

Inhibition of Carbonic Anhydrase III Exerts Paradoxical Influences on the Soleus Muscle of Hypo and Hyperthyroid Rats

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Abstract

Carbonic anhydrase III (CA III) inhibition with methazolamide (METH) is associated with an increased performance of the slow-twitch soleus (SOL) muscle during a standardized fatigue test. To further challenge this intriguing observation, the fatigue test was applied to SOL muscles of intact, hypothyroid (HYPO-T) and hyperthyroid (HYPER-T) female rats and the influence of METH ascertained in each experimental condition. Without METH in the bathing solution, the SOL from both experimental groups showed an increased resistance to fatigue. METH cancelled the gain in resistance of HYPER-T SOL while no influence was recorded in HYPO-T SOL. A mathematical model representing the fatigue curves of intact and METH-exposed SOL is presented as a tool to help further elucidate the influence of METH inhibition. Very significant changes in the SOL phenotype were observed in both HYPO and HYPER-T rats. CA III activity was respectively increased and decreased in these two groups while numerous metabolic changes occurred in several energy related metabolic pathways. An inversed relationship was found between changes in CA III activity and those of three representative cellular enzyme markers (PFK, GAPDH and CS) of muscle metabolism. These data provide additional indications that CA III harbour complex relationships with other cellular enzymes during prolonged contractile activity.

Key words: soleus, carbonic anhydrase III, methazolamide, fatigue test, cellular enzymes.

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Several authors have questioned the physiological role of carbonic anhydrase III (CA III; EC 4.2.2.1), one of the numerous carbonic anhydrase isozymes which is very abundant (20% of the soluble muscle protein mass [18]) in type I slow-twitch skeletal muscle fibers. Tashian [31] has recently summarized the well documented and/or putative implications of these isozymes in diverse biological processes, but as stated by Silverman and Lindskog [30], it is difficult to understand why such a "less than perfect" isozyme has survived during million years of evolution close to a "perfectly evolved" and efficient CA II isozyme in several tissues. Notwithstanding the modest performance of CA III in terms of its capacity for interconversion of CO_2 and HCO_3^- , this isozyme is widely distributed in the muscle apparatus, its activity and content are modified by physical exercise [32], in the course of muscular diseases [24] and under neuronal [23] and hormonal influences [10]. Over the past years, studies conducted by our

group on the link between cytoplasmic CA III and muscle contractility using an *in vitro* system have culminated in one unorthodox conclusion: "resistance to fatigue is decreased in the soleus (SOL) muscle when CA III remains active during a 30 min fatigue test" [7]. To further challenge this result, we have exploited initial data published in 1985 [12] clearly indicating that CA III was up and down-regulated in the course of hypo and hyperthyroidism, respectively. Thyrotoxicosis affects histochemical [8], biochemical [27], physiological [29] and contractile [9] properties of rat skeletal muscles such that the slow-twitch SOL muscle becomes a fast-twitch muscle with regards to its contractile properties. Hypothyroidism induces just the opposite effect and prolongs isometric contraction and relaxation times [14]. The aim of our study was thus to drastically modify the SOL muscle phenotype using T_3 -induced hyperthyroidism or surgical thyroidectomy in order to first evaluate the working performance of

the SOL with a well standardized fatigue protocol performed at 25°C without and with methazolamide (METH), a CA specific inhibitor. Secondly, the activity of several marker enzymes of the major muscle energy metabolic pathways were determined in basal, hypo- and hyperthyroidism conditions and correlated with the CA III activity of the muscle under study.

Materials and Methods

Female Wistar rats (mean body weight 135 g) were used for these experiments. Surgically thyroidectomized (HYPO-T) rats were purchased from Charles River (St-Constant, Québec) while hyperthyroidism (HYPER-T) was induced in another group of thyroidectomized rats by injecting daily 100 µg of triiodothyronine (T3) for 18 days.

Measurement of contractile properties and fatigability

Rats were anesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg) and the SOL muscle was carefully dissected. Contractile properties were measured "in vitro" in a buffered physiological salt solution (Krebs-Ringer) maintained at 25°C and supplemented with curare (20 mg/ml) and glucose (11 mM) unless otherwise specified. The pH of the bathing solution was stabilized at 7.4 by equilibration with a gas mixture of 95% O₂ - 5% CO₂. Muscles were placed in a vertical bath and secured at each end with silk sutures. One tendon was attached to a rigid support at the bottom of the bath while the other end was connected to an isometric force transducer (Grass Instr., Model FT03) through a stainless steel hook. Muscles were stimulated with 25 V square pulses of 0.2 ms duration delivered through platinum field electrodes and only when force was recorded. Current was supramaximal.

Muscles were first adjusted to their optimum length, defined as the length at which maximal isometric twitch tension was produced. Muscles were then allowed to equilibrate with the incubation medium for 30 min with or without METH (1 mM). This concentration represents the highest amount that can be solubilized in the physiological solution used for these experiments. METH 1 mM inhibits 100% of the SOL CA III activity. According to Maren [21], METH is still one of the best all-purpose compounds for most physiological experiments because: 1) its penetration rate through cell membranes is much higher than most other sulphonamides [16], 2) it is much more specific than cyanates in general and 3) it is also quite soluble in aqueous solutions. We previously showed that incubation times of 5 to 10 min are required before one can see a significant effect of METH on the fatigue profile of a SOL muscle at 25°C [11].

