MECHANISM OF HORMONE ACTIONS (III)

THE CHANGES IN THE CONCENTRATIONS OF FREE AMINO ACIDS IN RAT LIVER AND MUSCLE AND RATES OF AUTOLYSIS OF MUSCLE PROTEIN AFTER FASTING AND HORMONE TREATMENT

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1. Introduction

The cause of the profound effect of glucocorticoids on carbohydrate metabolism in intact and adrenalectomized animals has been extensively investigated in recent years [1–6]. These studies were primarily confined to the changes in the activities of certain liver enzymes that are involved in gluconeogenesis, synthesis of RNA, its separation into different fractions (species) and their activities in amino acid incorporating system [7–10]. Although it has often been suggested that amino acids are the major precursors of carbohydrate synthesis in hormone-treated fasting rats and that the action of triamcinolone can be simulated by a mixture of amino acids [6], very little attention has been paid as to the source of their origin and the mechanism of their formation.

Recently Sutherland et al. [11] demonstrated an increased release of amino acids from thymic tissue slices of cortisone-treated rats on incubation in borate buffer at pH 7.4. The catabolism of tissue proteins is generally very low at neutral pH, e.g., Koszalka and Miller [12] found maximum autolysis of muscle protein at pH 9.0 and very little at neutral pH. Neither did Sarkar and Dounce [13] find any degradation of hemoglobulin by calf thymus nuclei at neutral pH. This and the possibility that muscle tissue might be a better source than thymus tissue to supply amino acids for gluconeogenesis in hormone-treated rats, led to the present investigation. The changes in the concentrations of free amino acids in the liver and muscle have also been studied. The results are presented in this paper.

2. Materials and methods

For the present study, intact and adrenalectomized rats from 110 to 120 g and 120 to 130 g body weight were purchased from Canadian Breeding Company, St. Constance La Prairie, Quebec. The detailed information regarding the treatment with hormone can be obtained from previous papers [6,14].

A 10% muscle homogenate prepared from hind limbs by the method of Snoke and Neurath [15] was used for the protein catabolism study (autolysis) employing the method described by Koszalka and Miller [12].

The concentrations of the free amino acids in the liver and muscle were measured by the modified method of Moore, Spackman and Stein [16] in an automatic amino acid analyzer (Model VG200B – Phoenix Precision Instruments) from the extract obtained after the addition of an equal volume of 10% TCA to a 10% homogenate of liver or muscle, centrifugation and removal of TCA with ether.

3. Results

The rate of protein catabolism as measured by the increase in optical density at 280 mμ was found to be higher in the initial stages of incubation (first 4 hr) than that which was noted between 4 to 6 hr. An in-

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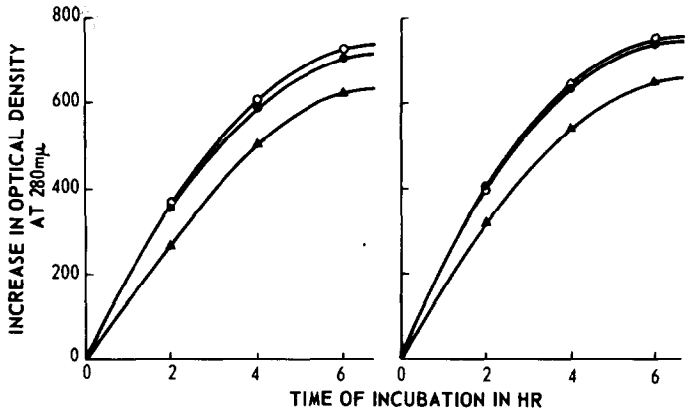

Figs. 1 and 2. Autolysis of muscle (endogenous) proteins obtained from fed, starving and hormone-treated starving intact and adrenalectomized rats, measured by determining the increase in optical density at 280 mμ of the perchloric acid extracts obtained by adding an equal volume of 0.6 N cold perchloric acid to muscle homogenates, after incubation for 2, 4, and 6 hr in glycine buffer pH 9.0 at 37°C. All values are the average of results from 4 animals. Fed (▲), starving (●) and hormone-treated starving (○). Fig. 1 for intact animals and fig. 2 for adrenalectomized animals. For experimental details see section 2 of the text.

crease in autolysis by 20 to 30% was observed in fasting rats as well as in hormone-treated fasting rats over the control values. A slightly higher initial rate of autolysis was noticed after adrenalectomy, if the results obtained from 2 hr incubation were compared. These results are shown in figs. 1 and 2.

