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Repartitioning Effect of a Mixed β-Agonist on Body Composition

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Subcutaneous administration of a mixed β-agonist to young rats induced no changes in animal growth and food conversion efficiency. However, a repartitioning effect was found with increases in lean tissue and decreases in body fat. The enhancement of muscle protein deposition was attributed to a fall in protein breakdown as muscular cathepsin A activity was lower in treated rats. A reduction of muscle reduction-oxidation state is associated to those changes in protein metabolism.

Key words: β-Agonist, Body composition, Muscle protein breakdown, Reduction-oxidation state.

The regulatory systems of animal homeostasis include neurotransmitters and hormones (31). The β-adrenergic agonists have both the properties of neurotransmitters of the sympathomimetic system with effects on cardiovascular, respiratory, gastrointestinal and metabolic functions (30) and also of hormones with circulatory and metabolic activities (13). Thus, the effects on carbohydrate and fat metabolism as well as on energy expenditure have been long recognized (17), but only recently a novel anabolic effect of some β-adrenergic agonists have been found. It has been reported that compounds with specificity for β-receptors may have a so-called repartitioning effect by increasing lean tissue and decreasing body fat (4, 16).

In this context, protein deposition in animal tissues results from a fine balance between the processes of synthesis and breakdown, depending upon dietary, hormonal and nervous factors (33), which, in turn, are mediated by intracellular messengers or signals as well as by changes in other biochemical or physiological processes (19).

The purpose of this study was focussed in the assessment of growth rate, body composition and measurements of several biochemical measurements as indicators of the possible mechanisms involved, includ-

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ing the muscle redox state in rats treated with the β-agonist metaproterenol.

Materials and Methods

Male Wistar rats weighing about 90 g were assigned into two groups of eight animals each. They were fed *ad libitum* and housed in a temperature regulated room at about 22 °C. Metaproterenol (1 mg/kg) or vehicle (saline) was s.c. administered twice a day (9 h a.m. and 5 h p.m.) for 23 days.

Organs were carefully dissected, weighed and stored as well as blood samples at −20 °C after cervical dislocation.

Body composition. — Determinations of protein, fat, water and ash were carried out by using standard analytical procedures (1).

Plasma measurements. — Plasma glucose levels were evaluated by using the glucose-oxidase colorimetric technique (8), fatty free acid levels according to the FAHLOT method (12) protein levels by using the LOWRY technique (18) and alanine-aminotransferase (ALAT) with a commercial Kit of Boehringer-Mannheim.

In order to measure plasma urea, 25 µl of plasma were mixed with 0.5 ml of 2 % diacetyl-monoxime and 5 ml of a reagent prepared with 44 ml of concentrate H_2SO_4, 66 ml of 85 % H_3PO_4, 100 ml of distilled water, 50 mg of thiosemicarbazide, 2 g of $CdSO_4$ 8 ml H_2O and 10 ml of urea solution (26 mg/l). After a period of incubation of 12 min at 100 °C, samples were placed into a cold water bath. The developed colour was read at 540 nm.

Indices of protein breakdown. — For cathepsin A (EC. 3.4.12.2) activity determinations, 1 g of the gastrocnemius muscle was homogenated with 1 ml of 0.1 mM EDTA and 4 ml of 0.25 M saccharose. After a centrifugation at 1,100 g for 10 min at 4 °C the pellet was discarded and the supernatant was collected (15).

Enzymatic activity was determined by using N-carboxibenzoxy-α-L-glutamil-L-tyrosine (N-CBZ-Glu-Tyr) as substrate with a colorimetric ninhydrin procedure (20). In this method, 0.1 ml of the supernatant was mixed with 0.2 ml of substrate solution and 0.7 ml of 0.2 M acetate buffer, pH = 5, with 0.2 % Triton X−100 and later incubated at 37 °C for 30 min. The reaction was stopped by the addition of 1 ml of 10 % trichloroacetic acid (TCA). The TCA mixtures were heated at 50-55 °C and centrifuged for 10 min at low speed. The supernatant solutions were used for the ninhydrin determination with the appropriate level as standard. Controls without substrate were treated in the same way. Activity was expressed in µmoles of released aminoacid after 30 min of incubation.

Another portion of the gastrocnemius muscle (1 g) was homogenized in 5 ml of 1 N $HClO_4$ and centrifuged at 5,000 g for 10 min, for lactate and piruvate measurements. The supernatans were analyzed espectrophotometrically as described elsewhere (5, 14).

