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Free intracellular and protein bound amino acids in tissues as affected by a mixed β-adrenergic agonist

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Abstract. The administration of metaproterenol induced an increase in gastrocnemius muscle weight without change in body growth rate or tissue protein concentrations, while epididymal fat was reduced. This effect was accompanied by an enhancement in the levels of intracellular amino acids in muscle. By contrast, liver amino acids were unaffected by treatment with the mixed β-adrenergic agonist.
Key words. Amino acids; β-adrenergic agonist; protein turnover.

The regulation of some physiological pathways controlling the availability of nutrients for specific purposes such as growth, protein deposition, etc.[1], is under neurohormonal and nutritional control[2,3]. For example, several different hormones control carbohydrate, lipid and protein turnover[4–7]. On the other hand, the stimulation of the autonomous sympathetic branch of the nervous system also induces changes in nutrient metabolism and thermogenesis[8,9]. The β-adrenergic agonists can mimic the properties of neurotransmitters with effects on cardiovascular, respiratory, gastrointestinal and metabolic functions, and also may act as hormones affecting circulatory and metabolic activities[10].
Recently, it has been reported that some β-adrenergic agonists have the ability to change body composition by increasing lean tissue deposition at the expense of fat content[11,12]. The in vivo responses to β-agonists have been attributed either to direct interaction with tissues mediated by cAMP or to overall changes in the levels of circulatory hormones and nutrient profile[13]. However, little is known about the molecular mechanisms of action at the cellular level[14], nor about the role of β-agonists in directing nutrients to support developmental changes during growth[1,2].
Protein turnover can be estimated by measuring body and tissue/organ weight gain[15], tissue protein and nucleic acid (RNA and DNA) content (as indicators of metabolic units activity)[16], the activity of enzymes involved in protein metabolism such as cathepsins or calpains[17,18], and the level of bound or free intracellular amino acids, as protein precursors[19].
The aim of the present study was to evaluate the influence of a mixed β-adrenergic agonist, metaproternol, which has potential for use in medicine or as a promoter of muscle growth in animal production, when administered subcutaneously. The effect on protein metabolism was assessed by the measurement of daily gain in body weight, food intake, tissue weight, and determination of muscle and liver protein content and of bound and free amino acids, which have been used in other studies as indices of protein metabolism[20,21].

Material and methods

Experimental animals and diet. Male Wistar rats weighing about 90 g were assigned to two groups of eight animals each and housed in individual cages in a temperature regulated room at about 22 °C. Animals had free access to a standard laboratory diet. Control and treated groups were injected s.c. twice daily (09.00 h and 17.00 h) with metaproterenol (1 mg/kg b.wt) or vehicle (saline) for 23 days.
Rats were killed by cervical dislocation. Gastrocnemius muscle and liver were carefully dissected, weighed and stored frozen at − 20 °C.
Measurement of tissue amino acids. Liver and gastrocnemius muscle amino acid content were assessed by HPLC as previously described[21–24] and their protein content by the Lowry method[25].
Statistical analysis. Data (mean ± SE) were statistically evaluated by the two-tailed Student's t-test, with the level of significance set at $p < 0.05$.

Results

Animal growth rate and food intake were similar in control and β-agonist-treated rats (table 1). However, gastrocnemius muscle weight showed a statistically significant increase ($p < 0.01$), while epididymal fat was reduced ($p < 0.05$). Liver weight remained unchanged and perirenal fat was only slightly decreased in the metaproterenol-treated group as compared with the control animals.
In liver, neither tissue protein content nor the total and intracellular liver amino acids were affected by the treat-

Table 1. Values of body weight, daily weight gain, food intake and organ (liver, gastrocnemius muscle, epididymal fat and perirenal fat) weight in control and β-agonist treated rats

Measurement	Treatments Vehicle	β-Agonist
Body weight (g)		
Initial	99.5 ± 2.3	101.4 ± 2.5
Final	276.7 ± 7.5	283.4 ± 6.1
Daily gain (g/d)	7.7 ± 0.3	7.9 ± 0.3
Food intake (g/d)	23.0 ± 0.5	23.5 ± 0.4
Organ weight (g)		
Gastrocnemius muscle	1.56 ± 0.03	1.73 ± 0.04**
Liver	14.19 ± 0.57	14.00 ± 0.53
Epididymal fat	1.20 ± 0.03	1.03 ± 0.07*
Perirenal fat	0.41 ± 0.08	0.37 ± 0.05

Value are the mean ± SE for 8 animals per group. *$p < 0.05$**$p < 0.01$ compared to the control.

ment (table 2, fig. 1 and fig. 3), although the levels of free glycine were markedly increased ($p < 0.05$).