Significant decreases in the concentrations of such free amino acids as alanine, serine (plus threonine), and aspartate in the decreasing order, were observed in hormone-treated fasting rat liver. Glutomate showed an increase of 100%. On the other hand, glycine, leucine, iso-leucine, lysine, proline and valine did not show any change. The levels of tyrosine and phenylalanine were too low for accurate quantitative determinations. A significant decrease in the concentration of alanine and a slight increase in glutamate concentration were found in fasting rat liver. The concentrations of other amino acids in rat liver did not show any significant change after fasting. The results are shown in table 1.

No significant differences in the concentrations of lysine, histidine, arginine, aspartate, glycine, valine, glutamate, and tyrosine in the pool, prepared from hind limbs of fed, fasting and hormone-treated fasting

Table 1

The effect of triamcinolone administration on the changes in the concentrations of free amino acids in the livers from fasting rats. The concentrations were determined in an automatic amino acid analyzer by the method of Moore, Spackman and Stein [16]. The amino acid concentrations are expressed as μmoles/g fresh weight of liver tissue, and the results are mean values (± S.E.). The number in brackets is the number of animals used to determine the mean. For experimental details see section 2 of the text.

Amino acid	Fed (4)	Starved (4)	Hormone-treated starved (6)
Lysine	0.52 ± 0.16	0.58 ± 0.20	0.66 ± 0.23
Histidine	0.98 ± 0.24	1.02 ± 0.28	1.08 ± 0.26
Glycine	2.04 ± 0.44	1.80 ± 0.36	1.92 ± 0.33
Aspartate	0.96 ± 0.22	1.04 ± 0.28	0.70 ± 0.18
Alanine	3.42 ± 0.54	1.66 ± 0.32	1.12 ± 0.27
Serine (plus threonine)	1.46 ± 0.38	1.12 ± 0.26	0.90 ± 0.23
Glutamate	2.34 ± 0.31	2.66 ± 0.29	4.03 ± 0.54
Valine	0.28 ± 0.12	0.27 ± 0.14	0.24 ± 0.18
Leucine	0.34 ± 0.18	0.36 ± 0.21	0.30 ± 0.16
Iso-leucine	0.16 ± 0.08	0.17 ± 0.06	0.14 ± 0.08

Table 2
The effect of triamcinolone administration on the changes in the concentrations of free amino acids in the muscle from fasting rats. For details see table 1.

Amino acids	Fed (6)	Starved (5)	Hormone-treated starved (5)
Lysine	0.56 ± 0.12	0.62 ± 0.14	0.58 ± 0.15
Histidine	2.08 ± 0.22	1.88 ± 0.20	1.96 ± 0.26
Arginine	0.26 ± 0.03	0.26 ± 0.04	0.28 ± 0.06
Glycine	2.84 ± 0.18	2.64 ± 0.24	2.88 ± 0.21
Aspartate	0.20 ± 0.04	0.18 ± 0.04	0.14 ± 0.05
Alanine	3.90 ± 0.31	2.84 ± 0.26	2.64 ± 0.28
Valine	0.24 ± 0.06	0.24 ± 0.04	0.30 ± 0.06
Glutamate	1.10 ± 0.18	1.20 ± 0.14	1.16 ± 0.20
Leucine	0.18 ± 0.06	0.30 ± 0.04	0.32 ± 0.04
Iso-leucine	0.10 ± 0.05	0.18 ± 0.06	0.22 ± 0.06
Methionine	0.06 ± 0.09	0.10 ± 0.05	0.12 ± 0.03
Tyrosine	0.16 ± 0.06	0.20 ± 0.05	0.18 ± 0.08
Phenylalanine	0.10 ± 0.08	0.14 ± 0.08	0.22 ± 0.06

rats was observed (table 2). However, a significant decrease in the concentration of alanine and a slight increase in leucine, isoleucine and phenylalanine were noticed.

