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SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 841–847

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Response of protein synthesis to hypercapnia in rats: independent effects of acidosis and hypothermia

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Received 23 August 2004; accepted 24 January 2005

Abstract

Acute metabolic acidosis has been shown to inhibit muscle protein synthesis, although little is known on the effect of acidosis of respiratory origin. The aim of this study was to investigate the effect of acute respiratory acidosis on tissue protein synthesis. Rats (n = 8) were made acidotic by increasing the CO_2 content of inspired air to 12% for 1 hour. Similar rats breathing normal air served as controls (n = 8). Muscle and liver protein synthesis rates were then measured with L-[$^2H_5$]phenylalanine (150 μmol per 100 g body weight, 40 mol%). The results show that protein synthesis is severely depressed in skeletal muscle (−44% in gastrocnemius, −39% in plantaris, and −24% in soleus muscles, $P < .01$) and liver (−20%, $P < .001$) in acidotic animals. However, because breathing CO_2-enriched air was found to lower body temperature by approximately 2°C, in a second experiment (n = 10), the difference in body temperature between treated and control animals was minimized by gently wrapping rats breathing CO_2-enriched air in porous cloths. This second experiment confirmed that respiratory acidosis depresses protein synthesis in muscle (−22% in gastrocnemius, $P < .001$; −19% in plantaris, $P < .01$; and −4% in soleus, P = NS). However, no effect on liver protein synthesis could be detected, suggesting that liver protein synthesis may be sensitive to changes in body temperature but is not affected by acute respiratory acidosis for 1 hour. The results show that respiratory acidosis inhibits protein synthesis in skeletal muscle and indicates that acidosis, whether of metabolic or respiratory origin, may contribute to loss of muscle protein in patients with compromised renal or respiratory function.

1. Introduction

Metabolic acidosis has been shown to have detrimental effects on growth and nitrogen balance and to promote loss of muscle mass both in animal and human studies (ie, Refs [1-3]). Muscle protein loss due to acidosis is the result of an elevation of muscle protein degradation and amino acid oxidation (eg, Refs [3-5]). Recent studies have also shown that metabolic acidosis inhibits muscle protein synthesis [6,7], which can further contribute to muscle loss. A reduction in muscle protein synthesis has been shown in human volunteers made acidotic by administration of NH_4Cl for 48 hours [6], and similarly, we found that acidosis induced by administration of NH_4Cl or a cation (H^+) exchange resin to rats inhibits muscle protein synthesis after 24 hour [7]. However, all these studies have investigated the effect of acidosis of metabolic origin and very little is known of the effect of respiratory acidosis on protein metabolism. Both metabolic and respiratory acidosis are important in clinical practice, but although they might both result in the same change in the hydrogen ion concentration, the associated changes in other blood gas parameters (eg, bicarbonate and PCO_2) will be different. To be sure that the inhibition of protein synthesis can be attributed solely to the change in hydrogen ion concentration, it is therefore important to demonstrate that these 2 forms of acidosis result in the same inhibition of protein synthesis.

Respiratory acidosis derives from accumulation of CO_2 in body fluids (ie, hypercapnia) because of impaired respiratory function, and it is frequently observed in patients with chronic obstructive pulmonary disease (COPD), especially in those with more severe disease [8]. Patients with COPD also often suffer from weight loss and muscle atrophy [9], which not only worsen their quality of life, but also increase morbidity and mortality and have been shown to be independent negative prognostic factors [9,10]. The causes of weight and muscle wasting in these patients are not clear. We hypothesize that similarly to metabolic acidosis, respiratory acidosis can negatively affect muscle protein

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doi:10.1016/j.metabol.2005.01.026

metabolism. Acidosis itself may therefore play an important role in promoting body protein loss in patients with COPD and other conditions accompanied by respiratory acidosis.

The aim of this study was to investigate the effect of acute respiratory acidosis on body protein metabolism, and in particular, to test whether acidosis of respiratory origin has an inhibitory effect on protein synthesis of muscle and other tissues. Respiratory acidosis was induced in rats by increasing the content of CO_2 in inspired air using a specifically designed apparatus, whereas controls were breathing normal air. Protein synthesis in skeletal muscles and liver was then measured with the flooding method using L-[$^2H_5$]phenylalanine [7].