Results

Administration of metaproterenol (1 mg/kg, s.c.) to young rats for 23 days induced no changes in animal growth as indicated by final body weights and daily weight gain. However, muscle weight was significantly increased and also a cardiac hypertrophy was found ($p < 0.01$). In contrast, adipose stores, represented by back fat and perirenal fat were decreased. Differences in liver weight had no statistical signification. These results were observed without changes in food intake and food conversion efficiency ratio (table I).

Body composition determinations showed an increase in carcass protein content ($p < 0.05$) and a decrease in carcass

Table I. *Final body weight, daily weight gain, food intake, food conversion efficiency ratio (FCE; g food/b.w.) and weight of some organs from control and metaproterenol (1 mg/kg, s.c.) administered rats for 23 days.*

Mean values ± SEM are given with the number of animals in parentheses. Student «t» test was used. NS: not significant; *p < 0.05; **p < 0.01.

	Control (8)	Treated (8)
Final body weight (g)	251.3 ± 13.29	253.1 ± 12.97 NS
Daily weight gain (g/day)	7.0 ± 0.54	7.1 ± 0.49 NS
Food intake (g)	530.5 ± 26.17	549.3 ± 10.58 NS
Food conversion efficiency (g/g)	3.42 ± 0.11	3.45 ± 0.09 NS
Gastrocnemius muscle weight (%)	0.49 ± 0.01	0.53 ± 0.01*
Liver weight (%)	4.04 ± 0.10	3.78 ± 0.14 NS
Heart weight (%)	0.34 ± 0.02	0.39 ± 0.006**
Back fat weight (%)	0.33 ± 0.01	0.28 ± 0.01*
Perirenal fat weight (%)	0.14 ± 0.01	0.10 ± 0.01*

Table II. *Plasma measurements from control and metaproterenol (1 mg/Kg, s.c.) administered rats for 23 days.*

*p < 0.001. Legend as in table I.

	Control (8)	Treated (8)
Glucose (mg/100 ml)	89.5 ± 3.83	84.6 ± 3.27 NS
Free fatty acids (μmol/ml)	0.42 ± 0.01	0.57 ± 0.03*
Proteins (g/100 ml)	7.8 ± 0.13	8.0 ± 0.14 NS
Urea (mg/100 ml)	0.36 ± 0.02	0.35 ± 0.02 NS
ALAT (Units/l)	42.5 ± 2.23	42.9 ± 2.34 NS

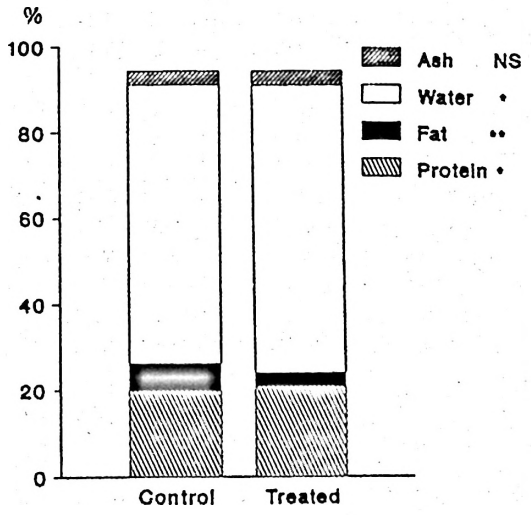

Fig. 1. *Body composition, expressed as a percentage of body weight from control and (1 mg/kg, s.c.) metaproterenol administered rats for 23 days.* Bars represent the mean values from groups of eight animals. Student «t» test was used. NS: not significant; *p < 0.05; **p < 0.01.

fat content. The higher water content in the treated rats was associated to the increased protein deposition while no changes in ash were found (fig. 1).

Some plasma measurements were made as indicators of the biochemical effects produced by the β-agonist. No variations were observed unless for free fatty acid levels, which were raised in the treated rats (table II).

Muscle cathepsin A activity, an index of

Table III. *Capthepsin A activity, lactate and piruvate levels and lactate/piruvate ratio from g astrocnemius muscle of control and metaproterenol (1 mg/Kg, s.c.) administered rats.* ***p < 0.001. Legend as in table I.

