Effect of sympathetic denervation on the rate of protein synthesis in rat skeletal muscle

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Navegantes, Luiz Carlos C., Neusa M. Z. Resano, Amanda M. Baviera, Renato H. Migliorini, and Isis C. Kettelhut. Effect of sympathetic denervation on the rate of protein synthesis in rat skeletal muscle. Am J Physiol Endocrinol Metab 286: E642–E647, 2004; 10.1152/ajpendo.00371.2003.—Rates of protein synthesis were investigated in skeletal muscles from rats submitted to chemical and surgical sympathectomy. Three models of sympathetic denervation were used: 1) treatment with guanethidine (100 mg·kg⁻¹·day⁻¹·sc); 2) lumbar sympathetic denervation (surgical excision of the second and third lumbar ganglia of the sympathetic chain, from which arises the postganglionic fibers to the skeletal muscles of rat hindlimb); and 3) adrenomedulladulation. Protein synthesis was estimated in isolated soleus muscle by the rate of incorporation of [¹⁴C]tyrosine (0.1 mM, 0.05 μCi/ml) into total protein. Soleus isolated after 2 and 4 days of chemical sympathectomy or after 3 days of lumbar denervation showed a 17–20% statistically significant decrease in in vitro rates of protein synthesis. These effects were reverted by addition of 10⁻⁵ M isoproterenol or epinephrine in vitro. Neither clenbuterol nor isoproterenol (10⁻⁷, 10⁻⁶, or 10⁻⁵ M) in vitro affected the rate of protein synthesis in soleus from normal rats. On the other hand, clenbuterol or epinephrine (10⁻⁵ M) increased by 20% the rate of protein synthesis in soleus muscles from adrenomedullated rats and prevented its decrease in muscles from fasted rats. The data suggest that the sympathetic nervous system stimulates protein synthesis in oxidative muscles, probably through the activation of β₂-adrenergic receptors, especially in situations of hormonal or nutritional deficiency.

THE PHYSIOLOGICAL ROLE OF PLASMA CATECHOLAMINES AND ADRENERGIC NERVE TERMINALS

The physiological role of plasma catecholamines and adrenergic nerve terminals that make close contact with striated muscle fibers in protein metabolism is far from being completely established. Numerous studies suggest that endogenous catecholamines may induce anabolic, protein-sparing effects on skeletal muscle protein metabolism in humans (29) and in rats (14, 20). In growing animals, the oral administration of β₂-adrenergic agonists not only stimulates body weight gain but also increases muscle mass (4, 19). β-Agonist-induced hypertrophy seems to be specific for striated muscles, since the smooth muscles of gut, liver, and kidney (28) do not increase in size in response to these agents. It has also been reported that treatment with β₂-adrenergic agonists reduces muscle wasting in different experimental catabolic situations (20). In contrast, it has recently been shown that long-term β-blockade with propranolol decreases lean-mass catabolism in severely burned children (12). The biochemical mechanism of these in vivo effects of β₂-adrenergic agonists and antagonists is not completely understood.

The accretion of muscle protein reflects the net balance between the two opposing processes of protein synthesis and protein degradation. In previous work (21, 23), we provided evidence indicating that plasma catecholamines exert an anabolic effect through an inhibition of Ca²⁺-dependent proteolytic process and that this effect is modulated by β₂-adrenoceptors and cAMP-dependent pathways (22). However, the possibility could not be excluded that a stimulation of skeletal muscle protein synthesis by catecholamines also contributed to their anabolic effect. Experiments have been carried out in several species to find out whether an increased rate of protein synthesis contributes to the muscle hypertrophy induced by β₂-agonist administration (4, 19). The results obtained in vivo have been conflicting because of differences in the technique used for measurement of protein synthesis and in the experimental design, such as dose, route of drug administration, and timing of measurements (26). The finding in some experiments that muscle protein synthesis was not affected in clenbuterol-treated animals (28) led to the suggestion that the muscle hypertrophy induced by the β₂-agonist is exclusively dependent on the inhibition of protein degradation. However, it has been found in other studies that the hypertrophy promoted by β₂-agonists is accompanied by increases in the fractional rate of protein synthesis in skeletal muscles of rats (30), lambs (5), and pigs (11). In rats, the onset of the stimulatory effect of clenbuterol on protein synthesis is very rapid, usually within the first 3 days of treatment, but is markedly attenuated after 14 days (14). The reason for this attenuation is not completely understood, but it may be due, at least in part, to a downregulation of β-adrenergoreceptors (15). In contrast to these in vivo studies, no clear stimulatory effect of β₂-agonists on protein synthesis has been reported in incubated muscles or in muscle cell cultures (3, 15).

