Inverse Correlation between the Nitrogen Balance and Induction of Rat Liver Serine Dehydratase (SDH) by Dietary Protein

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Rats of different ages (3 to 15 wk-old) were fed on a 25% casein diet for one week, and the nitrogen balance and liver serine dehydratase (SDH, EC 4.2.1.13) activity were then determined. The value for nitrogen balance decreased with the age of the rats, while the liver SDH activity increased. A statistical analysis showed clear inverse correlation between the two factors (R² = 0.7372, p < 0.01). This result suggests that SDH was induced by response to the amount of surplus amino acids from dietary protein taken beyond the body’s requirement. The increase in SDH activity was accompanied by an increase in the level of SDH mRNA. Since the half-life of this mRNA did not change significantly, the induction was mainly controlled at the level of transcription. In addition, the induction seems not to be related to gluconeogenesis, since the mRNA levels of tyrosine aminotransferase (TAT, EC 2.6.1.5) and phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32), other gluconeogenic enzymes, were not changed under these experimental conditions.

Key words: serine dehydratase; dietary induction; protein requirement; nitrogen balance; rat

Serine dehydratase (SDH, EC 4.2.1.13), the enzyme localized specifically in the liver and kidney, catalyzes the deamination of serine and threonine (thus, it is also termed threonine dehydratase, EC 4.2.1.16). It has long been known as a gluconeogenic enzyme, since its activity is dramatically induced by starvation, diabetes mellitus and feeding of a high-protein diet.1-3 The induction mechanism has been extensively studied on a molecular basis in vivo and in vitro, and it has been found that glucagon, glucocorticoids and insulin mainly regulated the gene expression at the level of transcription; i.e., glucagon and glucocorticoids stimulated the transcription while insulin counteracted it.2-4 The hormonal regulation of SDH induction is very similar to that of other gluconeogenic enzymes such as tyrosine aminotransferase (TAT, EC 2.6.1.5) and phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32).5,6,7 However, we recently found in mature rats that the induction of liver SDH began with a casein diet as low as 9% by which the rat can maintain nitrogen balance.8 This induction was accompanied by an increase in the mRNA. As far as we know, there are no other reports of SDH being induced by such a low-protein, high-carbohydrate diet. This suggests that hormones such as glucagon and glucocorticoids might not be involved in the induction under this dietary condition. On the other hand, SDH induction in young growing rats began when they were fed a casein diet above 25% by which maximum growth was obtained.8,9 We hypothesize from these results that SDH might be induced for both gluconeogenesis and for catabolizing surplus amino acids to maintain nitrogen homeostasis in the body and that a factor(s) other than hormones may be involved in this induction.

If the primary role of SDH is to catabolize surplus amino acids, the amount of dietary protein necessary for the induction would vary during the development of an animal, since the protein requirement largely depends on the growth rate of the body. To examine this hypothesis, we fed the same 25% casein diet to rats of different ages to determine the relationship between SDH induction and nitrogen balance. In addition, we compare the induction of TAT and PEPCK with that of SDH in these animals and in starved rats to examine the relationship between SDH induction and gluconeogenesis and the mechanism for SDH induction by protein nutrition.

Experimental Procedures

Animal care. Male Sprague-Dawley rats, aged from 3

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Abbreviations: SDH, serine dehydratase (EC 4.2.1.13); TAT, tyrosine aminotransferase (EC 2.6.1.5); PEPCK, phosphoenolpyruvate carboxykinase (EC 4.1.1.32); PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; N-balance, nitrogen balance
to 15 weeks, were purchased from Nihon SLC (Hamamatsu, Japan). They were housed individually in metabolic cages in an air-conditioned room at 25 °C with a half-day light/dark cycle.

