EFFECT OF MALNUTRITION ON CORTISOL-BINDING PROTEIN IN THE LENS OF CYNOMOLGUS MONKEYS

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Summary A cortisol-binding protein exists in the lens of cynomolgus monkeys as in other species. The cortisol-binding capacity decreased markedly with a diet either moderately or severely depleted of protein, even if the diet was later changed to a normal diet during infancy. This suggests that malnutrition may cause insufficient synthesis of the cortisol-binding protein in the lens and that malnutrition is one of the important factors controlling the inactivation of steroid hormone in the lens.

We have demonstrated that cortisol binding by lens protein is carried out fairly actively leading to inactivation of steroid hormone in the lens (1). The factors influencing the synthesis of cortisol-binding protein in the lens have been reported (2, 3). However, it is still not clear how nutritional insufficiency during early infancy affects the inactivation of cortisol in the lens of animals.

We are well aware of the report by CRAVITO et al. (4) indicating that malnutrition during infancy may sometimes cause irreversible and permanent retardation of physical and behavioral development and that the younger the infant, the more severe the retardation. Therefore, in order to better understand the role of nutrition on the inactivation of steroid in the lens, we investigated the effects of malnutrition during infancy on the synthesis of cortisol-binding protein in the lens of cynomolgus monkeys.

This study was designed to determined the effect of protein deficiency on the cortisol-binding capacity in the lens of cynomolgus monkeys immediately after birth.

MATERIALS AND METHODS

Animals used in the present investigation were male cynomolgus monkeys

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(Macaca fascicularis) bred in the laboratory of the National Institute of Health in Tokyo. All infant monkeys were transferred within a week after birth to an individual incubator cage designed for immature new born infants. The temperature in the incubator was fixed at 25°C and the relative humidity at 50 to 60 %. The experimental animals were divided into 5 groups of 2 monkeys each, A, B, C, D and E. Group A (control) was fed on its mother's milk for 2 months. Group B was fed on its mother's milk for 2 weeks followed by a change to a low-protein diet consisting of 194 cal, 5.0 to 5.5 g of protein and 330 ml of water per kg body weight. The frequency of suckling was 4 times a day. These animals showing a body weight three-fifths of the control infant monkey were extremely underdeveloped. Group C was fed on its mother's milk for 2 weeks followed by a change to a very low-protein diet consisting of 250 cal, 3 g of protein and 420 ml of water per kg body weight. The frequency of suckling was 4 times a day. The daily increase of body weight for the infant monkey fed on a low-protein diet was 1.2 g at 4 weeks of age, 6 g at 6 weeks of age, 1 g at 8 weeks of age, with a doubling of the birth body weight at about 21 weeks of age. Group D was fed on its mother's milk for 2 weeks prior to changing the diet to an artificially modified milk. The suckling was 6 times a day for the first 1 month after birth, 5 times for the next month then 4 times, 3 times and 2 times in proportion to the increased intake of the weaning diet. Group E was fed on artificially modified milk only. The frequency of suckling was the same as in group D. After the monkeys in all groups finished their weaning period of about 100 days, they were fed on apples, bananas, strawberries, mandarin oranges, sweet potatoes and an Oriental solid food of a biscuit type in place of milk. They took more or less 200 to 400 cal, 6-7 g of protein and 400 ml of water per kg. After weaning, they were fed on a variety of food, consisting chiefly of biscuits. Groups A, B and C were sacrificed by exsanguination under anesthesia with sodium pentobarbital 8 months after birth. Groups D and E were sacrificed at 24 and 30 months of age, respectively. It is rather difficult to get milk from the cynomolgus monkey but we have made an effort to collect and chemically analyze cynomolgus monkey milk. The modified milk used to feed the cynomolgus monkeys is a commercial product “p7a” used for human infants. The composition of “p7a” is as follows: cow’s milk (63.0 %), saccharose (8.0 %), modified fat (15.0 %), lactose (10.0 %) and soluble polysaccharide (4.0 %). It represents 500 cal per 100 g and contains 13.3 % of protein, 59.2 % of carbohydrate, 23.3 % of fat and 2.2 % of minerals as well as 200 I.U. of vitamin A, 0.25 mg of vitamin B₁ (hydrochloride), 0.2 mg of vitamin B₁₂-phosphate ester, 1.4 mg of vitamin B₂-phosphate ester, 0.2 mg of vitamin B₆, 2.0 μg of vitamin B₁₃, 400 mg of nicotinamide and 0.3 mg of folic acid. The mineral additives include 330 mg of calcium, 270 mg of phosphorus, 450 mg of potassium, 160 mg of sodium and 6.0 mg of iron.

Determination of cortisol-binding capacity in the lens of cynomolgus monkeys. Lenses from excised eyes of the male cynomolgus monkeys were carefully
removed by a posterior approach and homogenized in phosphate buffered (pH 7.4) Krebs-Ringer solution to give a final concentration of 20 mg wet weight per ml. Two ml of this homogenate were then incubated, for 60 min at 37°C, with 0.04 ml of cortisol-4-14C solution containing 0.02 μCi radioactivity and 0.125 μg of cortisol. Following incubation the solution was cooled to 4°C and centrifuged at 12,000×g for 60 min to remove insoluble protein and particulate matter. The cortisol-binding activity of the lens protein was assayed using a Sephadex G-50 column as reported previously (1). Cortisol-4-14C, with a specific activity of 57.0 mCi/m mole, was purchased from Radio Chemical Centre, England. Protein concentration of the lens was determined according to LOWRY et al. (5) with egg albumin was used as a standard.