To gain more information about the role of the sympathetic nervous system (SNS) in muscle protein synthesis, we have investigated in the present work the effect of chemical or surgical sympathectomy on the rate of protein synthesis in isolated soleus muscle in vitro as well as the effect of addition, in the same conditions, of catecholamines, isoproterenol (a β-adrenergic agonist), or clenbuterol (a selective β₂-adrenergic agonist). Three models of sympathetic denervation were used: 1) treatment with guanethidine (100

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mg·kg⁻¹·day⁻¹ sc), 2) lumbar sympathectomized denervation, and 3) adrenomedullation.

In addition to the experiments with sympathectomized rats, we have examined the effect of catecholamines and of clenbuterol, added in vitro, on the rate of incorporation of [¹⁴C]tyrosine in protein in soleus isolated from rats fasted for 24 h. The concentration of catecholamines of muscle, plasma, or adrenal medulla in sympathectomized and fasted rats is 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 a normal lab chow diet. A group of rats was left without food for 24 h. Rats of similar body weight (65–70 g) were used in all experiments, which were performed at 8:00 AM.

Models of Sympathectomy

Guanethidine administration. Animals were injected subcutaneously with guanethidine sulfate for 1, 2, or 4 days to block adrenergic neurons. Previous experiments showed that a daily dose of 100 mg/kg body wt of guanethidine (dissolved in 0.9% NaCl, pH adjusted to 7.4) is sufficient to completely deplete the skeletal muscles of norepinephrine (21). Control rats received 0.9% NaCl. Although a 16% reduction in food consumption was observed during the 1st day of guanethidine treatment, food consumption was not altered in the following 3 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.

Adrenomedullation. Adrenomedullation was performed under ether anesthesia 1, 2, or 4 days before the animals were utilized in the experiments. The medulla of each adrenal was squeezed through a nick made on its capsula. The animals did not require saline in drinking water after surgery. Sham-operated rats were used as controls. Food consumption was not altered by surgery.

Lumbar sympathetic denervation. Lumbar sympathetic denervation consisted of the surgical excision, with the help of a dissection microscope, of the bilateral second and third lumbar ganglia of the paravertebral sympathetic chain, from which arises the sympa-thetic fibers to the skeletal muscles of the rat hindlimb (2). The lumbar sympa-thetic chain on both sides lies embedded in connective tissue in front of the vertebral column behind the aorta and vena cava. Frozen sections of the ganglia were stained and confirmed histologically. Control rats were submitted to a sham operation. The animals were used 3 days after surgery. Food consumption was not altered by surgery.

Incubation Procedure

Rats were killed by cervical dislocation for muscle excision. The soleus muscles 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. They were incubated at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4, equilibrated with 95% O₂-5% CO₂, containing glucose (5 mM) and all amino acids at concentrations similar to those of rat plasma (31). In the experiments with β-agonists and catecholamines in vitro, 10 mM ascorbic acid was added to the incubation medium to prevent drug oxidation. After a 1-h equilibration period, tissues were incubated for a total of 6 h, with replacements of fresh medium after 2 and 4 h.

Measurement of Rates of Muscle Protein Synthesis

At the end of the initial 4 h of incubation, L-[U-¹⁴C]tyrosine (0.05 μCi/ml) was added to the replacement medium in which the muscles were incubated for the next 2 h. At the end of this period, the specific activity of acid-soluble tyrosine (intracellular tyrosine pool) in each muscle was measured by measuring in this pool the radioactivity and the concentration of tyrosine, which was determined by the method of Waalkes and Udenfriend (35). After measurement of the radioactivity incorporated into protein of the same muscle, the rate of synthesis was calculated using the specific activity of the intracellular pool of tyrosine, assuming that there was no recycling of the label during the incubation period (8, 33). Because of day-to-day variations in the rates of protein synthesis, measurements in muscles from control and sympathectomized animals were always made in parallel.

β-Agonists, Catecholamines, and Muscle Protein Synthesis

In these experiments, the incubation procedure and muscle protein synthesis measurements were exactly as described above, with epinephrine, norepinephrine, isoproterenol, or clenbuterol present in the incubation medium from the beginning of the 6-h incubation period. Drug concentration and conditions of the rats (fed, fasted, or sympathectomized) from which the soleus was isolated are specified in RESULTS.

Catecholamine Concentration Measurement

Muscle norepinephrine and plasma catecholamines were determined after guanethidine treatment, adrenomedullation, and lumbar sympathectomy. Muscle norepinephrine, adrenal medulla dopamine, and plasma catecholamines were measured in rats fasted for 24 h. The animals were killed by decapitation, and tissues and plasma were stored at −70°C until assayed as previously described in detail (9).