Maximum twitch tension (Pt), contraction time (CT), and 1/2 relaxation time (1/2 RT) were first obtained from a twitch contraction. Maximum tetanic tension (P_o) was then measured followed by a 10 min rest period which preceded the fatigue protocol. The fatigue test consisted in one tetanic contraction at 10 Hz every 5 s for 30 min, each single train of pulses lasting 0.5 s. This frequency of

stimulation produces a partly fused tetanic contraction generating approximately 50 to 60% of P_o at the beginning of the fatigue test. Following the fatigue protocol, the muscles were removed from the chamber, blotted and weighed. The mean cross-sectional area of each muscle was estimated by dividing muscle mass by fiber length and the density of mammalian skeletal muscle (1.062 g/cc). Under the experimental conditions used for these experiments, P_o was stable for at least 4 hours. Muscles were then frozen in liquid nitrogen and stored at -80°C.

Preparation of cytoplasmic extract and determination of CA III activity

The soluble cytoplasmic fraction of the SOL muscle was prepared essentially according to Lebherz et al. [20] with the exception that the homogenization buffer was composed of 2.5 mM HEPES (4-2-hydroxyethyl-1-piperazineethane sulfonic acid), 1 mM MgCl₂, 1 mM EDTA (ethylene-diamine tetraacetate), 1 mM DTT (dithiothreitol), pH 7.4. Fresh preparations were used to determine the CA III activity which was measured using a procedure similar to the one used by Bruns et al. [1]. Each individual reaction contained 50 µl of the diluted muscle extract, 50 µl of reaction buffer (75 mM Na₂CO₃, 50 mM NaHCO₃), 100 µl of the pH indicator (2.5 mg phenol red per 100 ml of 5 mM NaHCO₃). The reaction was initiated by the addition of 200 µl of CO₂ saturated H₂O. The entire procedure was performed at 1°C and resulted in an uncatalyzed reaction time of approximately 90 s.

CA III content by Western analysis

The denaturated proteins of the soluble cytoplasmic fraction from the SOL muscles under study were analysed using one-dimensional gel electrophoresis and the CA III content of the gel was visualized by Western blotting with an antibody developed and characterized in our laboratory [23].

Measurement of total cellular enzyme activities

Total cellular enzyme extracts were obtained by homogenizing small muscle samples (10-15 mg) in Na KPO₄ followed by sonication on ice 5 s, 6 times with an 85 s pause between each sonication as previously described [17]. Composition of the assay mixture for the measurement of phosphofructokinase (PFK; EC 2.7.1.11), glyceraldehyde-phosphate dehydrogenase (GAPDH; EC 1.2.1.12) and citrate synthase (CS; EC 4.1.3.7) was as previously described [2, 25, 26]. All the above enzymatic activities were determined spectrophotometrically at 30°C and expressed as U (mmol/min) per g of wet weight.

Statistical analysis

All results are expressed as mean ± standard error of the mean (S.E.M.). Data on contractile properties were analysed by a one-way analysis of variance followed by a Fisher's protected LSD test when a significant F ratio was obtained. Data on fatigue were analysed by a 2 way analysis of variance for repeated measures (split plot design).

Until now, our data have been analysed in such a way; however, we introduce for the first time a mathematical model which will be briefly described in this paper. Although the model adequately represents the fatigue curves in all experimental conditions, its validation has been limited to control muscles in the present study. Further details related to the application of the model will be published later.

Results

Effect of METH on the time course of fatigability of control SOL muscle of the rat

In order to visualize the overall effect of a complete inhibition of all CA isozymes with METH (1 mM) in intact SOL, Fig. 1 summarizes previously published results [8, 15] and those of the present study. The decrease of initial tension of the SOL is adequately represented by a mathematical model whose structure results from the combination of two processes: the fatigue (F) and the resistance (R) to fatigue. Ability of this composite structure to fit the experimental data is presented in Fig. 1, Panel A and B, by a dark and solid line. F and R are illustrated in Fig. 1 by broken lines and these two components of the model correspond to exponential functions which are delineated by the following equations:

$$F = A_1 \exp(-a_1 t) + (100 - A_1) \exp(-a_2 t)$$

$$R = B_1 (1 - \exp(-b_1 t))$$

Calculation of the parameters is based on a procedure of weighted non linear regression performed according to Marquardt's algorithm [22]. That the model insures the best fit of the experimental data is demonstrated by the low values (0.02 to 0.35) of the reduced chi-squares [19].