2. Methods

Male Sprague-Dawley rats (body weight approximately 300 g) (Taconic Farms, Germanatown, NY) were placed in individual cages and adapted to the laboratory conditions for at least a week, with free access to water and standard rodent chow (Purina Mills, Richmond, Ind). Food was removed at 10:00 PM the night before each experiment. All the protocols were approved by the Institutional Animal Care Committee of the State University of New York, Stony Brook, NY.

2.1. Experimental protocol

Acute respiratory acidosis was induced by increasing CO_2 content of inspired air. An apparatus was specifically designed for inducing different levels of respiratory acidosis by altering the CO_2 content of inspired air. This apparatus consisted of a chamber in which animals could be kept comfortably for extended periods with minimal distress and without anesthesia, which might itself alter protein synthesis [11]. Air from a cylinder was first humidified then delivered to the chamber through a flowmeter. Air composition was modified by addition of CO_2, also delivered from a cylinder through a flowmeter, before going to the chamber. The composition of the air mixture in the chamber was continuously monitored with a CO_2/O_2 analyzer (Deltatrac metabolic monitor; SensorMedics, Yorba Linda, Calif).

Preliminary experiments were performed to test the optimal proportion of CO_2 to be added to the air to achieve a rapid and consistent level of respiratory acidosis. These involved groups of rats (n = 4) that first underwent surgery for implantation of carotid arterial catheters and then were given a week to recover from surgery. Animals were placed in the chamber, and arterial blood gases were measured (ABL System 615; Radiometer Medical, Copenhagen, Denmark) during ventilation with air initially and then with mixtures of air and CO_2 at different concentrations (4%, 8%, 12%).

2.1.1. Experiment 1. Effect of acute respiratory acidosis on tissue protein synthesis

After an overnight fast, a group of rats (n = 8, acidosis) were placed in a chamber in which the air mixture delivered was enriched with 12% CO_2, as described in the previous sections. A similar group of rats (n = 8) given normal air served as controls. After 1 hour, tissue protein synthesis was measured with the flooding method using L-[$^2H_5$]phenylalanine [12]. A solution containing L-[$^2H_5$]phenylalanine (150 μmol per 100 g body weight, 40 mol%) (MassTrace, Woburn, Mass) was injected via a tail vein while animals were in the chamber. After 10 minutes, the animals were rapidly removed from the chamber and killed. Skeletal muscles (gastrocnemius, plantaris, and soleus) and liver were harvested and quickly frozen in liquid nitrogen as previously described [7]. Tissue samples were then stored at −70°C until further analysis.

2.1.2. Experiment 2. Effect of acute respiratory acidosis on tissue protein synthesis with temperature control

Observations after experiment 1 indicated that the body temperature in rats breathing air enriched with 12% CO_2 was lower than in controls (see Results). Because a drop in body temperature may have an independent effect on tissue protein synthesis and represent a confounding variable in interpreting results from experiment 1, a second experiment was designed attempting to minimize differences in body temperature between the treated and control groups (n = 10). The protocol of this second experiment was similar to that described in experiment 1, except that animals' torsos were gently wrapped in porous cloths just before being placed in the chamber with 12% CO_2 to reduce body heat dispersion. Rectal body temperature was also monitored in all animals throughout the experiment. Protein synthesis in skeletal muscle (gastrocnemius, plantaris, and soleus) and liver was measured with a flooding amount of L-[$^2H_5$]phenylalanine 1 hour after placing the animals in the chamber, similarly to experiment 1.