Protein-bound amino acids in muscle were significantly increased ($p < 0.05$) by the metaproterenol treatment as were the total free muscle amino acids ($p < 0.05$). Free non-essential amino acids were more enhanced than were the essential amino acids (table 2).

Table 2. Values of protein and tissue (liver and gastrocnemius muscle) amino acids grouped according to different criteria in control and β-agonist-treated rats

Measurement	Treatments Vehicle	β-Agonist
Protein (mg/g)		
Liver	200.10 ± 4.16	201.10 ± 3.62
Gastrocnemius muscle	203.64 ± 2.69	204.89 ± 3.03
Free liver amino acids (μmol/g)		
Essential	7.44 ± 0.37	7.55 ± 0.47
Non essential	22.28 ± 0.95	22.40 ± 1.02
Free muscle amino acids (μmol/g)		
Essential	3.71 ± 0.39	4.27 ± 0.31
Non essential	14.75 ± 0.71	18.71 ± 0.98**
Total free amino acids (μmol/g)		
Liver	29.72 ± 1.23	29.95 ± 1.46
Gastrocnemius muscle	18.46 ± 1.04	22.44 ± 1.22*
Total protein amino acids (μmol/g)		
Liver	9342.7 ± 574.1	9420.1 ± 646.2
Gastrocnemius muscle	1579.8 ± 73.0	1804.7 ± 98.96*

Each value is the mean ± SE for 8 animals per group. *$p < 0.05$; **$p < 0.01$ compared to the control.

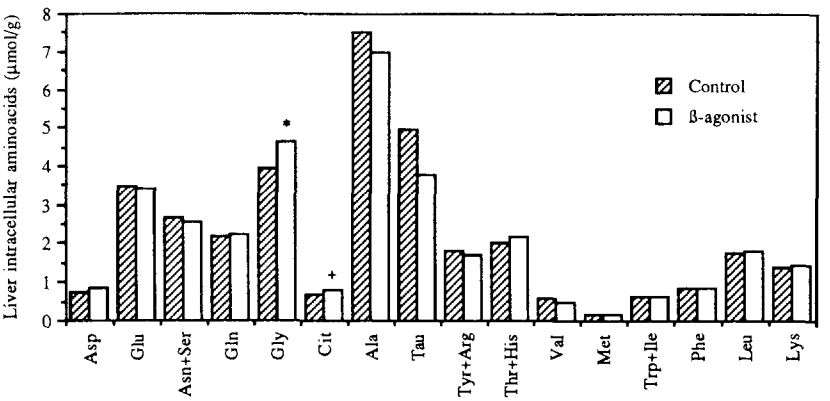

Figure 1. Mean individual intracellular amino acid levels in liver of control and β-agonist-treated rats (n = 8 for each group). +$p < 0.06$, *$p < 0.05$ compared to the control.

