Effect of guanethidine-induced adrenergic blockade on the different proteolytic systems in rat skeletal muscle

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Navegantes, Luiz Carlos C., Neusa M. Z. Resano, Renato H. Migliorini, and Isis C. Kettelhut. Effect of guanethidine-induced adrenergic blockade on the different proteolytic systems in rat skeletal muscle. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E883–E889, 1999.—Overall proteolysis and the activity of skeletal muscle proteolytic systems were investigated in rats submitted to guanethidine-induced adrenergic blockade for 4 days. In soleus, overall proteolysis increased by 15–20% during the first 2 days of guanethidine treatment but decreased to levels below control values after 4 days. Extensor digitorum longus (EDL) did not show the initial increase in total proteolysis, which was already reduced after 2 days of guanethidine treatment. The initial rise in the rate of protein degradation in soleus was accompanied by an increased activity of the Ca$^{2+}$-dependent proteolytic pathway. In both soleus and EDL, the reduction in overall proteolysis was paralleled by decreased activities of the Ca$^{2+}$-dependent and ATP-dependent proteolytic processes. No change was observed in the activity of the lysosomal proteolytic system. Overall proteolysis in soleus and EDL from nontreated rats was partially inhibited by isoproterenol, in vitro. The data suggest an acute inhibitory control of skeletal muscle proteolysis by the adrenergic system, well evident in the oxidative muscle, with an important participation of the Ca$^{2+}$-dependent pathway. catecholamines; isoproterenol; lysosomal proteolytic activity; calcium-dependent proteolysis; ATP-dependent proteolysis

Although many studies have demonstrated that the sympathetic innervation of skeletal muscle is not restricted to the vasculature and that adrenergic nerve terminals make close contact with striated muscle fibers in mammals (3, 4), the physiological role of this innervation is far from being established. The actions of catecholamines are generally associated with catabolic processes, promoting the breakdown of both glycogen and fat for use as fuel, but a growing body of evidence suggests that the sympathetic nervous system may have an anabolic effect on muscle protein metabolism (8). Numerous studies, reviewed in Refs. 18, 21, and 32, show that the dietary administration of β$_2$-adrenergic agonists, e.g., clenbuterol and cimaterol, to many species markedly stimulates hypertrophy of skeletal muscle. In addition, treatment with clenbuterol has been reported to retard atrophy in denervated muscles (33) and to reduce muscle waste in hepatoma Yoshida AH-130-bearing rats (6). The precise mechanism through which β$_2$-adrenergic agonists produce these effects is not known, but they seem to be due, at least in part, to a reduction in the rate of muscle protein breakdown. Indeed, it has been shown that the skeletal muscle hypertrophy induced by β$_2$-adrenergic agonist administration is accompanied by a reduced μ-calpain activity and an increase in the activity of calpains, an endogenous inhibitor of calpains, suggesting a suppression of the Ca$^{2+}$-dependent proteolysis (2). Also, it has been recently reported that the hyperactivation of the ATP-ubiquitin-dependent proteolytic system that occurs in skeletal muscle of tumor-bearing rats is effectively antagonized by clenbuterol (6). On the other hand, it has been found that intravenous infusion of epinephrine, in an amount that simulates the plasma increase observed in stress, causes a decline in circulating amino acids of similar magnitude in normal and diabetic subjects (27). Furthermore, this fall occurs in the absence of changes in plasma insulin levels and can be totally prevented by propranolol, a β-adrenergic antagonist (27). Epinephrine infusion into healthy humans has also been shown to induce a decrement in the rate of whole body proteolysis that is still evident when endogenous insulin secretion is blocked by somatostatin infusion (7). In agreement with these studies in human subjects, it has been found in experiments with rat perfused hindquarters that epinephrine infusion induces a sharp decrease in tyrosine release (an index of protein degradation), which can be prevented by the concomitant administration of propranolol (15). In addition to suggesting that epinephrine inhibits proteolysis via β-adrenergic receptors, these in vitro experiments indicate that its effect is not mediated by other hormones (15).

The present study was undertaken to examine in rat isolated skeletal muscles the changes in the activities of the different proteolytic systems that occur after short-term adrenergic neuronal blockade. Guanethidine treatment for a few days was used because it has been shown to specifically produce blockade of norepinephrine release from peripheral nerves without affecting central adrenergic neurons in adult rats (24). The major systemic effect of chronic administration of guanethidine is a decrease of the blood pressure, which has been shown to be relatively small during the 1st wk of treatment (13). We could not find in the literature any information about changes in metabolite or hormone levels induced by short-term guanethidine treatment.