2.2. Analytical methods

2.2.1. Enrichment of L-[$^2H_5$]phenylalanine in tissue protein and of free L-[$^2H_5$]phenylalanine in tissue fluid

The enrichment of protein-bound and tissue fluid–free L-[$^2H_5$]phenylalanine was measured as previously described [7,13]. Briefly, frozen powdered tissue protein was precipitated with cold 0.2 mol/L concentration of perchloric acid and solubilized in 0.3 mol/L concentration of NaOH at 37°C for 1 hour. After several washes with 0.2 mol/L concentration of perchloric acid, it was hydrolyzed with 6N HCl. Phenylalanine was then enzymatically converted to phenylethylamine and solvent extracted. The enrichment of L-[$^2H_5$]phenylalanine was measured with an MD800 gas chromatography mass spectrometer (Fisons Instruments, Inc, Beverly, Mass) run under electron impact condition and in splitless mode by monitoring the ions at mass-to-charge ratio (m/z) 106 (m + 2) and 109 (m + 5) of the n-heptafluorobutyryl derivative [13].

Tissue-free amino acids were purified by cation-exchange chromatography (AG Resin, 100-200 mesh, hydrogen form

BioRad Laboratories, Richmond, Calif) after precipitation of tissue protein with perchloric acid. The enrichment of the butyldimethylsilyl derivative of phenylalanine was measured on a MD800 gas chromatography mass spectrometer (Fisons Instruments) by monitoring the ions at m/z 336 and 341 [13].

2.2.2. Amino acid analysis

Plasma amino acid concentration was determined on blood samples collected at death. After precipitation of plasma protein with acetonitrile, amino acids were derivatized with *o*-phthaldehyde. Amino acid concentration was then measured on a Waters 2696 high-performance liquid chromatography system (Waters, Milford, Mass) using 6-amino-*n*-caproic acid as internal standard.

2.2.3. Calculations

Fractional rates of protein synthesis (FSR) were calculated from the enrichment of L-[$^2H_5$]phenylalanine in protein and in tissue-free amino acids (precursor pool) described by Garlick et al [12]:

$$FSR(\%/d) = \frac{E_P}{E_F \cdot t} \cdot 100$$

where E_P is the enrichment of phenylalanine in tissue protein, E_F is the enrichment of phenylalanine in the tissue-free amino acid pool (precursor pool), and t is the time expressed in day.

2.2.4. Statistics

All the values are presented as mean ± SE. Two-tailed *t* tests for unpaired data were used for comparisons between acidotic and control groups. A P value <.05 was considered to be statistically significant.

3. Results

3.1. Blood gas analysis

Arterial blood pH consistently decreased in rats breathing air enriched with CO_2, and the extent of the drop was related to the amount of CO_2 added to the air mixture. Results of preliminary experiments in rats that had previously undergone surgery for placement of arterial carotid catheters showed a progressive mild, moderate, and more severe acidosis by increasing CO_2 concentrations from 4% to 8% and 12%, as illustrated in Table 1. Because animals hyperventilated with elevated inspired CO_2, the values for P_{O_2} were raised relative to control values, but this did not lead to significant changes in O_2 saturation of hemoglobin (Table 1). These data confirmed that modification of air/CO_2 mixtures produced consistent and reproducible changes in blood gases, and could be used to examine the effects of respiratory acidosis on protein metabolism. Because the drop in blood pH was more rapid and marked with a gas mixture containing 12% CO_2, a concentration of 12% CO_2 in the inspired air was used to induce acute respiratory in all subsequent experiments.

In experiment 1 and 2, animals did not have arterial catheters in place to avoid any interference of surgical stress on protein metabolism, and therefore, arterial blood gases could not be measured in these rats. However, blood gas analysis performed on mixed artero-venous blood collected at death confirmed a significant decrease in blood pH in rats breathing 12% CO_2 (7.478 ± 0.011 vs 7.313 ± 0.009, P < .001) (experiment 2), although the decrease was smaller than that observed in animals with catheters (Table 1), as they were removed from the chamber immediately before killing. The drop in pH was accompanied by an increase in P_{CO_2} (53.3 ± 1.6 vs 33.3 ± 1.2 mmol/L, P < .001) and O_2 (75.8 ± 4.3 vs 58.9 ± 3.1 mmol/L, P < .01) but no changes in O_2 saturation of hemoglobin (85% ± 2.5% vs 83% ± 1.4%), bicarbonate (26.2 ± 0.5 vs 24.3 ± 0.5 mmol/L), or base excess (0.7 ± 0.4 vs 1.1 ± 0.5 mmol/L) concentrations.