The values of the coefficients (A_1 and B_1) and of the time constants (a_1 , a_2 and b_1) are characteristics of the experimental conditions. The acceleration of the fatigue in the early and late phases of the experiments is expressed by a_1 and a_2 , respectively. A_1 is thus the relative contribution of the early phase to the overall process of fatigue. The time course of resistance to fatigue depends on the rate of increase b_1 and the plateau reached asymptotically B_1 . For sake of illustration, the equations representing the data showed in Fig. 1 are proposed for comparison but will be discussed in a companion paper:

Panel A

$$F = 42 \exp(-1.34t) + (100 - 42) \exp(-0.18t)$$

$$R = 35 (1 - \exp(-0.49t))$$

Panel B

$$F = 10 \exp(-2.5t) + (100 - 19) \exp(-0.12t)$$

$$R = 55 (1 - \exp(-0.15t))$$

A detailed analysis of the fatigue data reveals that as soon as 1 min after initiation of the fatigue protocol, the average curves corresponding to Control and METH-exposed muscles become significantly divergent but they follow a similar pattern of decrease, expressed in %, during the fatigue test (Fig. 1, panel A and B); at 10 and 25 min, differences ranging between 20 and 10% are respectively observed between both groups.

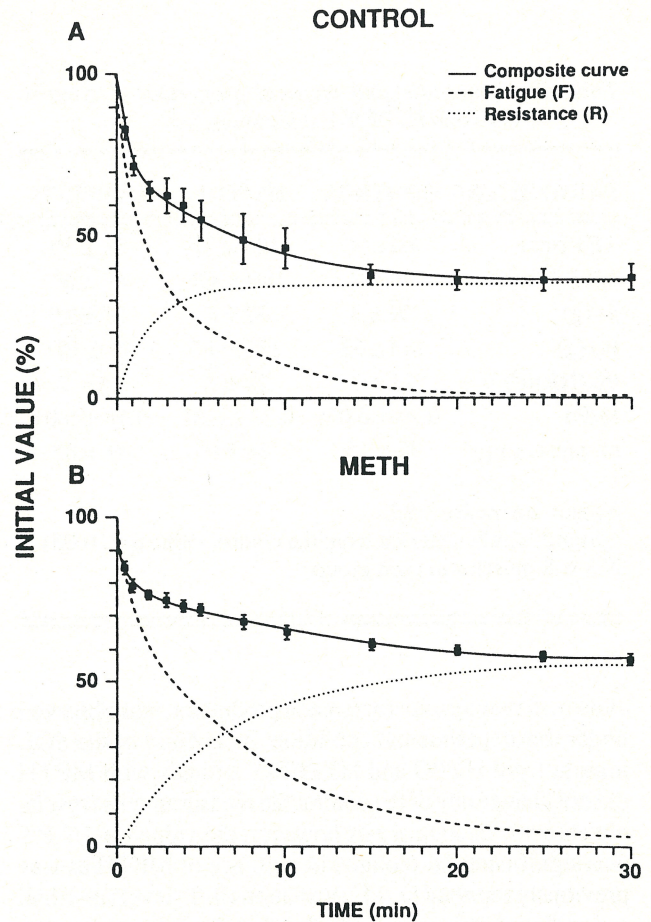


Figure 1. Mathematical modeling of the time course for the effect of fatigue (F) and of resistance to fatigue (R) of rat SOL muscles submitted to a standardized fatigue protocol. Both variables were studied in CTR (Panel A) and METH (Panel B) exposed muscles. Dark squares and their vertical bars represent the S.E.M. ($N = 24$). A highly significant difference ($p < 0.001$) was rapidly observed after 2 min of stimulation in CTR and METH groups. The dark line represents the best fit of our experimental data with a mathematical model which allows the dissociation of the composite curve in two equations characterizing respectively the fatigue (F) and the resistance to fatigue (R).

Effect of HYPO and HYPER-T on contractile properties measurements and CA III activity

As expected, the SOL from HYPER-T rats was drastically modified to become a fast-twitch muscle. All speed related measurements were indeed significantly faster than those of control SOL (Table 1). Also previously observed was the fact that maximum twitch and tetanic tensions were significantly reduced in this same group compared to control SOL. This is obviously related to the significant loss of muscle mass observed in this group of animals. In the HYPO-T rats, the SOL became significantly slower than the control muscle as shown by very significant increases in contraction and half-relaxation times. No

Table 1. Contractile and physical properties of control, HYPO and HYPER-T soleus muscles

PARAMETERS	CONTROL	HYPO-T	HYPER-T
TPT (ms)	62 ± 7	105 ± 7*	38 ± 5*
1/2 RT (ms)	94 ± 7	180 ± 32*	47 ± 6*
Pt (g)	29 ± 3	32 ± 4	18 ± 2*
Po (g)	143 ± 23	137 ± 17	98 ± 10*
Po (N/cm ²)	21 ± 3	24 ± 2	18 ± 2
Pt/Po	0.21 ± 0.02	0.22 ± 0.01	0.18 ± 0.01
m. musc. (mg)	95 ± 15	88 ± 9	81 ± 8*

Values are means ± S.E.

* Significantly different from the control value ($p \leq 0.05$)

N = 6-8 muscles in each group

change in the capacity for tension production was observed under this experimental condition. Incubation of the SOL muscle from HYPO and HYPER-T animals with METH did not change any of the contractile measurements (results not shown). In control rats however, the value for 1/2 RT was significantly prolonged in presence of METH as was previously reported [7, 11]. Variations in T_3 levels also had a significant influence on the level of CA III activity: CA III specific activity was 135.7 ± 12 , 171.3 ± 17 and 71 ± 12 U/mg prot, respectively, in control, HYPO and HYPER-T. Comparable changes were also noticed for CA III content determined by quantitative immunoblotting (results not shown).