In addition to the effect of guanethidine treatment on the rate of overall proteolysis and on the activity of four proteolytic systems (lysosomal, Ca$^{2+}$-dependent, ATP-dependent, and energy-independent systems) (16), we...
examined the in vitro effect of isoproterenol (a β-adrenergic agonist) on the proteolytic rate of skeletal muscles isolated from nontreated rats. The concentration of catecholamines in muscle, plasma, and adrenal medulla in guanethidine-treated rats and plasma levels of insulin, testosterone, and corticosterone are also reported.

MATERIALS AND METHODS

Animals

Because the incubation procedure required intact muscles sufficiently thin to allow an adequate diffusion of metabolites and oxygen, young rats were used in all experiments. Male Wistar rats were housed in a room with a 12:12-h light-dark cycle and were given free access to water and normal lab chow diet for ≥1 day before the beginning of the experiments. Rats of similar body weight (65–70 g) were used in all experiments, which were performed at 8:00 AM.

Guanethidine and Muscle Proteolysis Studies

Guanethidine administration. Animals were injected subcutaneously with guanethidine sulfate for 1, 2, or 4 days to block adrenergic neurons. Preliminary experiments showed that a daily dose of 100 mg/kg body weight of guanethidine (dissolved in 0.9% NaCl, pH adjusted to 7.4) was sufficient to completely deplete the skeletal muscles of norepinephrine (see RESULTS). Control rats received 0.9% NaCl. Although a 16% reduction in food consumption was observed after 1 day of guanethidine treatment, food consumption was not altered after 2 and 4 days of treatment. To avoid any interference of the reduced food intake in the results, rats of the “1 day” group were killed for the experiments 10 h after guanethidine or saline injection. Guanethidine treatment did not affect body weight gain or skeletal muscle weight at any of the experimental periods (data not shown).

Incubation procedure. Rats were killed by cervical dislocation for muscle excision. The soleus and extensor digitorum longus (EDL) were rapidly dissected, care being taken to avoid damaging the muscles. Soleus muscles were maintained at approximately resting length by pinching their tendons in aluminum wire supports, and EDL muscles were maintained by pinning them on inert plastic supports. Tissues were incubated at 37°C in Krebs-Ringer bicarbonate buffer (pH 7.4, equilibrated with 95% O2-5% CO2) containing glucose (5 mM) and in the presence of cycloheximide (0.5 mM) to prevent protein synthesis and the reincorporation of tyrosine back into proteins. Tissues were preincubated for 1 h and then incubated for 2 h in fresh medium of identical composition.

Measurement of rates of protein degradation. The rate of overall proteolysis and of the different proteolytic systems was determined by measuring the rate of tyrosine release in the incubation medium. Because muscle cannot synthesize or degrade tyrosine, its release reflects the rate of protein breakdown. Tyrosine was assayed as previously described (31). Preliminary experiments showed that, as previously reported for normal animals (1), the intracellular pools of tyrosine of the guanethidine-treated rats were not significantly affected by all the incubation conditions used here. Therefore, rates of amino acid release into the medium reflect rates of protein degradation.

To measure the intralysosomal proteolysis, muscles from one limb were incubated in the absence of insulin and branched-chain amino acids, a condition in which the lysosomal process is activated. Contralateral muscles were incubated in the presence of insulin (1 U/ml), amino acids (leucine, 170 μM; isoleucine, 100 μM; valine, 200 μM), and with methylamine (10 mM), a weak base that raises intralysosomal pH and inhibits lysosomal proteolysis (16). The difference in tyrosine release between the two muscles reflects the activity of the lysosomal proteolytic component.

To test the activity of the Ca2+-dependent proteolysis, muscles from one limb were incubated in a Ca2+-free medium that contained, in addition to insulin and branched-chain amino acids (to block lysosomal process), E-64 (50 μM) and leupeptin (25 μM), inhibitors of thiol proteases (calpains) (1, 16). The contralateral muscles were incubated in the presence of Ca2+ and in the absence of E-64 and leupeptin. The difference between the two muscles represents the Ca2+-dependent proteolytic process.