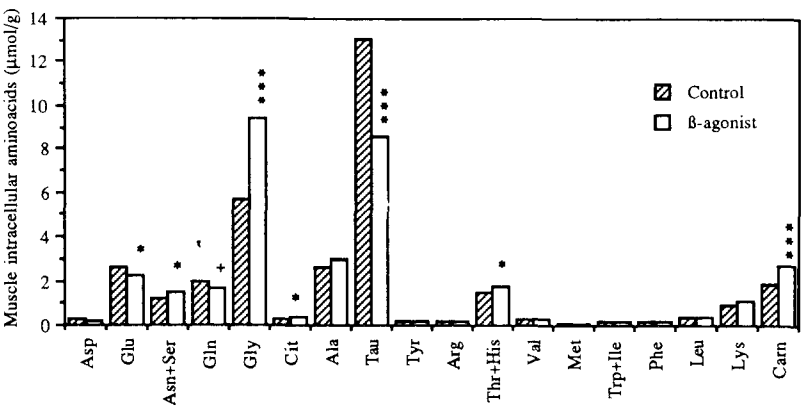

Figure 2. Mean individual intracellular amino acid levels in gastrocnemius muscle of control and β-agonist-treated rats (n = 8 for each group). '$p < 0.06$, *$p < 0.05$, ***$p < 0.001$ compared to the control.

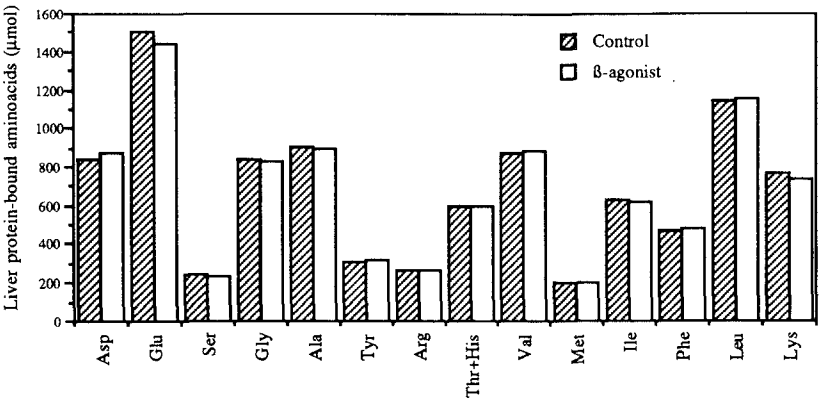

Figure 3. Mean individual protein bound amino acid levels in liver of control and β-agonist-treated rats (n = 8 for each group).

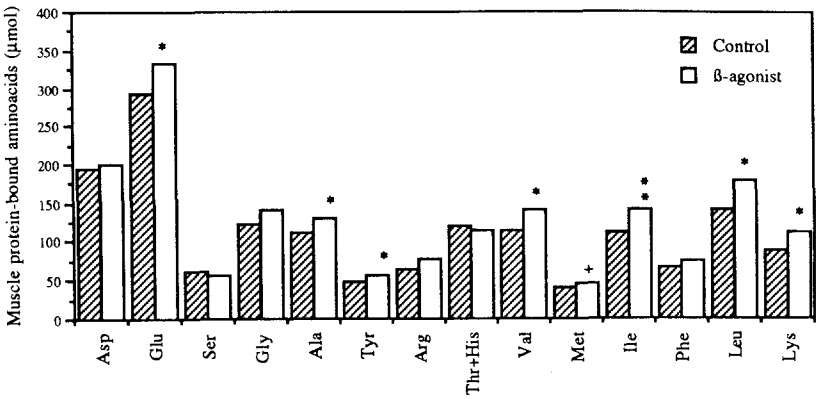

Figure 4. Mean individual protein bound amino acid levels in gastrocnemius muscle of control and β-agonist-treated rats (n = 8 for each group). $^{+}p < 0.06$, $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control.

By contrast, muscle intracellular amino acids were significantly increased, although the levels of glutamate and taurine amino acids decreased in the β-agonist-treated animals. The metaproterenol treatment increased some of the protein-bound muscle amino acids as compared with the placebo group (fig. 4).

Discussion

Dietary amino acids and their uptake or release from splachnic and peripheral tissues are the major factors affecting the level of plasma amino acids, which may, eventually, be involved in the regulation of protein turnover[5,20,26]. It has been reported that β-adrenergic agonists increase protein accretion at the expense of fat content[11]. This repartitioning effect has been attributed to changes in muscle protein degradation rather than changes in protein synthesis, while either a reduction in lipogenesis or an increase in lipolysis have been described[27], although other mechanisms of action such as changes in hormonal profiles or blood flux could not be ruled out[13].