	Control (8)	Treated (8)
Cathepsin A activity (Units/g)	80.8 ± 1.50	70.3 ± 2.45***
Lactate (mmol/g)	0.027 ± 0.001	0.024 ± 0.001***
Piruvate (μmol/g)	0.33 ± 0.008	0.20 ± 0.011***
Lactate/piruvate ratio (mmol/μmol)	80.3 ± 4.08	119.5 ± 4.30***

proteolysis, was reduced after metaproterenol administration. This effect was associated to a statistically significant increase of the lactate/piruvate ratio, which means a more reduced muscle reduction-oxidation state (table III).

Discussion

The mixed β-adrenergic agonist metaproterenol had similar effects on the rats to those seen in other trials with productive animals or other sympathomimetic compounds (2, 24, 28). Thus, no changes in growth rate and food efficiency have been noted although some β_2-selective agonists, but not all, have been shown to promote growth and improve nutrient utilization (9).

The treatment with metaproterenol caused a marked increase on gastrocnemius muscle and cardiac weights, which has been previously noted with clenbuterol (25, 27), while no changes in liver weight were found (16).

The effects on carcass composition were evident, with a dramatic fall in lipid content and reduction in back and perirenal fat, which has been originally attributed to an increased lipolysis (16), although recent observations have suggested that an inhibition of lipogenesis also may occur (7). These findings are associated to increases in body protein composition and decreases in carcass fat content, which is characteristic of these repartitioning agents (2, 23, 30, 34).

Measurements of plasma glucose, urea, ALAT, protein and fatty acids were carried out in order to evaluate their possible involvement in the mechanism of action of these substances. Thus, changes in plasma free fatty acid levels should be ascribed to an action on lipomobilization either on lipogenesis or lipolysis as discussed earlier (5, 25).

Surprisingly, plasma glucose levels were similar in both groups, despite the known effects of these compounds on glucogenolysis (17). This observation could be explained by the fact that chronic treatments with sympathomimetic agents can modify the insulin response (3, 6) or show a phenomenom of tachyphylasis (21). The remaining plasma variables were unaltered, as previously reported with other β-adrenergic compounds (3).

The effects or β-agonists on lipid mobilization are reasonably well documented (13, 30) since compounds with β-adrenergic properties apparently act on β-receptors of the adipocyte through changes in cAMP intracellular levels (29), However, few studies have been reported concerning the effects on protein turnover. The possible involvement of endogenous hormones pattern (17), alterations in intracellular signals (19) and vascular changes (6) or direct effect on muscle has not been discarded (26).

Our measurements of muscle cathepsin A gave an indirect indication that a reduction in muscle proteolysis, al least partially, participates in the anabolic effect, which is in good agreement with other authors, following different methodological approaches (24, 34).

Additionally, changes in the reduction-oxidation state of skeletal muscle have been reported to correlate with alterations in the rate of protein degradation in this tissue (22, 32). Thus, the couple lactate/piruvate has been widely used under different situations as index of the reduction-oxidation state (with piruvate more oxidized) (10, 11). An explanation of the link between redox state and proteolysis has been proposed, in such a way, that the increased formation of glutathion-protein disulphide links would act as a signal of the initiation of proteolysis (10, 19).

It has been suggested (19) that since PGE_2 and $PGF_{2\alpha}$ form a redox couple, a possible role for prostaglandins could be played in the reduction of proteolysis. Our values of the lactate/piruvate ratio, with an increase of the reduced state are therefore in good agreement with the fall in muscle protein degradation, as assessed by the muscle cathepsin A activity.

It is concluded that the repartitioning effects of this mixed β-adrenergic agonist with increases in protein deposition and reductions in body fat are not accompanied by changes in growth rate and food conversion efficiency, while a decrease in muscle protein breakdown and an increase in reduction-oxidation couple (lactate/piruvate) were found.

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Resumen

La administración de un agonista β-adrenérgico no selectivo a ratas jóvenes no altera el crecimiento ni la ingesta de alimento. Sin embargo, se observa un efecto «repartidor», aumentando la masa muscular y disminuyendo los depósitos grasos. El aumento de tejido magro puede deberse a una disminución de la degradación proteica puesto que, en las ratas tratadas, se produce una disminución de la actividad del enzima catepsina A muscular. Estos cambios en el metabolismo proteico pueden estar relacionados con la aparición de un estado redox más reducido, representado por la disminución de la relación lactato/piruvato.

Palabras clave: β-Agonista, Composición corporal, Degradación proteica muscular, Estado redox.

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