In muscles maintained at resting length in the presence of insulin and amino acids, most protein breakdown occurs by a nonlysosomal Ca2+-independent process that requires ATP (16). To measure the ATP-dependent and energy-independent processes, muscles were first incubated under conditions that prevent activation of the lysosomal and Ca2+-dependent proteolytic systems by use of Ca2+-free medium and different inhibitors, including methylamine, insulin plus branched-chain amino acids, E-64, and leupeptin (16). The proteolytic activity measured in contralateral muscles incubated with dinitrophenol (DNP) (0.5 mM), 2-deoxyglucose (5 mM), and without glucose (to deplete them completely of intracellular ATP) must represent an ATP-independent proteolytic process. This residual process represents a distinct energy-independent degradative system and not just a failure to block completely the ATP-requiring process, because it varies in a distinct fashion (9, 16). The difference in tyrosine release between the two contralateral muscles (with and without ATP depletion) reflects the activity of the ATP-dependent proteolytic system.

Isoproterenol and Muscle Proteolysis Studies

To investigate the in vitro effect of isoproterenol on the rates of overall proteolysis, soleus and EDL muscles from nontreated rats were incubated in the presence of 0.25 mM of this nonselective β-adrenergic agonist by use of the same procedure described for the guanethidine experiments.

Catecholamine Measurements

For the determination of catecholamine plasma levels and of muscle and adrenal medulla catecholamine content, a group of rats was killed by decapitation after 1, 2, and 4 days of guanethidine or saline treatment. Tissues and plasma were stored at −70°C until assayed. Catecholamines were assayed as previously described (11) using HPLC (LC-7A, Shimadzu Instruments) with a 5-μm Spherisorb ODS-2 reversed-phase column (Sigma-Aldrich).

Metabolite and Hormone Measurements

In a group of rats treated for 2 days with guanethidine or saline, blood was collected by decapitation to determine the plasma concentrations of glucose, insulin, testosterone, and corticosterone. Glucose concentration was determined with glucose oxidase with a glucose analyzer (Beckman). Muscle glycogen content was measured by the method of Carrol et al. (5). Hormone levels were determined by radioimmunoassays.

Statistical Methods

Means of muscle samples from different groups of animals were analyzed using Student's nonpaired t-test. In the experi-
ments with isoproterenol, means of samples from contralateral legs were compared using Student's paired t-test. P < 0.05 was taken as the criterion of significance.

RESULTS

Metabolic Effects of Guanethidine Treatment

As shown in Table 1, muscle glycogen content and plasma levels of glucose, insulin, testosterone, and corticosterone after 2 days of guanethidine treatment did not differ significantly from levels in saline-injected controls.

Effect of Guanethidine Treatment on Muscle, Plasma, and Adrenal Medullary Catecholamine

Guanethidine treatment for 1, 2, or 4 days induced a 90% reduction in norepinephrine content of soleus and EDL muscles; (Fig. 1 shows the data for 2 days). Plasma levels of norepinephrine and epinephrine were significantly reduced by 40–80% during the experimental period (Fig. 2). Dopamine was not detected in plasma samples from either group at any time. Adrenal medullary norepinephrine and epinephrine levels were not altered by guanethidine treatment, but dopamine content increased by >80% during the three experimental periods (Fig. 2). Dopamine content of chromaffin cells is thought to be a useful in vivo index of the rate of catecholamine synthesis and correlates well with the previously observed increase in tyrosine hydroxylase activity (22) and with the faster adrenal medulla epinephrine turnover induced by adrenergic blockade (29). This suggests that guanethidine treatment leads to a compensatory activation of catecholamine synthesis by the medulla, secondary to the blockade of peripheral sympathetic nerve terminals (29).

Evaluation of Different Proteolytic Processes

Skeletal muscle proteolysis in guanethidine-treated rats varied according to the type of muscle and duration of treatment. In soleus muscle, a 15–20% increase in the rate of total protein degradation was observed after 1 and 2 days of treatment (Fig. 3). However, after 4 days, proteolysis in soleus reverted to levels below control values. No increase in EDL proteolysis was detected after guanethidine administration. After 2

Table 1. Muscle glycogen content and plasma levels of glucose, insulin, testosterone, and corticosterone in rats treated with guanethidine or saline for 2 days