Effect of HYPO and HYPER-T on the fatigability of the SOL muscle

As stated previously, the above two experimental conditions were thought to help exploiting new hypotheses regarding the biological role of CA III in skeletal muscles and to ascertain the influence of METH in a putative mechanism of intervention of CA III in relation with other muscular enzymes.

A. The fatigue test without METH inhibition

As illustrated in Fig. 2, the percentage of initial tension follows a similar pattern of decrease during the first 5 min of stimulation in control (CTR) and HYPER-T animals; from this time on however, HYPER-T SOL shows a significantly higher tension production than CTR muscles, the mean difference between both groups progressively increasing until the completion of the protocol. In HYPO-T SOL the decline in initial tension is abrupt during the first min, followed by an impressive tension production during the next 4 min; from this time on fatigue invades the muscle so that the % of initial tension of HYPER and HYPO-T SOL reaches similar values (60%) at 25 min, values still 15% higher than in control SOL. Therefore both

experimental conditions lead to an interesting and intriguing improvement in SOL performance during the fatigue test, but obviously for quite different reasons if one takes into account the initial contractile and physical properties of control and experimental SOL at 25°C (Table 1).

B. The fatigue test with METH inhibition

The improvement in fatigue resistance of HYPER-T SOL over CTR muscle was globally abolished when carrying out the fatigue protocol in presence of METH such that the profile of both fatigue curves was rather similar (Fig. 3). It should be noted that METH exerts then its inhibitory action on a decreased content of CA III. In the case of HYPO-T, tension production by SOL muscle was well above the intact SOL muscle and it was not at all influenced by METH which in this case acts on a much larger pool than normal of cytosolic CA III (Fig. 4). METH did not significantly influence the initial physical properties of SOL muscles whose contractile and biochemical profiles had been drastically modified by thyroid-induced disorders.

Thyroid status of the animals and relationship between representative enzymes of three energy metabolic pathways and CA III activity of the soleus

HYPO and HYPER-T were used as models that can lead to rapid phenotypic changes in skeletal muscles. If CA III function in the cell is linked to one or more specific metabolic pathways, one would expect that changes in the activities of these putative pathways should be matched by quantitatively comparable changes in CA III activity. In the HYPO-T animals, a 25% increase in CA III activity is accompanied by a 20% decrease in the capacity of the glycolytic and free fatty acid (FFA) pathways and by a 40% decrease in mitochondrial oxidative capacity, as depicted by the change in CS activity (Fig. 5). HYPER-T induced a 42% decline in CA III activity that was matched by a 40% increase in glycolytic capacity and a 20% increase in FFA related pathways; aerobic oxidative capacity was improved by 40% in this case. A regression analysis including all individual values for muscles from CTR, HYPO and HYPER-T animals showed that very highly significant negative correlation coefficients are obtained between changes in CA III activity and those of PFK and GAPDH. A highly significant negative correlation is also observed between changes in CA III and CS activity levels.

Discussion

Contractile properties

Contractile properties measurements are in agreement with previously published values for rat SOL *in vitro* [3, 4, 28]. The mean values for maximum specific tetanic tension in control and HYPO-T SOL muscles also indicate that muscle integrity was well within the normal range. As previously demonstrated by several groups [8, 14], the SOL from HYPO and HYPER-T groups were drastically modified and became slower and faster, respectively, than