All animals were maintained on a 25% casein diet for 7 days. The composition of the diet (in w/w) was 25% casein (Oriental Yeast Co., Tokyo), 5% soybean oil (Fuji Oil Co., Osaka), 5% cellulose powder (Oriental Yeast Co., Tokyo), 1% vitamin mix (Oriental composition; Oriental Yeast Co., Tokyo), 5% mineral mix (Oriental composition; Oriental Yeast Co., Tokyo) and 59% cornstarch (Kansai Denpun Co., Osaka). The compositions of the vitamin mix and mineral mix have been described elsewhere.10) Food and water were available ad libitum. The food intake and body weight were measured every day. Urine and feces were collected on the last two days of the experiment to determine the nitrogen balance (N-balance). The animals were anesthetized by intraperitoneal administration of sodium pentobarbital (50 mg/kg of body weight) and were killed by cardiac puncture after blood had been withdrawn from the inferior vena cava. The liver was excised, frozen in liquid nitrogen and kept at −80 °C until needed. The experiment was conducted twice. In the first experiment, we used 4, 6, 8, 10 and 15-wk-old rats, and in the 2nd, 3, 4, 5, 6, 8, 10 and 12-wk-old animals. Three rats were used in each age group.

To induce SDH by starvation, twenty 5-wk-old rats were fed on the 25% casein diet for 3 days, and then food was withdrawn. Starvation was continued for up to 4 days, and 4 rats were killed each day as already described. Water was available ad libitum. This experimental method was approved by the Animal Experiment Committee of Kyoto Prefectural University. All the rats were managed in line with the Guidelines for Care and Use of Laboratory Animals.

**Determination of the half-life of mRNA.** Actinomycin D (Sigma-Aldrich Fine Chemicals, MO, U.S.A) in a 5% EtOH-PBS solution (2.5 mg/ml) was injected into the abdominal cavity (2.5 mg/kg BW), and then the rats were killed at the indicated times. As a control, the rats received only the vehicle. The level of mRNA in the liver was quantified by the RT-PCR method described later. The values obtained are expressed as percentages and were plotted on a logarithmic scale against the time after the injection, the half-life then being calculated from the regression curve. Three rats were used for each time point.

**N-balance.** The nitrogen contents in the diet, urine and feces were determined by the micro-Kjeldahl method. The apparent N-balance was calculated by subtracting the nitrogen contents in the urine and feces from that in the diet consumed.8)

**Enzyme assay.** Preparation of the liver extract and the assay for SDH activity were performed as previously described.11) One unit of SDH activity is defined as the formation of 1 μmol of pyruvate per minute at 37 °C. The protein content was determined by the Lowry method, with bovine serum albumin used as a standard.

**Measurement of mRNA.** The expression of SDH, TAT, PEPC and β-actin mRNA in the liver was determined by the RT-PCR method as previously described.8) The primer pair sequences used were as follows: SDH, 5'-CTCTGTTGAAAGGAGCTGAAGG-3', 5'-CCACCAAGATCTTCTCAGTCG-3'; TAT, 5'-TACACTCCTGTCAGCTAAGATGGG-3', 5'-CGAGAAGTCC-TTGGAAATTGACC-3'; PEPC, 5'-CTTGGGAATGAGCCTGTGGACG-3', 5'-AGGTATATTTCCA-TCTGGCAGCGG-3'; and β-actin, 5'-CTACAATGAGCTGCGTGTTGG-3', 5'-ATGGCTACGTACATGGCCTGGG-3'. The annealing temperature was 55.0 °C for SDH and β-actin, 61.5 °C for TAT and 64.0 °C for PEPC. PCR was performed for between 24 and 27 cycles depending on the signal intensity, and the linearity of the signal was confirmed to compare the result obtained from 2 to 3 different cycles of PCR.

**Statistical analysis.** Each result is expressed as the mean ± SD, and significant difference among groups was analyzed by one-way ANOVA. If the difference was significant, the values were compared by Sheffe’s test for the growth rate, food intake, N-balance value and SDH activity, or by the Tukey-HSD test for the levels of SDH, TAT and PEPC mRNA. The correlation between the SDH activity and N-balance value, and the half-lives of SDH, TAT and PEPC mRNA were determined by a regression analysis. A difference is considered significant at p < 0.05. The analysis was performed by using SPSS 6.1J for the Macintosh computers (SPSS Japan, Tokyo).