3.2. Body temperature

When rats were placed in the chamber containing air enriched with 12% CO_2, body temperature declined. Rectal temperature measured in experimental conditions comparable to those used in experiment 1 showed a drop by 2°C after placing animals in the chamber containing 12% CO_2 for 1 hour (38.1°C ± 0.3°C vs 36.1°C ± 0.2°C, before vs 1 hour after breathing 12% CO_2, P < .001, n = 7). No change in body temperature could be detected in rats in chambers breathing normal air for the same length of time (38.3°C ± 0.2°C vs 38.1°C ± 0.2°C, before vs 1 hour after breathing normal air, P = NS, n = 7). The drop in body temperature was likely caused by increased heat dispersion

Table 1
Arterial blood gas analysis of rats before (control) and 1 hour after breathing air enriched with 4%, 8%, or 12% CO_2 for 1 hour

	4% CO_2		8% CO_2		12% CO_2	
	Control	1 h	Control	1 h	Control	1 h
pH	7.50 ± 0.01	7.46 ± 0.01*	7.51 ± 0.01	7.29 ± 0.01**	7.52 ± 0.01	7.18 ± 0.01**
P_{CO_2} (mm Hg)	36 ± 1.2	41 ± 0.7*	34 ± 0.5	62 ± 0.4**	35 ± 0.8	86 ± 0.4**
P_{O_2} (mm Hg)	97 ± 0.6	107 ± 5.3*	87 ± 2.3	119 ± 1.9**	90 ± 2.1	122 ± 1.4*
HCO_3^-	28 ± 1.0	29 ± 0.7	27 ± 0.4	30 ± 0.7*	29 ± 0.7	32 ± 0.6
O_2 sat (%)	97 ± 0.6	98 ± 0.1	97 ± 0.4	98 ± 0.2	98 ± 0.2	97 ± 0.1

Values are means ± SE (n = 4).

* Significantly different from control, P < .05.

** Significantly different from control, P < .005.

Table 2
Plasma amino acid concentrations (micromole per liter) in the control and acidosis groups in experiment 2

	Control	Acidosis
Leu	133 ± 4	132 ± 6
Lys	266 ± 9	367 ± 22*
Tyr	304 ± 16	276 ± 11
Val	175 ± 4	177 ± 6
Ile	88 ± 3	83 ± 3
Glu	152 ± 7	118 ± 44*
Gln	743 ± 34	841 ± 28*
Asp	18 ± 1	16 ± 1
Met	55 ± 2	62 ± 3
Ala	235 ± 15	392 ± 32*
His	61 ± 2	81 ± 3*
Arg	132 ± 5	148 ± 6
Trp	120 ± 5	99 ± 5*
Thr	243 ± 14	281 ± 15
Ser	213 ± 8	271 ± 14*
Asn	55 ± 2	70 ± 4*
Gly	324 ± 15	341 ± 22

Values are means ± SEM (n = 10).
The concentration of phenylalanine is not reported because this amino acid was injected for measurement of tissue protein synthesis.
* Significantly different from control, $P < .05$.

after hypercapnia, suggesting that it could be minimized by reducing body heat dissipation. Therefore, in experiment 2, differently from experiment 1, rats that were placed in chambers with CO_2-enriched air were gently wrapped in porous cloths to reduce body heat dispersion. This experimental procedure minimized the drop in body temperature in the group treated with 12% CO_2 (difference in body temperature over 1 hour: $-0.3^{\circ}C \pm 0.1^{\circ}C$ vs $-0.3^{\circ}C \pm 0.2^{\circ}C$, normal air vs 12% CO_2 groups) and maintained the body temperature in the 2 groups comparable during the experimental period (body temperature before vs after 1 h: $38.8^{\circ}C \pm 0.1^{\circ}C$ vs $38.5^{\circ}C \pm 0.1^{\circ}C$ in normal air group; $38.5^{\circ}C \pm 0.1^{\circ}C$ vs $38.2^{\circ}C \pm 0.2^{\circ}C$ in 12% CO_2 group).