Other Biochemical Methods

Plasma glucose was determined with glucose oxidase by use of a glucose analyzer (Beckman), and corticosterone was measured by radioimmunossay.

Statistical Analyses

Means of muscle samples from different groups of animals were analyzed using Student’s non-paired t-test. In the experiments with catecholamines and β-agonists, means of samples from contralateral legs were compared using Student’s paired t-test. P < 0.05 was taken as criterion of significance.

RESULTS

Body weight gain and skeletal muscle weight of rats were not affected by chemical or surgical sympathectomy (data not shown).

Effect on Muscle and Plasma Catecholamines

Guanethidine treatment for 1, 2, or 4 days resulted in a marked (90%) reduction in the content of muscle norepinephrine and in plasma norepinephrine (83%) and epinephrine (59%) levels (Fig. 1 shows day 2 values), whereas 3 days after lumbar denervation only muscle norepinephrine was reduced (85%; Fig. 1A). Adrenomedulladulation induced a reduction of plasma epinephrine (94%) and norepinephrine (40%) concentrations after 1, 2, or 4 days (Fig. 1 shows day 2 values) but did not affect the content of muscle norepinephrine during the same experimental period (Fig. 1A). Dopamine was not detected in plasma samples from any group, at any time.
Characterization of Fasting State

Soleus muscle weight ($n/N_{11005}$ 8) from 1-day-fasted rats (32 $/N_{11006}$ 0.8 mg) was 11% smaller than that of muscles from fed rats (36 $/N_{11006}$ 0.7 mg). As expected, plasma levels of glucose were decreased and corticosterone increased in fasted rats (Table 1).

Effect of Isoproterenol and Clenbuterol on Muscle Protein Synthesis from Fed Rats

Figure 2 shows that neither isoproterenol nor clenbuterol, at the different concentrations tested, affected the rate of muscle protein synthesis from nontreated rats.

Protein Synthesis in Sympathectomized and Fasting Rats

Skeletal muscle protein synthesis varied according to the type of sympathetic denervation. A 20% decrease in the rate of soleus muscle protein synthesis was observed after 2 and 4 days of guanethidine treatment (Fig. 3). These effects were prevented in the contralateral muscles by addition of isoproterenol ($10^{-5}$ M) to the incubation medium (Fig. 3). A similar anabolic effect was observed in soleus muscles from rats treated with guanethidine for 2 days incubated with $10^{-5}$ M epinephrine (8 rats, 0.321 $/N_{18528}$ 0.009 nmol Tyr incorporated $/N_{18528}$ mg $/N_{11002}$ 2h) compared with their contralateral muscles (8 rats, 0.320 $/N_{18528}$ 0.007). The rate of protein synthesis in isolated muscles from rats submitted to lumbar denervation (8 rats, 0.321 $/N_{18528}$ 0.020 nmol Tyr incorporated $/N_{18528}$ mg $/N_{11002}$ 2h) was 17% lower than in sham-operated rats (7 rats, 0.388 $/N_{18528}$ 0.020). Protein synthesis was not significantly affected by adrenodemedullation, at any of the experimental intervals (Fig. 4), but addition of clenbuterol ($10^{-5}$ M) to the incubation medium of muscle isolated from adrenodemedullated rats 2 and 4 days before induced a 22% increase in protein synthesis (Fig. 4). Rates of muscle protein synthesis decreased 26% in fasted rats (Fig. 5). This reduction was prevented in contralateral muscles incubated in the presence of $10^{-5}$M epinephrine or $10^{-5}$M clenbuterol, but not of $10^{-5}$M norepinephrine (Fig. 5).

DISCUSSION

To study the anabolic effect of catecholamines secreted by the adrenal medulla and sympathetic adrenergic fibers, the rate of protein synthesis was assessed in soleus muscles from rats submitted to different models of sympathetic denervation. The present data show that guanethidine-induced adrenergic blockade for 2 and 4 days results in a 20% decrease in the rate of [14C]tyrosine incorporation in soleus muscle protein, which was abolished by addition of isoproterenol (Fig. 3) or epinephrine (see RESULTS) to the incubation medium. The decrease in the rate of protein synthesis induced by guanethidine treatment occurred without any change in the plasma levels of insulin, testosterone, and corticosterone (21) and was probably a direct consequence of the depletion of muscle norepinephrine and/or reduction of plasma catecholamines. This view is consistent with the similar results obtained after lumbar denervation, which induced a similar 17% decrease in the rate of protein synthesis. Decreased muscle norepinephrine content by guanethidine treatment or lumbar sympathetic ganglia surgical