<table>
<thead>
<tr>
<th></th>
<th>Glycogen, mg/100 mg</th>
<th>Glucose, mg/dl</th>
<th>Insulin, µU/ml</th>
<th>Testosterone, ng/dl</th>
<th>Corticosterone, µU/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.55 ± 0.03</td>
<td>145 ± 2</td>
<td>17 ± 3.1</td>
<td>30 ± 7</td>
<td>4.7 ± 1.2</td>
</tr>
<tr>
<td>Guanethidine</td>
<td>0.49 ± 0.06</td>
<td>133 ± 5</td>
<td>16 ± 1.2</td>
<td>39 ± 5</td>
<td>5.2 ± 1.2</td>
</tr>
<tr>
<td>EDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.60 ± 0.04</td>
<td>145 ± 2</td>
<td>17 ± 3.1</td>
<td>30 ± 7</td>
<td>4.7 ± 1.2</td>
</tr>
<tr>
<td>Guanethidine</td>
<td>0.63 ± 0.05</td>
<td>133 ± 5</td>
<td>16 ± 1.2</td>
<td>39 ± 5</td>
<td>5.2 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE from 5–7 animals. EDL, extensor digitorum longus.

Fig. 1. Norepinephrine content of soleus and extensor digitorum longus (EDL) muscles from rats treated with guanethidine or saline for 2 days. Similar results were obtained in muscles from rats treated for 1 or 4 days. Values are means ± SE of 7–9 muscles. †P < 0.01.

Fig. 2. Adrenomedullary content (A) and plasma concentration (B) of catecholamines from guanethidine-treated rats. Values are means ± SE of 6–8 animals expressed as % of control values. Adrenomedullary content and plasma concentration of catecholamines in control rats did not differ significantly in the different experimental periods. Control average values were as follows: in the adrenal medulla, dopamine (ng/medulla), 27.4 ± 25; norepinephrine and epinephrine (µg/medulla), 1.9 ± 2.0 and 3.9 ± 2.0, respectively; in the plasma (dopamine not detected), norepinephrine and epinephrine (ng/ml), 5.1 ± 0.4 and 6.4 ± 0.6, respectively. †P < 0.01.
and 4 days of treatment, rates of protein degradation in EDL were reduced by ~15% (Fig. 3). Isoproterenol added to the incubation medium of soleus or EDL isolated from nontreated rats reduced the overall rate of tyrosine release by 13 and 27%, respectively (Fig. 4).

Lysosomal proteolytic activity. Lysosomal proteolytic activity amounted to ~18–25% of total proteolysis in soleus and EDL at the three experimental intervals, with no difference between treated and nontreated rats (Fig. 5).

Calcium-dependent proteolytic activity. Calcium-dependent proteolytic activity in soleus muscle increased by 30% in the 1st day of treatment and by 45% after 2 days (Fig. 5). However, after 4 days of guanethidine treatment, the activity of this pathway reverted to levels below control values. In EDL from rats treated for 2 and 4 days, the decrease in the rate of overall proteolysis was accompanied by a reduction in the activity of the Ca\(^{2+}\)-dependent proteolytic process (Fig. 5).

ATP-dependent proteolytic activity. In both soleus and EDL muscles, the activity of the ATP-dependent proteolytic system in guanethidine-treated rats did not differ from that of control muscles after 1 day but showed a 20–30% decrease after 2 and 4 days of treatment (Fig. 5). In EDL muscles, the ATP-independent proteolytic process was not affected by guanethidine treatment at any of the experimental intervals. However, a significant 19% reduction in the activity of this process was observed in soleus muscles from rats treated for 4 days (Table 2).

ATP-dependent proteolytic activity. In both soleus and EDL muscles, the activity of the ATP-dependent proteolytic system in guanethidine-treated rats did not differ from that of control muscles after 1 day but showed a 20–30% decrease after 2 and 4 days of treatment (Fig. 5). In EDL muscles, the ATP-independent proteolytic process was not affected by guanethidine treatment at any of the experimental intervals. However, a significant 19% reduction in the activity of this process was observed in soleus muscles from rats treated for 4 days (Table 2).
from rats treated with guanethidine or saline for 4 days

Table 2. ATP-dependent and ATP-independent proteolytic activity in soleus and EDL muscles from rats treated with guanethidine or saline for 4 days