4. Discussion

Because amino acids are the only major precursors of carbohydrate synthesis in hormone-treated fasting rats, a continuous flux of amino acids to liver cells is to be expected in order to ensure the high rate of gluconeogenesis in these animals, and they must arise from extrahepatic tissue protein catabolism. Yatvin and Wannemacher [17] found more free amino acids in the livers from corticoid-treated rats than in the livers from normal rats. Smith and Long [18] reported that the lowered plasma amino acid nitrogen in adrenalectomized rats can be restored to normal levels in animals with cortisol. Their work does not however indicate the source of the origin of these amino acids nor suggests how they are produced. The results presented in this paper demonstrate an increase of autolysis in muscle protein by 20 to 25% after fasting and after fasting and hormone treatment over the control. However, no difference in the rates of autolysis has been noticed between fasting and hormone-treated fasting rats. On the basis of this limitation, it is difficult to explain the observed high rate of gluconeogenesis in fasting rats after hormone treatment. The activities of the gluconeogenic enzymes are markedly increased and as a result, the amino acids in the liver pool are rapidly utilized for the synthesis of carbohydrate. To maintain an adequate supply of amino-acids for gluconeogenesis, a continuous flux of amino acids from muscle pool to liver cell is warranted, and this in turn induces endogenous protein catabolism in muscle. It is not certain how far the results obtained with experiments carried out with broken cells at unphysiological pH 9.0 can be extended to explain the rates of gluconeogenesis in intact animals. At any rate, the existence of a possible relationship between the rate of muscle autolysis and the extrahepatic release of amino acids in vivo must be assumed in order to ensure the high rate of gluconeogenesis in hormone-treated fasting rats. Further work is now in progress.

The results presented in this paper revealed a significant reduction in the concentrations of alanine, serine (plus threonine) and aspartate and an increase in glutamate concentration in the pool of livers of hormone-treated fasting rats. The concentrations of aspartate and glutamate did not show any appreciable change in rats after fasting, although that of serine (plus threonine) was reduced. Williamson [19] like Kirsten et al. [20], also reported lowered values for aspartate, alanine, serine (plus threonine) and glutamate in the livers of rats with alloxan diabetes. The results presented in this paper are in accord with those reported by other workers [19,20] except that an increase in glutamate concentration was found in the experiments described in this paper. Feigelson et al. [21] found an elevation in the concentrations of these amino acids in the livers of rats 4 hr after the administration of cortisone. The changes reported in this paper were observed 16 hr after the administration of hormone to fasting rats. These conflicting results can be explained if it is assumed that the rate of formation of amino acids by protein catabolism exceeded the rate of their utilization in the experiments described by Feigelson et al. [21], whereas the results presented in this paper were obtained under the conditions when the rates of the two processes are either equal or reversed. It is interesting to

note that blood glucose level was not significantly elevated (< 15%) in rats 4 hr after the administration of hormone, nor any significant increase in hepatic glycogen and protein contents was detected. On the other hand, blood glucose level was markedly elevated and the glycogen and protein contents of the liver were greatly increased 16 hr after hormone treatment [6]. Whether or not the increased concentrations of amino acids found by Feigelson et al. [21] were due to increased rate of catabolism of liver or extrahepatic tissue proteins cannot be said with any certainty, but it should be mentioned that all attempts to show an increased rate of autolysis of liver proteins at neutral pH failed (results not shown). The results presented in this paper indicate the importance of amino acids as the precursors of carbohydrate synthesis and suggest a possible source of their origin. The results presented in this paper also indicate that although the changes in the concentrations of free amino acids in the liver and muscle and their release from the latter tissue can be related to the effect of hormone on gluconeogenesis, it is not certain how they are produced.

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