**Results**

The groups of rats aged between 3 and 15 weeks were maintained on a 25% casein diet for 1 week in a metabolic cage. They had free access to the diet and water. Their growth curve was almost linear, and the daily food intake per 100 g of body weight did not change significantly during the experimental period in any individual group (data not shown). To show the relationship between the growth rate and food intake, the mean values of the daily weight gain and daily food intake in the respective group is indicated in Table 1. The growth rate was calculated to divide the difference of body weight in two successive days by the body weight of the first day and expressed in a percentage. The growth rate at 3 wk old was 11.1 ± 0.8% and gradually decreased week by week to 0.6 ± 0.2% at 15 wk old. Although the daily food intake per rat at 15 wk old was 3 times that at 3 wk old, it was less than one-half if the food intake is compared on the basis of per 100 g of body weight. The growth rate and food...
intake decreased almost in parallel until 6 wk old, after which the food intake remained virtually unchanged while the growth rate seemingly continued to reduce. This may suggest that the rats had begun to accumulate body fat. Table 1 also shows that 5–6 g of the diet per day per 100 g of body weight might be enough to maintain the body composition after growth.

Figure 1a shows the apparent nitrogen balance (N-balance) of the rats at different ages. The N-balance value decreased with the age of the rats, the change in N-balance value being roughly parallel with the change in growth rate (Table 1). In contrast, the liver SDH activity markedly increased with the age of the rats (Fig. 1b). Thus, the activity in a rat of 16 wk old was about 8–9 times higher than that when 4 wk old. When the SDH activity of the respective rat was plotted against the N-balance value being roughly parallel with the change in growth rate, an inverse correlation was apparent (R² = 0.7448, P < 0.01) between the two factors (Fig. 1C).

Figure 2a shows the relative abundance of SDH, PEPCK and TAT mRNA in the liver of rats at different ages that had been maintained on the 25% casein diet for 1 week. The body weight and food intake of each rat were measured daily, and the growth rate and daily food intake per 100 g of BW were calculated as described in the text. Data were taken from two different experiments.

The decay rate of SDH mRNA in the starved animals (5-wk-old) is shown in Fig. 3a. The result demonstrates that the mRNA half-life of 13 wk-old-rats (t½ = 7.2 h) was a little longer than that of 5-wk-old rats (t½ = 5.0 h). These values are within the range reported in the previous papers (t½ = 5–10 h). Without actinomycin D, the levels of mRNA in both groups did not change markedly during the experimental period of 8 hours. This indicates that the synthesis and degradation rates of the mRNA were in equilibrium.

The decay rate of SDH mRNA in the starved animals (5-wk-old) is shown in Fig. 3b. The mRNA half-life of the starved rats was 3.2 h, being shorter than that of the fed animals, although the starvation increased the level of SDH mRNA by several fold (Fig. 2b). Table 2 compares the half-lives of SDH, TAT and PEPCK mRNA in the liver of rats at different ages under the fed and starved conditions. The starved rats seemed to have a shorter mRNA half-life than that of the fed animals.

**Discussion**

SDH as well as TAT and PEPCK have been recognized as gluconeogenic enzymes and were therefore induced under similar physiological conditions such as starvation, diabetes mellitus and feeding of a high-protein diet. Many studies have demonstrated that the induction of these enzymes was controlled mainly at the level of transcription by glucagon, glucocorticoids and insulin. However, we have demonstrated here that liver SDH, but not TAT and PEPCK, was specifically induced by dietary protein in response to the change in N-balance value during the development of the rat.