<table>
<thead>
<tr>
<th>Addition</th>
<th>Tyrosine Release, nmol·mg wet wt⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>DNP</td>
<td>Glucose</td>
</tr>
<tr>
<td>− − +</td>
<td>Soleus</td>
</tr>
<tr>
<td>+ − −</td>
<td>ATP independent</td>
</tr>
<tr>
<td>Difference</td>
<td>ATP dependent</td>
</tr>
<tr>
<td>− − +</td>
<td>EDL</td>
</tr>
<tr>
<td>+ − −</td>
<td>ATP independent</td>
</tr>
<tr>
<td>Difference</td>
<td>ATP dependent</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7-9 muscles. DNP, dinitrophenol. Incubation conditions are indicated in MATERIALS AND METHODS. NS, not significant; *P < 0.05, †P < 0.01 vs. control; ‡effect of DNP addition was statistically significant (P < 0.01).

DISCUSSION

The present data show that short-term adrenergic blockade induces changes in the rate of overall proteolysis and in the activity of proteolytic systems in skeletal muscle, which are dependent on both the type of muscle and the duration of guanethidine treatment. The increase in the rates of total protein degradation in soleus observed during the first 2 days was followed by a decrease below control levels after 4 days of guanethidine treatment (Fig. 3). The early rise in proteolytic rate occurred without any change in the plasma levels of insulin, testosterone, and corticosterone and was probably a direct consequence of the depletion of muscle norepinephrine (Fig. 1) and/or of the reduction in plasma catecholamine concentration (Fig. 2) induced by guanethidine treatment. The acute increase in protein breakdown after adrenergic blockade suggests the existence of an inhibitory adrenergic tonus in skeletal muscles that restrains proteolysis. This view is consistent with the inhibition of proteolysis obtained here with isoproterenol in vitro (Fig. 4), and also with the finding that infusion of epinephrine in human subjects (27) and in perfused rat hindquarters (15) induces a rapid and similar 20% decrease in protein degradation that can be prevented by β-adrenergic blockade. This suggests that the anti-proteolytic effect of norepinephrine and epinephrine is mediated by β-receptors. β-Adrenergic receptors, predominantly of the β2-subtype, have been identified in rat skeletal muscles (19). Recently, the β2-adrenoceptor subtype, which has a higher affinity for norepinephrine than for epinephrine (28), has been found to be present in oxidative muscles, including soleus (18).

The subsequent decrease in the rate of overall proteolysis observed after 4 days of guanethidine can be interpreted as a restoration of muscle capacity to limit protein breakdown. The mechanism of the reestablishment of muscle anti-proteolytic activity cannot be explained on the basis of the present data. Chemical sympathectomy by 6-hydroxydopamine has been shown to induce an increase in the number of β-adrenergic receptors in the myocardium (12), and it is tempting to speculate that if a similar increase occurred in soleus, circulating catecholamines would now be able to effectively inhibit proteolysis despite the lower plasma levels. It is also possible that adrenergic blockade by guanethidine induces an increased sensitivity of the skeletal muscle to other anabolic hormones, such as insulin, growth hormone, or insulin growth factors (16).

It is interesting to note that the biphasic pattern of proteolysis observed in soleus from guanethidine-treated rats, with an initial increase followed by a decrease in the rate of protein degradation, has also been found in short-term and long-term catabolic states, including fasting (16) and diabetes (23). It would thus appear that the activation of regulatory mechanism(s) to prevent excessive breakdown of protein and spare muscle protein reserves is a characteristic feature of skeletal muscle. Maintenance of protein content is especially essential for red oxidative muscles such as soleus, which serve the important function of sustaining most of the postural work of the animal and therefore are continually active. On the other hand, there are indications that the control of muscle protein content may also actuate in the opposite direction, that is, to prevent excessive accumulation. Thus it is well established that the anabolic action of β2-adrenergic agonist treatment is efficient for only a limited period of time (18, 21, 32). In rats fed cimaterol, urinary excretion of 3-methylhistidine, an index of myofibrillar proteolysis, decreases by 31% after 3 days but increases by 42% after 18 days (18), suggesting a reversion of the initial anti-proteolytic response by prolonged treatment.

