂, nutrients, and metabolic by-products may be easier and more efficient in the smaller muscles, allowing them to perform better.

CA III and its influence on fatigue. CA III activity and content increased with age. The rate of increase was particularly important between 10 and 25 days of age. After 25 days of age, CA III activity continued to increase linearly until 100 days, but at a slower rate. These observations are in good agreement with the data of Riley et al. (19), who observed a 3.5-fold increase in the Sol muscle CA III activity between 30 and 100 days of age. They also showed that the activity level measured at 100 days remains constant for the rest of the animal's life. The faster rate of increase observed in the first 4 wk may be related to the drastic changes in fiber type composition as the proportion of oxidative and CA III-rich fibers (type I and IIa) increased from 55 to 93% (21). However, changes in fiber types cannot explain the increase in CA III activity after 4–6 wk of age. After this period, it could be hypothesized that the metabolic machinery is undergoing changes that require a higher level of CA III.

We previously showed and characterized the influence of Meth on Sol muscle fatigue and subsequently proposed that it may be linked to an effect on carbohydrate metabolism (5, 8). Meth at a dose sufficient to totally abolish CA III activity leads to an improved resistance to fatigue when muscles are submitted to the same fatigue test used in the present study. Such an effect was not seen with 5 μM Meth, a concentration that inhibits only the sensitive CA IV isozyme present in the SR and within the sarcolemma. Also, no effect on fatigue was seen with acetazolamide, another CA inhibitor with the same affinity for CA III as Meth but with a very low rate of penetration through the cell membrane (6). Collectively, these data strongly indicate that this effect on fatigue is due to inhibition of an intracellular CA isozyme with a low affinity for Meth, CA III being the sole candidate.

Although the last observations may appear quite convincing, there remains the unlikely possibility that inhibition of CA III is not responsible for the effect on fatigue. Because a specific inhibitor of CA III is still not available, the demonstration that the influence of Meth on fatigue and metabolism is, to some extent, proportional and linearly related to the level of CA III activity could be an inductive argument supporting the conclusion previously proposed. Indeed, the fact that Meth at a constant dose has a growing influence on fatigue resistance, as CA III activity and content steadily increase, argues strongly against this influence being related to nonspecific effects of the CA inhibitor. However, the relationship between the level of CA III activity and fatigue resistance and HMP accumulation is no more apparent at > 35 U/mg, which, not surprisingly, suggests that other factors also have an impact. As mentioned previously, the metabolic and contractile machineries are undergoing numerous changes in rats of this age, along with maturation of some morphometric variables such as fiber size and capillary density. It is therefore plausible that the influence of increasing CA III activity may be counteracted by the global impact of several other determining parameters.

The fact that no relationship could be identified between the level of sensitive CA activity and the effect on fatigue supports the relationship between CA III inhibition and fatigue resistance. Sensitive CA activity in the Sol muscle could possibly result from the presence of the CA IV isozyme found in the SR and sarclemma in the rat. However, because we used cytosolic extracts in our studies, we do not expect to see high levels of these CA activities, which should remain in the pellet. Another source of sensitive CA activity can be red blood cells trapped in muscle capillaries. Red blood cells are very rich in CA I and CA II, two isoforms with very high specific activities. There also remains the possibility that a CA II-like activity may also be present in the cytosol of some skeletal muscle fibers, even though the evidence against such a conclusion appears quite strong (10, 13). In any case, in our protocols the level of sensitive CA activity was constant from 40 to 70 days, which clearly suggests that regardless of the nature of this activity, it is not closely related to the observed effect of Meth.

G-6-P accumulation. As we and others have shown previously, CA inhibitor can also influence muscle, even under resting conditions (8). Indeed, the presence of 1 mM Meth (0.1 mM ethanamide has the same effect) during the 45-min equilibration period preceding the fatigue protocol leads to an accumulation of HMPs, i.e., G-6-P and fructose 6-phosphate, along with a slight drop in intracellular pH. Although a mechanistic explanation exists for the effect on pH, explanations for the effect on G-6-P have been proposed only recently (8) but are still speculative at this point and will not be the focus of this discussion. As was the case for the effect of Meth on fatigue, the extent of G-6-P accumulation induced by Meth compared with age-matched control muscles is partially dictated by the level of CA III activity. This again suggests that this effect on carbohydrate metabolism is likely related to the inhibition of CA III activity. However, the relationship is not as linear and extended as the one for fatigue, which may suggest that other metabolic characteristics of skeletal muscle, also changing during development, could also be relevant to this effect. Interestingly, basal and insulin-stimulated glucose uptake undergo marked changes in the rat Sol muscle between 21 and 35 days of age (2), and this effect is not due to variations in the concentration of the GLUT-4 glucose transporter. One question that arises is whether the increased resistance to fatigue with CA inhibitor is partially or totally...
related to the buildup of HMPs in these muscles at the onset of the fatigue test. Indeed, such an important pool of glycolytic substrates could facilitate and possibly accelerate the use of glycolysis as an ATP synthesis pathway, especially in the first minutes of the test. Testing of muscles incubated with Meth for shorter periods of time before the fatigue test should enable us to answer that question, since HMP accumulation should be decreased, inasmuch as the time of contact with CA inhibitor is shorter.

In summary, the content and level of activity of CA III in the type I Sol muscle are constantly increasing during development in the rat until stabilization occurs at ~100 days of age. The previously documented influence of CA inhibitors on fatigue resistance and HMP accumulation was very tightly correlated with the level of CA III activity, further supporting the conclusion that these two effects are indeed the direct result of CA III inhibition.

Perspectives

General-purpose CA inhibitors, such as Meth, have been used to study CA III function, and it is not always perfectly clear that the observed effects are the consequence of CA inhibition only. By confirming that the effects of Meth are quite specific, the present experiments further support the hypothesis that an active CA III in a type I muscle can modulate carbohydrate metabolism and could therefore be linked to the still debated glucose-FFA cycle. Glycogen sparing is very highly efficient in type I fibers, where the highest CA III activities are found. Whether CA III has a direct effect on the preferential utilization or an indirect action linked to the preferential use of FFA as oxidative substrate remains to be investigated. CA III is unique among CA isoforms, in that it can also act as a protein phosphatase, which could interact with numerous enzymatic cascades of lipid and carbohydrate metabolism. Type I fibers preferentially use triglycerides, and because the hormone-sensitive lipase is sensitive to pH fluctuations and activated through a cascade of phosphatase reactions, CA III could potentially modulate FFA metabolism. CA III is found in very high concentrations, specifically in the three tissues controlling energy metabolism, i.e., fat cells, skeletal muscle, and liver, suggesting that it could possibly be a metabolic modulator. Specific inhibitors and/or mutant animals for CA III would greatly help in investigating these possibilities.

F. Ambrosio was the recipient of a scholarship from la Faculté des Etudes Supérieures de l’Université Laval. Methazolamide was generously provided by Cynamid Canada.

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada.

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

Received 3 June 1998; accepted in final form 13 October 1998.

REFERENCES