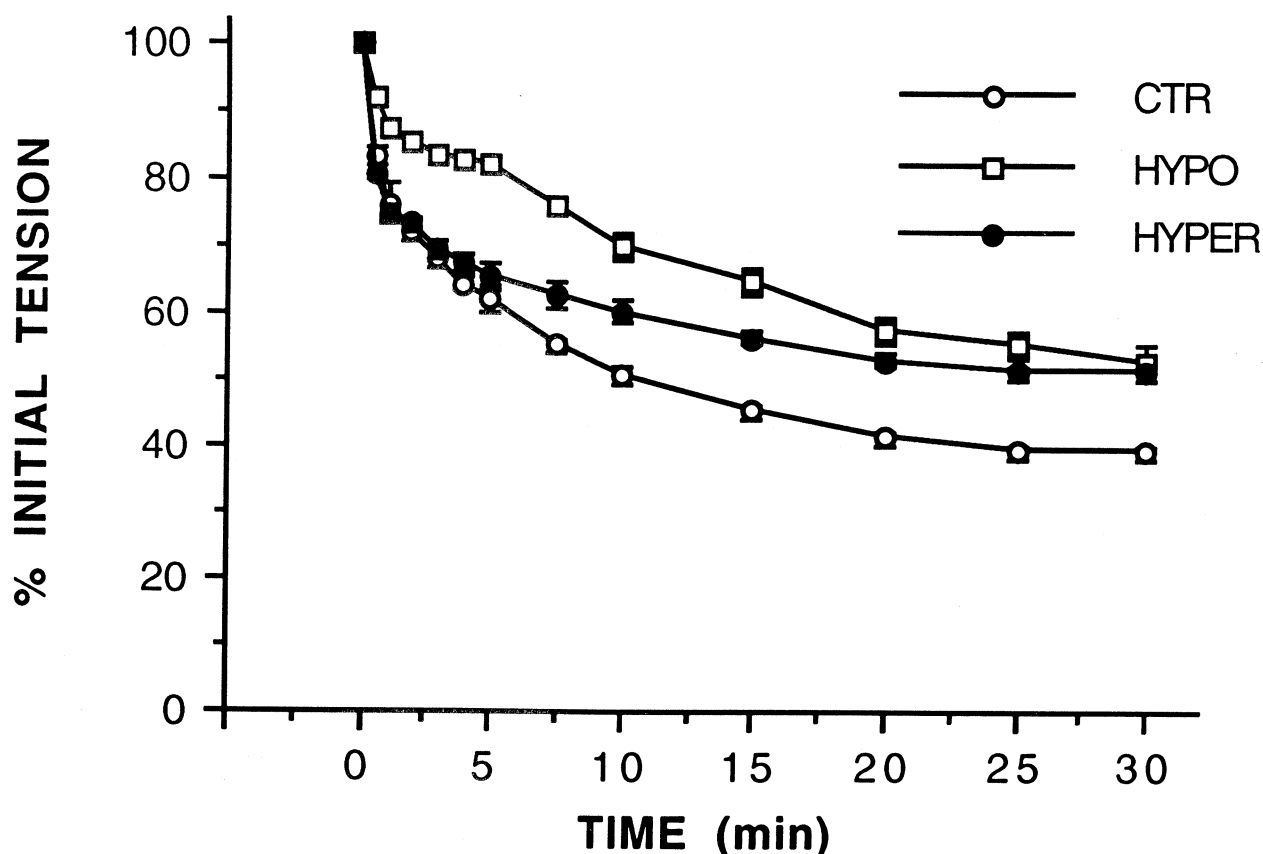


Figure 2. Effect of HYPO and HYPER-T on the resistance to fatigue of SOL muscles submitted to the standardized fatigue protocol ($N = 6$). Vertical bars represent the S.E.M. During the first 4 min of the test, the decrease in tension is similar in CTR and HYPER-T SOL. After that time point, both curves become significantly divergent. In HYPO-T SOL, the rapid decline in tension is followed by a short plateau and the process of fatigue, to the same extent as with HYPER-T SOL, is slower than in CTR muscles.

the control SOL. Previous experiments have shown that HYPO-T will transform the SOL into an histochemically pure and homogenous type I muscle compared to 85% type I fibers content in the control [8]. The contractile properties data reflects this change since all speed related parameters are very significantly prolonged. Quite to the opposite, HYPER-T SOL had contractile properties typical of rat fast-twitch muscles at 25°C. Twitch contraction and half relaxation times were approximately 50% shorter and this after only 18 days of treatment. Interestingly, SOL from HYPER-T had a significantly smaller muscle mass than SOL from both control and HYPO-T rats. This decrease in muscle mass was associated with significantly lower values for absolute and specific maximum tetanic tension. The high levels of circulating T_3 in these animals are known to produce loss in body weight due to the tremendous increase in the basal metabolic rate. The decrease in muscle mass is obviously related to an increased catabolism associated to the general loss in body weight observed in severely HYPER-T rats.

The addition of 1 mM METH in the incubation medium did not influence significantly any of the contractile meas-

urements in the HYPO and HYPER-T SOL muscles. Such a concentration has been shown to be sufficient to totally abolish the CA activity of a control SOL muscle [7]. In control SOL, the twitch relaxation rate is influenced by the presence of METH. This effect is also visible with 0.01 mM METH [7] and has been linked to the inhibition of the sulphonamide sensitive SR-CA isoform present in the SOL [6, 11]. Why such an effect is not seen in either HYPO or HYPER-T rats remains an opened avenue for investigation, since there are no data available on the influence of thyroid hormones on the SR-CA activity and content.

Fatigue and CA inhibition in control muscle

The fatigue protocol used for the present experiments is the same we have used over the years to investigate the physiological significance of CA III in type I skeletal muscle. It can be qualified as physiological since its intensity, frequency of contraction and duration are all moderate when compared to the muscle capacity. When METH is added to the *in vitro* incubation solution at a concentration high enough (1 mM) to totally abolish the CA III activity of the SOL, one sees a marked improvement in fatigue resistance compared to the control SOL muscle. This intri-