Unlike soleus, EDL muscle did not show the initial increase in the rate of protein degradation after guanethidine treatment, with the proteolytic rate already reduced after 2 days of treatment (Fig. 3). However, white muscles such as EDL are rich in type II fibers, which seem to be more responsive to β-adrenergic agonists than the type I fibers predominant in soleus (18, 32). Hence, it is possible that the increase in proteolysis occurred before 10 h (“1 day” group), which was the earliest time interval utilized in the experiments. This hypothesis is supported by the finding that only a few minutes of epinephrine infusion are needed to reduce muscle proteolysis, both in rat perfused...
hindquarters (15) and in microdialysis experiments with rat gastrocnemius (26). A higher sensitivity of EDL to changes in adrenergic activity could also explain the faster reduction in proteolysis (after 2 days, compared with 4 days in soleus). In fact, the reduction in proteolysis induced by isoproterenol in vitro was greater in EDL (27%) than in soleus (13%) (Fig. 4). Differences in the response of muscle fiber types to \( \beta \)-adrenergic stimulation have also been observed in catecholamine effects mediated by cAMP, as illustrated by the greater sensitivity of white muscles to the stimulating effect of epinephrine on glycogenolysis (14). Further experiments are needed to certify whether the different muscle fiber types display differences in the sensitivity also for \( \beta \)-adrenergic-mediated effects on proteolysis.

With respect to the role played by the different proteolytic pathways in the changes in skeletal muscle overall proteolysis induced by adrenergic blockade, the data of the present work indicate that the lysosomal proteolytic system does not participate in any of the changes observed, because the activity of this system remained unchanged in soleus and EDL throughout the experimental period (Fig. 5). In agreement with these results, it has been found that the reduction in the rate of protein degradation induced by \( \beta \)-adrenergic agonists in vitro in chick skeletal muscle (25) and in rat myocardium (30) is not affected by inhibitors of lysosomal proteolysis. The present data also show that the initial increase in the rate of overall proteolysis (detected only in soleus during the first 2 days) was accompanied by a parallel increase in the activity of the Ca\(^{2+}\)-dependent proteolytic system (Fig. 5). In line with what was just discussed in relation to the mechanism of the acute proteolytic effect of adrenergic blockade, these data suggest that the restraining effect of the adrenergic tonus on skeletal muscle proteolysis is exerted, at least in part, by keeping the Ca\(^{2+}\)-dependent pathway inhibited. Evidence for a close association between adrenergic activity and Ca\(^{2+}\)-dependent proteolysis has also been obtained in numerous studies showing that the activity and gene expression of \( \mu \)-calpain are decreased and those of calpastatin are increased after \( \beta \)-adrenergic agonist administration to rats (2), rabbits (10), lambs (20), and cattle (17). The periods in which the rate of overall proteolysis was reduced (after 4 days in soleus and after 2 and 4 days in EDL) were accompanied by decreased activities of both the Ca\(^{2+}\)-dependent and the ATP-dependent pathways (Fig. 5). As we have pointed out, an increased sensitivity to anabolic hormones may also contribute to the reestablishment of the muscle capacity to reduce proteolysis after adrenergic blockade, and recent studies from this laboratory have shown that insulin can regulate the Ca\(^{2+}\)-dependent and the ATP-dependent proteolysis in rat skeletal muscle (23). In soleus, but not in EDL of guanethidine-treated rats, the activity of the ATP-independent pathway was also decreased (Table 2), probably contributing to reduced proteolysis in this muscle. The activity of this little-studied degradative process has also been reported to be decreased in soleus from diabetic rats, at a time when muscle proteolysis was reduced (23).

In summary, the present work shows that an acute guanethidine-induced adrenergic blockade induces time-dependent changes in the nonlysosomal proteolytic systems in rat skeletal muscles. An initial rise in the rate of overall proteolysis in soleus accompanied by an increased activity of the Ca\(^{2+}\)-dependent pathway, together with a reduction in the rate of overall proteolysis in soleus and EDL induced by isoproterenol in vitro, suggests that the sympathetic nervous system exerts an acute inhibitory control of skeletal muscle proteolysis, mediated by \( \beta \)-adrenergic receptors, with the participation of the Ca\(^{2+}\)-dependent pathway.

We are indebted to Elza Aparecida Filippin, Maria Antonieta R. Gardaflo, José Roberto de Oliveira, and Víctor Díaz Galbán for technical assistance.

This work was supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 97/3950–5) and from the Conselho Nacional de Pesquisa (CNPq 501252/91–6). During this study L. C. C. Navegantes received fellowships from the CNPq (830682/97–0) and FAPESP (98/02591–4).

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Received 11 March 1999; accepted in final form 16 June 1999.

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