RESULTS

Cortisol-binding protein exists in the lens of cynomolgus monkey and its ability to bind cortisol to the monkey lens protein was similar to that of other animals. The cortisol-binding capacity was reduced remarkably by feeding on a moderately low protein diet during the weaning period (Table 1). Moreover the loss of protein binding capacity was further promoted by a severely low protein diet. No significant differences were found in protein concentrations in the lens of cynomolgus monkeys fed on a either severely or moderately low protein diet.

Table 1. Effect of nutrition on the cortisol-binding capacity in the lens of cynomolgus monkeys.

| Group | Cortisol-binding capacity (μg) | P value |
|-------|-------------------------------|---------|
| A     | 2.12±0.16 d                   | —       |
| B     | 1.81±0.16                     | <0.05   |
| C     | 1.34±0.13                     | <0.01   |
| D     | 2.38±0.28                     | <0.05   |
| E     | 2.65±0.22                     | <0.01   |

a Each group was fed on the following conditions: A, mother’s milk for 2 months; B, mother’s milk for 2 weeks followed by a change to a moderate low protein diet; C, mother’s milk for 2 weeks followed by a change to a very low protein diet; D, mother’s milk for 2 weeks prior to changing diet to an artificially modified milk for 24 months; E, mother’s milk for 2 weeks prior to changing diet to an artificially modified milk for 30 months.
b The cortisol-binding capacity in the lens of cynomolgus monkeys was determined using gel filtration technique as reported previously. Each group consisted of 4 lenses.
c Determined by t-test.
d Average of 4 lenses with standard deviation.

DISCUSSION

We have demonstrated that the binding of cortisol to lens protein in rats is
one of the inactivation mechanisms for steroid hormone (1). However, there is no report on the presence of cortisol-binding protein in the lens of cynomolgus monkeys. In the present study, the authors found that a cortisol-binding protein exists in the lens of cynomolgus monkeys as it does in other species such as rat, pig, bovine and human lenses.

The most interesting finding was that, compared to the control cynomolgus monkeys, the cortisol-binding capacity was reduced by feeding on a either severely or moderately low-protein diet during the weaning period. These findings suggest that a low-protein diet in the weaning period may lead to a defective synthesis of cortisol-binding protein in the lens.

Protein deficiency due to feeding on a low-protein diet is frequently accompanied by symptoms of a deficiency of one or more of the other nutrients, including many of the minerals and vitamins as is seen in KWASHIORKOR (6). The primary cause appears to be a general failure of protein synthesis, so that the mechanisms for absorption, transport and utilization of protein are defective (6, 7).

KERR et al. reported that it is possible to produce any degree of malnutrition in a quantitative manner by using dilutions of milk diet or soybean protein in a subhuman primate model using Macaca mulatta and there are close correlations between physical growth or biochemical changes in these monkey’s tissues and quality of protein in the diet (8). ORDY et al. reported that infant rhesus monkeys fed on a low-protein diets showed lower physical growth levels than the control monkeys (9). KUMAR et al. reported that there were significant decrease in amino acid content in the serum of malnutritional young pig tail monkeys (10).

On the other hand, there are many investigations, experimentally and clinically, on the relationship between malnutrition and eye tissues, especially on the formation of cataracts in the lens due to reduction of protein synthesis (11). It has been demonstrated that changes in lens of young rats occur by feeding them several different amino acid depleted diets, but cataracts caused by the lack of tryptophan is the best authenticated (12). SCHAFFER and MURRAY suggested that this type of cataract was a manifestation of protein deficiency (13). MC LAREN demonstrated that various changes in the eye of the rat and pig occurred if they had been fed a protein-deficient diet (14). Deficiency of riboflavin, folic acid and vitamin E sometimes cause opacity in the lens of experimental animals (14–16). We have demonstrated that feeding on an excess glucose diet for a long period of time depresses the incorporation of ^14C-leucine into the protein-fraction of rat lens (17).

Recently considerable attention has been drawn to the occurrence of steroid cataracts as one of the side effects of oral administration of steroid hormone for a long period of time (18). The most striking effect of low protein diet on the cortisol-binding capacity was to repress it when the cynomolgus monkey was fed a low-protein diet during its weaning period even if the diet was later changed back to a normal diet. These results indicate that malnutrition is one of the important
factors in reducing the inactivation of steroid hormone in the lens and that malnutrition during infancy may bring about not only retardation of the physiological development as pointed out by CRAVIOTO et al., but also repress the synthesis of binding protein in the lens (4).

It should be noted that the liver is the main metabolic pool for amino acids and protein. Therefore, when the animal is fed on a low-protein diet, it may lead to insufficiency in the turnover of liver protein. Furthermore, the liver is the organ primarily responsible for the catabolism of the steroid hormone (19). It may be thought that changes in the liver function could either directly or indirectly influence the steroid hormone metabolism in the lens as reported previously (20), and finally it would bring about a reduction in the synthesis of cortisol-binding protein in the lens.

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