3.3. Plasma amino acids

Plasma amino acid concentrations measured in experiment 2 are shown in Table 2. Plasma concentrations of most amino acids were increased in acidotic animals. However, levels of glutamic acid and tryptophan were significantly lower ($P < .05$) in the acidotic group. No changes were detected in the concentration of branched-chain amino acids leucine, isoleucine, and valine between the 2 groups.

Table 3
Protein synthesis rates in skeletal muscles (gastrocnemius, plantaris and soleus) and liver in rats breathing air enriched with 12% CO_2 (acidosis) or normal air (control) for 1 hour (experiment 1)

	FSR (%/d)			
	Gastrocnemius	Plantaris	Soleus	Liver
Control	4.84 ± 0.47	5.42 ± 0.32	11.41 ± 0.46	73.90 ± 1.67
Acidosis	2.72 ± 0.22*	3.29 ± 0.21*	8.65 ± 0.32*	59.20 ± 1.66*

Values are means ± SE (n = 8).
* Significantly different from control, $P < .01$.

Table 4
Protein synthesis rates in skeletal muscles (gastrocnemius, plantaris and soleus) and liver in rats breathing air enriched with 12% CO_2 (acidosis) or normal air (control) for 1 hour (experiment 2)

	FSR (%/d)			
	Gastrocnemius	Plantaris	Soleus	Liver
Control	5.61 ± 0.16	6.04 ± 0.24	11.3 ± 0.47	74.7 ± 1.20
Acidosis	4.37 ± 0.20*	4.90 ± 0.21*	10.8 ± 0.47	73.6 ± 1.35

Values are means ± SE (n = 10).
Body temperature was maintained comparable in the 2 groups by minimizing heat loss in rats breathing CO_2-enriched air, as described in the "Methods" section.
* Significantly different from control, $P < .01$.

3.4. Effect of acute respiratory acidosis on tissue protein synthesis

3.4.1. Experiment 1

Breathing air enriched with CO_2 greatly affected protein synthesis in skeletal muscle. Fractional synthesis rates (FSR) were depressed by 44% in gastrocnemius muscle ($P < .01$), by 39% in plantaris ($P < .001$), and by 24% in soleus ($P < .001$) in acidotic animals compared to controls (Table 3). A similar inhibitory effect was observed on liver, in which protein FSR was found to be 20% lower in the acidotic group ($P < .001$) (Table 3).

3.4.2. Experiment 2

The drop in muscle protein synthesis during respiratory acidosis was also observed in experiment 2, in which a decline in body temperature in the acidotic and control groups was minimized. The results of experiment 2 show that muscle protein synthesis was inhibited by 22% in gastrocnemius ($P < .001$), 19% in plantaris ($P < .01$), and by 4% in soleus (P = NS) in animals breathing 12% CO_2 compared to controls (Table 4). Differently from experiment 1, no changes were observed in liver protein synthesis between the 2 groups (Table 4).

4. Discussion

The results of this study demonstrate that induction of acidosis is accompanied by a rapid and significant inhibition of protein synthesis in skeletal muscle. Breathing air enriched with 12% CO_2 for 1 hour induced a rapid decrease in blood pH and suppressed protein synthesis by approximately 20% in gastrocnemius and plantaris muscles ($P < .001$, Table 4), suggesting that respiratory acidosis may be an important factor in promoting muscle wasting. The inhibition of protein synthesis is comparable to that previously observed during acute metabolic acidosis [7], indicating that acidosis, whether originating from respiratory or metabolic factors, has a similar negative effect on muscle protein metabolism and can be an important contributor to muscle catabolism.

During acute metabolic acidosis, protein synthesis was inhibited in muscle and lymphocytes but no suppressive

effect could be detected on liver or other visceral organs [7]. Similarly, in the present study, acidosis depressed protein synthesis in gastrocnemius and plantaris muscles but not in liver or soleus muscle (Table 3). An inhibition of protein synthesis in the liver and soleus muscle in acidotic animals was observed in experiment 1 (Table 3). However, in this experiment, respiratory acidosis was also accompanied by a drop in body temperature of approximately 2°C. When the decrease in body temperature in animals breathing CO_2-enriched air was minimized (ie, experiment 2), the suppression of protein synthesis could be reproduced in gastrocnemius and plantaris muscles but not in the liver or soleus muscle (Table 4). These findings therefore suggest that the inhibition of liver and soleus muscle protein synthesis observed in experiment 1 was not directly caused by respiratory acidosis but rather was related to concomitant changes in body temperature. In contrast, in agreement with previous observations during acute metabolic acidosis [7], the suppressive effect on protein synthesis in skeletal muscle with mixed fiber types is induced by respiratory acidosis, and this effect is independent from changes in body temperature.

