Stimulation by Cortisol and Insulin of the 'Ureotelic' Arginase Activity of Chicken Liver

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The function of arginase in chicken liver is still unknown, and it is even doubtful whether it can hydrolyse endogenous arginine, since it has a high K_m for this amino acid (100mM) and a very low turnover number (2970mol/mol of enzyme per min at 37°C and pH9.5) (Grazi & Magri, 1972). This situation could be of advantage, since it avoids waste of arginine, which is an essential amino acid for the chicken (Albanese, 1959). However, in some strains of chickens starvation for 36–48h increases the activity of liver arginase 20–30-fold. The same effect is also obtained by feeding chickens with a diet rich in protein and lipid. In this case, however, the maximal response is displayed only after 3–4 days of treatment (Rossi & Grazi, 1969; Grazi *et al.*, 1969). In all the above conditions the increase of liver arginase activity is related to the appearance of a new arginase (arginase B) of lower molecular weight and with a lower K_m for arginine (10mM) than the pre-existing one (arginase A) (Rossi & Grazi, 1969; Grazi *et al.*, 1969). Arginase B also differs in its substrate specificity, since it hydrolyses only L-arginine, whereas arginase A hydrolyses both L-arginine and L-argininic acid.

Materials and methods

New Golden Leghorn chickens were purchased from the Stazione Sperimentale di Pollicultura, Rovigo, Italy. Cortisol (cortisol 21-hemisuccinate) was from Richter (Cinisello Balsamo, Italy). Insulin was from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Homogenization of livers, arginase assays and sucrose-density-gradient centrifugations were carried out as described by Grazi & Magri (1972). One unit of enzyme is defined as that amount which catalyses the formation of 1 μ mol of either ornithine or urea/h at 37°C and pH9.5.

Results

In the attempt to identify the factors involved in the regulation of liver arginase activity we have tested a number of metabolites known to accumulate under gluconeogenic conditions, and also a number of different hormones. We have now found that both insulin and cortisol promote a rapid increase in arginase activity. As shown in Fig. 1, the effect of insulin is maximal at 3h after administration, and

Fig. 1. Induction of arginase in chicken liver by cortisol and by insulin

The experiment was begun at 9:00 a.m. (zero time on the abscissa). The animals (weighing 260–300g), which had received their last meal at 5 p.m. of the day before, were injected either intraperitoneally with 8mg (as cortisol) of cortisol hemisuccinate/100g (a) or subcutaneously with 2.3 units of insulin/100g (b), or received no treatment. After 1, 2, 3, 6 and 7h from the injection three animals per group were killed and the livers were assayed for enzyme activity. Arginase activities, expressed as units/g of liver, represent the averages of three animals; vertical bars indicate S.E.M. Treated animals: arginase activity with arginine (●) and with argininic acid (▲) as substrate; untreated animals: arginase activity with arginine (○) and with argininic acid (△) as substrate.

Fig. 2. Sucrose-density-gradient-centrifugation analysis of the livers of untreated and of cortisol-treated chickens

Liver samples from untreated (a) and from cortisol-treated (b) animals to be analysed by sucrose-density-gradient centrifugation were homogenized, acetone-dried and extracted as described by Grazi & Magri (1972). The extracts were treated with $(\text{NH}_4)_2\text{SO}_4$ (70% saturation), and the protein precipitate was dissolved with 10mM-triethanolamine-HCl buffer, pH 7.5, containing 5mM- MnCl_2 (one-twelfth of the original volume). Then 0.15ml of the resulting solution was layered on a 5–20% (w/v) sucrose gradient. After 14h at 110 000g at 10°C the gradient was fractionated into 0.15ml fractions (meniscus at tube 29) and analysed for arginase activity with arginine (●) and with argininic acid (▲) as substrate. Analysis of the livers of animals treated with insulin showed the same pattern as that obtained with those treated with cortisol.

arginase activity is increased about 10-fold. A similar effect is displayed by cortisol, with a 12-fold increase in arginase activity at 6h after administration of the

hormone. The hydrolytic activity on argininic acid, which is peculiar to arginase A, is not affected by the hormonal treatments.

Analysis of the liver extracts by sucrose-density-gradient centrifugation confirmed the appearance of a new arginase activity after injection of the hormones. In the untreated animals the hydrolytic activities on arginine and on argininic acid are superimposed to form a single peak, whereas in the treated animals an additional, lighter, peak appears, which is characterized by its hydrolytic activity towards arginine only (Fig. 2).

The increase in arginase activity in the livers of the chickens treated either with cortisol or with insulin is therefore linked to the appearance of the new arginase B and not to a change in the activity of the pre-existing arginase A. Hormonal treatment therefore modulates only the activity of arginase B, an arginase that, in accordance with the proposal of Mora *et al.* (1965), can be classified among the ureotelic arginases on the basis of its K_m for arginine and of its sedimentation coefficient.

The effect of cortisol and insulin need not to be due directly to the hormones but could result from secondary metabolic effects. It is, however, likely that for chicken liver arginase the same situation holds as for rat liver tyrosine- α -oxoglutarate transaminidase and for tryptophan pyrrolase (Garren *et al.*, 1964; Holten & Kenney, 1967; Bradley-Hager & Kenney, 1968).

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- Albanese, A. A. (ed.) (1959) *Protein and Amino Acid Nutrition*, p. 152, Academic Press, New York
 Bradley-Hager, C. & Kenney, F. T. (1968) *J. Biol. Chem.* **243**, 3296
 Garren, L. D., Howell, R. R., Tomkins, G. H. & Crocco, R. M. (1964) *Proc. Nat. Acad. Sci. U.S.* **52**, 1121
 Grazi, E. & Magri, E. (1972) *Biochem. J.* **126**, 667
 Grazi, E., Rossi, N. & Sangiorgi, G. (1969) *Ital. J. Biochem.* **18**, 426
 Holten, D. & Kenney, F. T. (1967) *J. Biol. Chem.* **242**, 4372
 Mora, J., Tarreb, R., Martuscelli, J. & Soberón, G. (1965) *Biochem. J.* **96**, 588
 Rossi, N. & Grazi, E. (1969) *Eur. J. Biochem.* **7**, 348