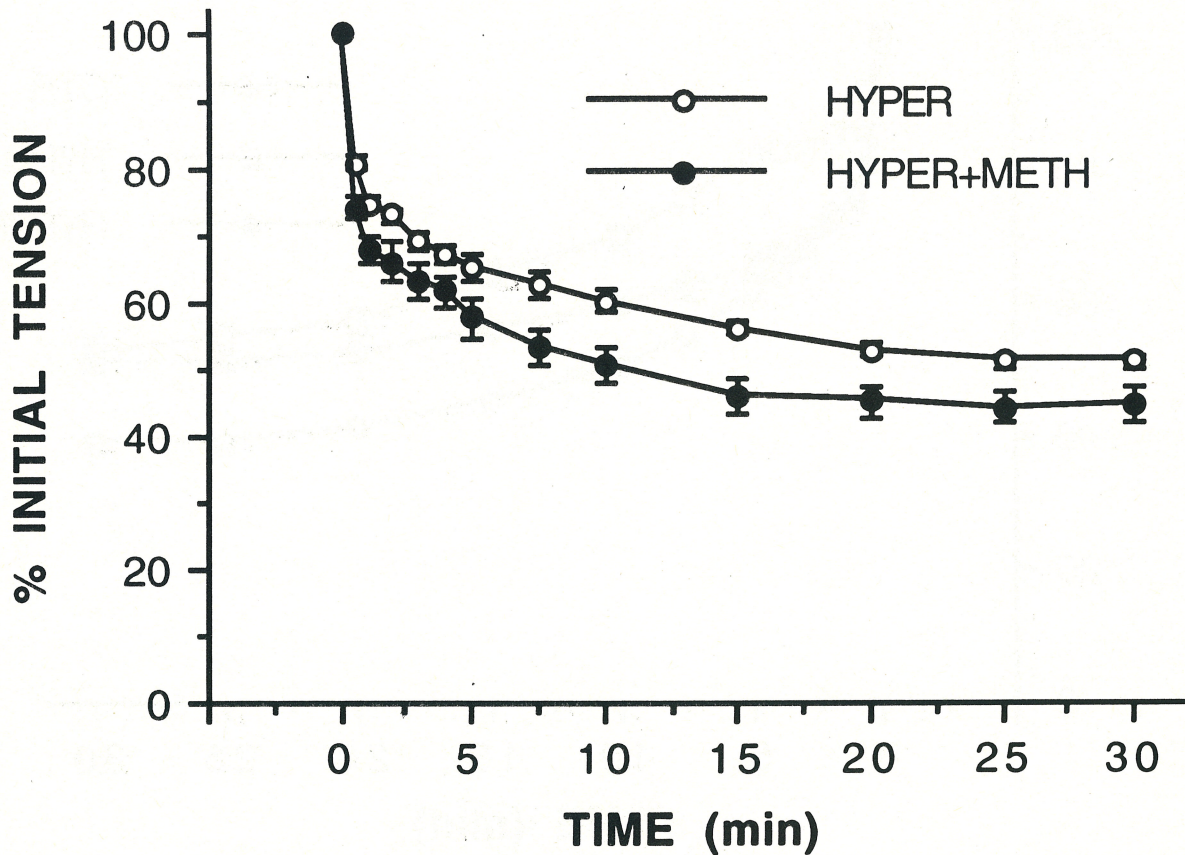


Figure 3. Effect of METH on the resistance to fatigue of HYPER-T SOL muscles ($N = 6$) submitted to a standardized fatigue protocol. Vertical bars represent the S.E.M. Addition of the CA III inhibitor significantly impairs the performance of HYPER-T SOL so that the profile of the curve representing the HYPER-T + METH becomes rigorously comparable to CTR SOL.

guing observation has been the basis of numerous subsequent experiments all dealing with the cellular function of CA III in skeletal muscle [6, 11, 23]. While we still do not have a definitive answer on this topic, we are nevertheless at the point where we have substantial evidence that this effect on fatigability is secondary to a primary influence of CA III inhibition on energy metabolism. Indeed, glycogen utilization, for one, appears to be "facilitated" when 1 mM METH is present in the solution. A detailed description and explanation of this rationale can be found elsewhere [6].

In the present experiments, HYPO and HYPER-T animals were used as an experimental model where, among other changes, higher and lower levels of CA III activity would be found, respectively, in the SOL compared to the value for control. In line with these facts, one would predict that the influence of METH on resistance to fatigue would be amplified in the HYPO-T group and decreased in the HYPER-T animals. However, such was not the case. Under the testing conditions used for the fatigue protocol, HYPO- and HYPER-T muscles were already more resistant to fatigue than the control group even in absence of METH. The SOL from HYPO-T rats are known to become

100% type I fibers; therefore an increase in resistance to fatigue is not surprising since these fibers show the highest resistance to fatigue under most isometric conditions. In the HYPER-T animals, contractile and histochemical data show that the SOL becomes a predominantly fast-twitch muscle, with IIa fibers replacing the slow contracting type I. Such a change should impact negatively on the resistance to fatigue if the relative level of contractile activity asked during the fatigue test is kept at the same level. Such was not the case here since we opted for the same stimulation frequency in all three groups. At 25°C, a 10 Hz pulse train results in a submaximal tetanic contraction reaching ~60% of maximum tetanic tension at the start of the fatigue protocol in control rats; however, since the SOL from HYPER-T animals is now a fast-twitch muscle, its force-frequency curve is heavily shifted to the right, and the same 10 Hz stimulation frequency results in a muscle working at a much smaller percentage of P_0 . Muscles are receiving the same number of stimuli, but this results in a very different work load because of the difference between control and HYPER-T SOL in terms of myosin composition. It is therefore conceivable that the absence of a positive effect of METH on the fatigue profile of the SOL