The results of this study highlight the potential impact of changes in body temperature on tissue protein synthesis. As discussed in the previous sections, a decrease in body temperature of 2°C during acidosis resulted in a 20% inhibition of liver protein synthesis (experiment 1, Table 3), which was not observed when the temperature change was minimized (experiment 2, Table 4). A comparable negative effect of a decrease in body temperature was also observed in skeletal muscle. The drop in protein synthesis in acidotic animals was in fact approximately 20% more pronounced in experiment 1 than in experiment 2 in all 3 muscles examined (Tables 3 and 4), inferring that a decrease in body temperature also depresses protein synthesis in muscle and that the effect is independent and additive to that of respiratory acidosis. The experimental results would therefore suggest that a drop of 2°C approximately results in a 20% inhibition of tissue protein synthesis rate. These changes in tissue protein synthesis as a result of temperature would correspond to a coefficient of temperature (Q_{10}) of approximately 3, which is comparable to that observed in fish [14], which appears to be the only other species in which the acute effect of temperature on protein synthesis has been examined in vivo.

This effect of temperature on protein synthesis may have considerable practical significance. The temperature of free-living rats varies by more than 1°C in a circadian fashion [15,16], and comparable or greater changes in body temperature than those observed in our study can result from experimental treatments such as food deprivation [16], inflammation [17], and hemorrhage [18]. Temperature might therefore be an unrecognized confounding variable in studies of the responses of protein metabolism involving treatments that unintentionally alter body temperature.

Although this is the first study specifically investigating the effect of acidosis of respiratory origin on tissue protein synthesis, there have been previous reports suggesting that impaired respiratory gas-exchange resulting in acidosis may have an inhibitory effect on muscle protein synthesis [19-21]. Preedy and Garlick [19], while investigating the effect of restraint on rates of muscle protein synthesis observed a decrease in muscle protein synthesis 1 and 6 hours after rats were wrapped in a linen towel. Because blood gas analysis showed that restrained animals were hypercapnic and acidotic but not hypoxic, the authors concluded that the likely cause of inhibition of protein synthesis was respiratory acidosis [19]. There is a remarkable similarity between the findings of the present study and that of Preedy and Garlick [19]. In the 2 studies, acidosis is accompanied by a comparable degree of hypercapnia and both studies show an inhibition of muscle protein synthesis of 20% to 30% after 1 hour [19] (Table 4).

Another study in rats highlighted the potential importance of acidosis due to central respiratory depression on tissue protein synthesis. Hashiguchi et al [20] showed that centrally administered morphine and morphine agonists depressed tissue protein synthesis in rats, and suggested that the mechanism might involve respiratory depression rather than a direct effect of the opioids themselves. This conclusion was further supported in a subsequent study in which different doses of the morphine agonist [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]-enkephalin (DAGO) were injected into the cerebroventricular space in rats with arterial catheters implanted to allow blood gas to be measured [21]. The results showed a progressive decline in protein synthesis with increasing doses of DAGO, in association with a progressive and parallel decline in pH and an increase in P_{CO_2} caused by respiratory depression. The depression of protein synthesis induced by central opioid administration was not limited to muscle, but involved liver and other visceral organs [21], differently from the result of our study, which showed that the suppression of protein synthesis by acidosis was mainly limited to muscle (experiment 2). However, administration of opioids was also accompanied by hypoxia and reduced hemoglobin O_2 saturation [20,21], which were not observed in our experimental model. Because hypoxia alone has been shown to inhibit protein synthesis [22], the severe suppression of tissue protein synthesis observed by Hashiguchi et al [20,21] may have been induced by a combination of respiratory acidosis and hypoxia. Furthermore, body temperature was not recorded during the experiments by Hashiguchi et al [20,21]. It is therefore not known whether central administration of opioids was followed by a drop in body temperature, which may have played an additive role in depressing protein synthesis in tissues (ie, liver) that were not affected by respiratory acidosis alone, as shown in our study.