Virtually all experiments conducted to data concerning the repartitioning effect by β-adrenergic compounds have been carried out with β-selective agonists (clenbuterol and cimaterol)[3], while the information on mixed β-agonists is rather scarce. This trial provides data concerning the influence of metaproterenol, a non-selective β-agonist, on the anabolic response and its mode of action. The growth-promoting activity of these compounds may have important applications in human health as antiobesity agents[9] or in muscle wasting diseases[28] and in animal production by increasing lean meat at the expense of fat[10].

The effects of sympathomimetic agents on growth are variable, depending on species, sex, age and dose or route of administration as well as the specificity for the receptor[29]. However, actions of β-adrenergic agonists on body composition have been shown to be rather uniform[11,13] with increases in muscle mass and reduction in fat. This experiment was performed in young rats because most studies concerning β-agonist treatment are carried out in growing animals, where anabolic actions on muscle would become more apparent[12,13]. The administration of metaproterenol induced no changes in body weight or growth rate. However, an anabolic response in muscle was observed, which was accompanied by a

decrease in fat content as indicated by the epididymal fat pad.

The changes and factors which govern the size of free amino acid pools are of interest to the study of the processes of protein synthesis and breakdown and the regulation of free amino acids[26]. Thus, the pool of free amino acids in tissues depends upon the input from food, protein turnover, biosynthesis, transamination, transport across cell membranes, and oxidation[19].

Total liver free amino acids remained unchanged after the administration of the β-adrenergic agonist. However, citrullline levels were markedly increased (20%, $p < 0.06$), which is in good agreement with lower urea levels[3], since the reaction between citrulline and aspartate limits urea synthesis[30].

On the other hand, total intracellular muscle amino acids were significantly enhanced by the experimental treatment when expressed per g of tissue, although the concentration of some individual amino acids involved in the urea cycle, such as glutamate, glutamine and taurine, were reduced. This situation could be associated with reduced muscle protein degradation as it has been found for leucine after the administration of the β_2-adrenergic clenbuterol[31].

The role of taurine remains unclear, although it is involved in the maintenance of intracellular levels of calcium[32], which, in turn, are associated with the regulation of lysosomal proteolysis and protein synthesis[33].

A reduction in muscle protein catabolism has been suggested by different reports using different indices such as the activity of cathepsins and calpains[18,34], amino acid incorporation into protein[35], or 3-methylhistidine excretion in urine[36]. A decrease in amino acid degradation could also occur as a consequence of the lipolysis associated with the β-adrenergic agonist administration, accompanied by the utilization of lipids as energy source instead of amino acids[37]. Additionally, a decreased glutamine production has been described after treatment with catecholamines[38,39].

The remaining amino acids could be increased either by a reduction in protein degradation, a decrease in the release to plasma, or an increase in the synthesis of non-essential amino acids[26]. The levels of glycine, which showed the most significant increase both in muscle and liver in response to sympathomimetic treatment, could be explained, at least in part, by the lower bone weights found in β-agonist-treated animals[40] and the fact that collagenous hydroxyproline is a precursor of this amino acid[30]. On the other hand, the β-adrenergic agonist treatment may affect specific pathways of gluconeogenesis, energy metabolism, biosynthetic processes or transport mechanisms for this amino acid[3,9,41]. It has been reported that clenbuterol increased net amino acid uptake in the muscle[42], which could be a sodium dependent process[43].

The increase in intracellular levels of amino acids could affect regulatory mechanisms which could then stimulate the protein synthetic machinery[44,46] or inhibit protein degradation[47].

No changes in muscle or liver protein nor in the level of amino acids incorporated into the liver or muscle protein were detected, although the absolute amount of amino acids was increased in muscle as a consequence of the growth promoting effect observed in this tissue.

Summing up, these results give further support to the hypothesis that β-adrenergic agonists have an anabolic effect by increasing muscle protein deposition, which, according to previous published evidence may be primarily due to changes in protein degradation as well as an increase in intracellular free muscle amino acids. These findings could not be interpreted without previous direct measurements of protein synthesis and degradation rates. No evidence has been obtained about any other possible mechanisms.

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