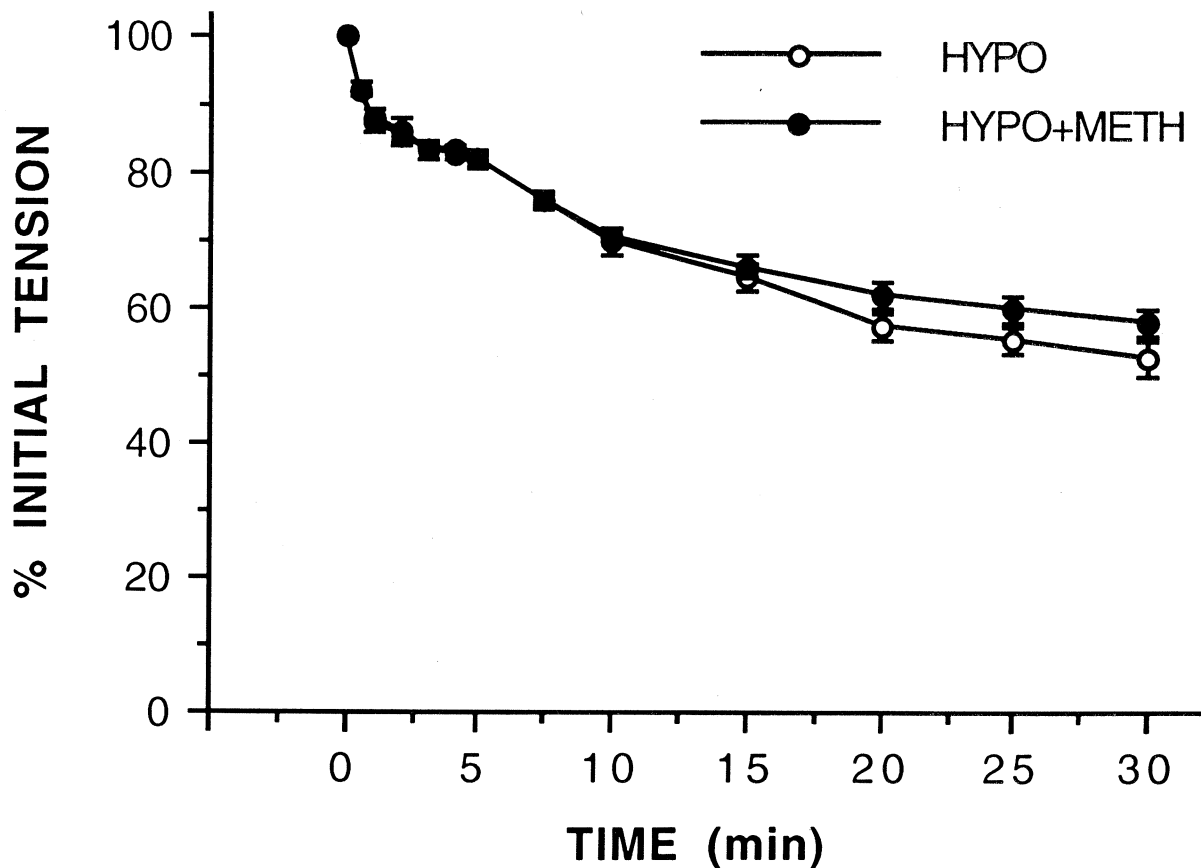


Figure 4. Effect of METH on the resistance to fatigue of HYPO-T SOL muscles ($N = 6$) submitted to a standardized fatigue protocol. Vertical bars represent the S.E.M. No significant difference was observed between both curves, both fatigue curves being significantly superior to the one for CTR muscles as shown in Fig. 2.

from HYPER-T rats results from the facts that much lower levels of CA III activity are found in these muscles and also because the working intensity during the fatigue protocol was much lower than the one for control muscle.

While the absence of a statistically positive influence of METH on fatigue in the HYPER-T SOL could be predicted, the same observation with the HYPO-T SOL was a surprise to us, since inhibition of an even higher than control CA III activity and content was expected to interfere even more dramatically with the muscle performance. Reasons for this are still obscure, but the fact that 1) the HYPO-T SOL were working at close to 100% of its maximum tetanic tension at the start of the protocol (compared to 60% for control) because of its much slower twitch contraction parameters (left-shift of the force-frequency curve) and 2) numerous metabolic changes are also occurring in these muscles in all energy related metabolic pathways and may have masked the potential influence of CA inhibition in these muscles. Further experiments will have to be performed with HYPO- and HYPER-T rats but with care being taken to adjust the frequency of stimulation such that all muscles will be tested at the same relative working intensity.

Relationships between the changes in CA III activity and those of enzyme markers of the energy metabolism.