Table 1. Muscle norepinephrine content and plasma levels of glucose, corticosterone, catecholamines, and adrenal medullary dopamine in 1-day-fasted rats

<table>
<thead>
<tr>
<th></th>
<th>Muscle Norepinephrine, ng/mg</th>
<th>Glucose, mg/dl</th>
<th>Corticosterone, $\mu$U/dl</th>
<th>Plasma Epinephrine, ng/ml</th>
<th>Plasma Norepinephrine, ng/ml</th>
<th>Medullary Dopamine ng/medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>110±14</td>
<td>133±4</td>
<td>4.7±1.2</td>
<td>6.9±0.7</td>
<td>4.8±0.8</td>
<td>33±1</td>
</tr>
<tr>
<td>Fasted</td>
<td>155±7*</td>
<td>86±0*</td>
<td>40±0.1*</td>
<td>6.1±1.5</td>
<td>3.2±0.4</td>
<td>62±11*</td>
</tr>
</tbody>
</table>

Values are means ± SE from 6–7 animals. *$P < 0.05$ vs. fed rats.
Excision (Fig. 1A) could have direct effects on protein flux machinery or could act indirectly by changing endogenous insulin responsiveness, cortisol activity, or regional blood flow. In fact, a significant increase in muscle blood flow following chronic lumbar sympathectomy has been reported in dogs (16). However, it has been shown that an increase in muscle protein synthesis in clenbuterol-treated rats is observed even in the absence of changes in muscle blood flow rates (30). Further studies are needed to clarify this point.

Adrenodemedullation did not affect protein synthesis significantly (Fig. 4), but addition of clenbuterol to the incubation medium of soleus isolated from adrenodemedullated rats induced a 22% increase in protein synthesis (Fig. 4), an effect not observed when either isoproterenol or clenbuterol was added to the incubation medium of soleus from intact control rats (Fig. 2). The fact that the in vitro anabolic actions of β-agonists was observed only in muscles from sympathectomized (guanethidine-treated or adrenodemedullated) rats 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 (10) and in the cAMP response to norepinephrine in the rat brain (25). It has also been found that adrenodemedullation may induce an increase in the sympathetic activity in rat pancreas and adipose tissue (32). Thus, if a similar compensatory increase occurred in muscle sympathetic activity in the guanethidine-treated and adrenodemedullated rats, agonists and catecholamines would now be able to effectively stimulate protein synthesis in vitro. Our results are also in agreement with the findings that catecholamines in vitro stimulate amino acid incorporation into protein of muscles isolated from hypophy-
septomized rats but not from control rats (24). It has also been shown that clenbuterol administration prevents entirely the loss of α-actin mRNA (1) that normally occurs in rat soleus muscles after sciatic nerve section, whereas the innervated contralateral muscles were unaffected (37). Taken together, these data suggest that protein synthesis in oxidative skeletal muscle may be stimulated by catecholamines, especially in situations in which the basal rate of protein synthesis is already reduced by a deficiency in anabolic hormones, such as nerve growth factor, growth hormone, and thyroid hormones. This hypothesis implies that the SNS may have an anabolic, protein-sparing action in other catabolic states, such as fasting and diabetes (24), an inference that is supported by data from the present study showing that reduction in the rate of protein synthesis in soleus isolated from fasting rats is reversed in the presence of epinephrine (Fig. 5). The fact that epinephrine, but not norepinephrine (Fig. 5), increased protein synthesis can be explained by differences in the relative potencies of catecholamines in stimulating anabolic processes. Norepinephrine in vitro is less potent than epinephrine in stimulating amino acid uptake in rat skeletal muscle (24). Also, the antiproteolytic effect of epinephrine in isolated skeletal muscle is more marked than that of norepinephrine (22).

The finding that clenbuterol, a selective β₂-adrenergic agonist, induced an increase in muscle protein synthesis similar to that of epinephrine (Fig. 5) strongly suggests that the stimulatory effect of catecholamines is mediated by β₂-adrenergic mechanisms. Indeed, clenbuterol treatment for 1 day induces a rapid and transient increase in actomyosin synthesis by myotubes. The beta-agonist cimaterol directly enhances chronic protein accretion in skeletal muscle. Am J Physiol Endocrinol Metab 259: E822–E827, 1990.


Hesketh JE, Campbell GP, Lobley GE, Maltin CA, Acamovic F, and Palmer RM. Growth factor, growth hormone, and thyroid hormones. This may also counteract catabolic effects of other hormones during severe stress.

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GRANTS

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REFERENCES