The inhibitory effect of respiratory acidosis appears to be specific to skeletal muscle, although the degree of response may vary among muscle types. The depression of protein synthesis rate in soleus (−4%, P = NS) was lower than that observed in gastrocnemius and plantaris muscles (Table 4).

A smaller response of soleus muscle was previously observed during acute metabolic acidosis [7], and we suggested that the differential response may be related to the muscle fiber composition [7]. Compared to plantaris and gastrocnemius, containing mixed fiber types, soleus is a predominantly oxidative slow-twitch–type muscle. Muscles with predominantly slow-twitch fibers, which have been shown to be less sensitive to nutritional or hormonal stimuli [21,23,24], also appear to be more resistant to the negative effect of acidosis.

During respiratory acidosis, low blood pH is associated with an increase of blood P_{CO_2}, that is, hypercapnia, and therefore, the observed inhibition of muscle protein synthesis could be directly caused by acidosis and/or be a consequence of hypercapnia. However, the fact that a comparable inhibition of muscle protein synthesis was also observed during metabolic acidosis, in which low blood pH was not accompanied by changes in blood CO_2 levels [7], suggests that the effect on muscle may be mediated by acidosis alone.

The effect of pH may be direct on the muscle cell and/or indirect, that is, mediated by action of hormones. Studies on the effect of acidosis on protein degradation have pointed out the important role of corticosteroids in mediating catabolic effects of acidosis on muscle protein metabolism [2,5,25]. However, although corticosteroid hormones have been shown to depress muscle protein synthesis both in vivo and in vitro [26,27], their effect is not immediate and can only be observed after several hours [26]. Because the inhibition of muscle protein synthesis by respiratory acidosis can be detected within an hour, it is unlikely that corticosteroids play an important role in mediating the inhibition of protein synthesis during respiratory acidosis.

The inhibition of protein synthesis by respiratory acidosis was not a consequence of a decrease in plasma amino acid concentration. On the contrary, similarly to what was previously observed after 24 hours of metabolic acidosis [7], the plasma concentration of most amino acids was higher in animals breathing CO_2-enriched air (Table 2). Because both groups of rats were in the postabsorptive state at the time of measurement, the increase in plasma amino acid concentration may be a consequence of the observed inhibition of muscle protein synthesis but also of the enhanced catabolism of endogenous body protein by acidosis, which has been consistently demonstrated in previous human and animal studies (ie, Refs [5,25,28,29]). However, higher plasma amino acid concentrations may also be the results of several other diverse metabolic processes induced by acidosis. These include an alteration in amino acid transport to/from specific tissues, leading to an overall increased release and/or to a decreased uptake of amino acids from blood circulation.

Although the concentration of many amino acids increased, the concentration of tryptophan and glutamic acid was significantly lower in acidotic animals. The decrease in plasma glutamic acid concentration may in part related to the use of this amino acid as a precursor for synthesis of glutamine, which is accelerated in some tissues during acidosis (eg, Refs [30-33]).

In summary, this study showed that acute respiratory acidosis for 1 hour depresses protein synthesis in skeletal muscle and that this effect is independent from changes in body temperature associated with hypercapnia. On the contrary, liver protein synthesis is not affected by respiratory acidosis but is inhibited by a drop of about 2°C in body temperature in animals breathing CO_2. The depression of protein synthesis due to respiratory acidosis is therefore specific to muscle and comparable to that previously observed during acute metabolic acidosis. These findings demonstrate that acidosis, whether of metabolic or respiratory origin, has a depressive effect on muscle protein synthesis and that it may contribute to loss of muscle protein in many clinical conditions associated with low blood pH.

Acknowledgments

This study was supported by National Institutes of Health grants DK54991 and M01RR10710. We thank Yuqun Hong for his technical assistance.

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