Another point in favour of the HYPO and HYPER-T approach is that it allows the investigators to look at how time-related are the numerous changes in the energy metabolic pathways. The rationale being that pathways whose activities are linked together or closely associated in control muscles should still show this tight coupling when undergoing major transitions. As mentioned previously, one of our hypothesis is that CA III is somehow related to the metabolic pathways responsible for the utilization of carbohydrates during prolonged contractile activity. Interestingly, the highest regression coefficient was obtained for the relationship between the variations in the activity levels of CA III and PFK, the key modulating enzyme of glycolysis. As was already observed using a comparative approach with the three types of skeletal muscle fibers, a very close inverse linear relationship can be seen between these two variables. Increases in CA III levels are matched by decreases in PFK very similar in magnitude and vice-versa: in other words, muscle fibers with low glycolytic potentials appear to have a strong need for CA III. The same can be said for the relationship between CA III and GAPDH, the enzyme marker for lipid metabolism. It is still

Inhibition of CA III in rat soleus muscle

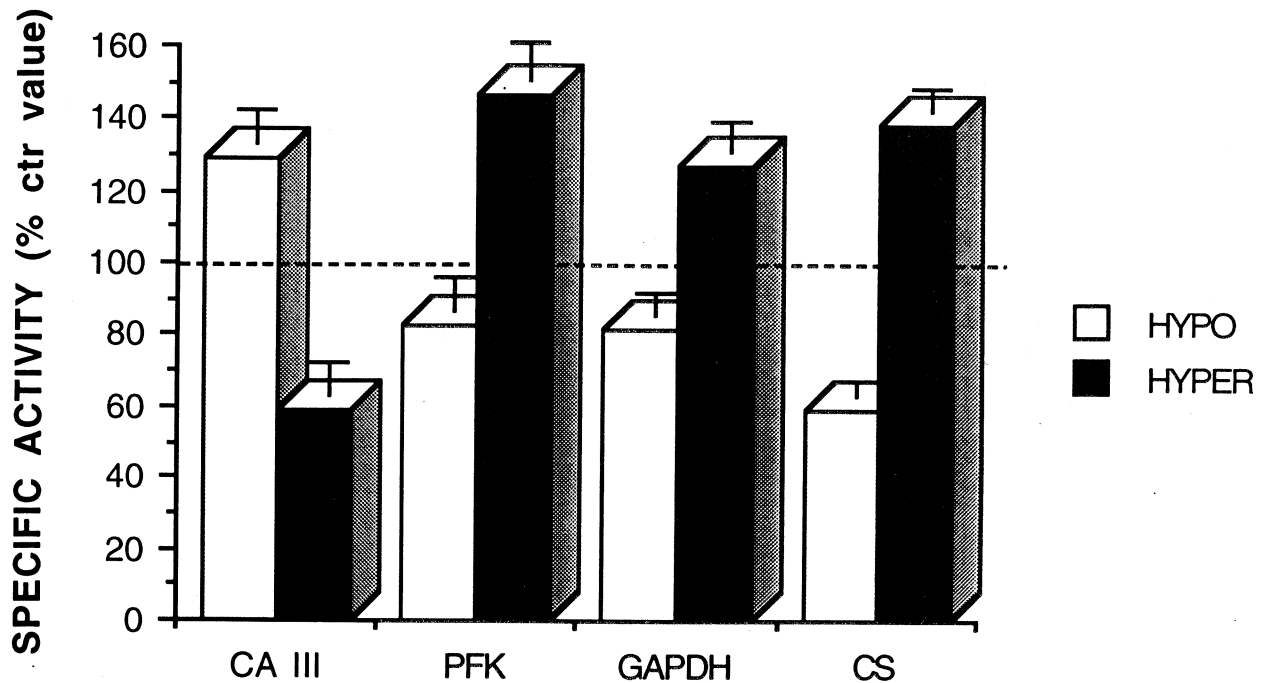


Figure 5. Effect of HYPO and HYPER-T on the specific activity of soluble CA III and of total cellular enzymes (PFK, GAPDH and CS) representative of the three major metabolic pathways of SOL muscles ($N = 6$). The dotted line corresponds to 100% and refers to the enzyme activity determined in the CTR group. Vertical bars correspond to S.E.M. An inverse relationship is observed between CA III activity and the three cellular enzymes.

too early to conclude firmly that a causal relationship exists between CA III and these two enzyme markers but these data can be added to the long list of evidences linking CA III and energy metabolism in skeletal muscle.

The relationship between CA III and CS changes is quite revealing. While a highly statistically significant correlation is found, the nature of the relationship is indeed paradoxical. The reported cellular function of CA III in skeletal muscle is to facilitate the CO_2 diffusion [13]. Therefore, increases in the capacity for oxidative metabolism should be matched by increases in the activity level of CA III in order to compensate for the newly improved capacity for CO_2 production. This is however not the case since an inverse relationship is observed, that is an increased capacity for CO_2 production, as observed in the HYPER-T rats, is accompanied by a quantitatively comparable decrease in CA III activity. This is a strong argument against the hypothesis frequently found in the literature stating that the sole cellular function of CA III in type I muscle fiber is indeed to facilitate the diffusion of CO_2 .

Why muscle fibers with low glycolytic capacities do indeed appear to have high CA III requirement remains an opened question. It could have to do with the need for a given amount of proton production in these fibers; indeed it has been frequently proposed that the function of glycolysis, once the initial minutes of a contracting protocol are over, is not to produce ATP for the muscle cell, but may

be to provide the cell with sufficient amount of lactate and protons under aerobic conditions to maintain an optimal cytosolic environment for support of oxidative phosphorylation [5]. The production of protons via glycolysis may indeed play an essential role in muscle metabolism [15]. The possible implication of CA III in this cellular function should be considered in the design of future experiments testing the physiological role of this abundant enzyme.

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