It seems likely that both the SDH activity and N-balance change in an age-related manner (Figs. 1a and 3a).
b). It is obvious that the decrease in N-balance value was due to the decrease in growth rate during the course of development of the animals (Table 1). When the SDH activity is plotted against the N-balance value, there is a clear inverse correlation between the two factors (Fig. 1c). We preliminarily determined the N-balance of young (4-wk-old) and mature (6-mo-old) rats that had been given different amounts of casein as a protein source. The result demonstrated that 20–25% (w/w) of casein was required to obtain the maximal growth of a young rat, while as low as 5% casein was enough to maintain the N-balance of a mature rat. SDH induction was invoked when they took protein above their requirement, i.e., SDH activity in the young rat could not be induced if the casein content was 25% or less, while induction in the mature rat could be observed from the 9% casein diet.8) The extent of induction by both ages of rat depended on the dietary protein content above their individual requirements. In the present experiment, all rats of different age groups were given the same 25% casein diet and, therefore, they would take excess protein relative to their requirements as the growth rate decreased. The inverse correlation observed between SDH and N-balance indicates that the age-related increase in SDH activity was induced by an increased amount of surplus amino acid due to the decreasing protein requirement of the rat during development. The induction of SDH activity was accompanied by an increase in the amount of SDH mRNA (Fig. 2a).

RNA was extracted from the liver. The amount of mRNA was determined by RT-PCR, and the expression of respective mRNA was normalized with that of β-actin mRNA as described in the “Experimental procedures” section. Each result is expressed as the mean ± SD, and bars not sharing the same letter were significantly different at p < 0.05. (a) Rats at different weeks of age were maintained on the 25% casein diet for 1 week. Age indicated in the figure was at the end of the experiment (n = 3). (b) Rats at 5 wk old were fed on the 25% casein diet for 3 days and then starved for the indicated numbers of days (n = 4).
exclude the possibility that stabilization of the mRNA was involved in the induction (Fig. 3a and Table 2). However, the difference seems too small to explain the more than 8-fold increase in the level of the mRNA. Thus, transcriptional stimulation of the SDH gene mainly contributed to the induction. As a gluconeogenic enzyme, SDH as well as TAT and PEPCK gene transcription are stimulated under similar physiological conditions by glucagon and glucocorticoids as already mentioned. In fact, we confirmed that the mRNA levels of these three enzymes increased in parallel when the rats were starved (Fig. 2b). However, only SDH mRNA, and not TAT and PEPCK mRNA, showed “age-related” or “protein requirement-dependent” induction (Fig. 2a).

If glucagon and/or glucocorticoids were involved in this SDH gene expression, the levels of TAT and PEPCK mRNA might have increased simultaneously, as was the case with the starved rats. Table 2 also shows that the half-lives of these three types of mRNA were not related to mRNA levels, and therefore transcriptional stimulation rather than post-transcriptional stabilization would have regulated the expression of these mRNAs. Taking these results together, we suggest that the “protein requirement-dependent” induction of SDH was mainly controlled at the transcriptional level, and that hormones such as glucagon and glucocorticoids were not involved in this induction.

Pitot et al. have demonstrated that intubation of a casein hydrolysate, amino acid mixture or single amino acid such as tryptophan to the rat induced liver SDH activity.18) On the other hand, Nakagawa et al. have also observed SDH induction in a rat fed on a synthetic diet containing l-serine as the sole source of dispensable amino nitrogen.19) These types of induction resulted from the de novo synthesis of enzyme protein and inhibitors of RNA synthesis such as puromycin blocking the induction. Ogawa et al. have examined a series of DNase I-hyper sensitive sites in the 5'-flanking region of the SDH gene and found specific sites in which DNase I hypersensitivity altered in response to a high-protein diet. Consensus sequences responsible for hormones such as glucagon, glucocorticoids and insulin are not present in these sites.12,20,21) Although the mechanism(s) is not known at this moment, it is considered that a specific amino acid such as serine or tryptophan induces the gene expression of SDH when its amount exceeds the requirement.

As far as we know, there has been no report of a gene changing its expression in response to protein requirement. The physiological significance of this induction is not clear at this moment. Interestingly, however, our preliminary results indicate that the plasma concentration of Ser was no different between 5-wk- and 13-wk-old rats (218 ± 58 and 213 ± 49 μmol/l, n = 3, respectively), while the Thr concentration was significantly lower (n = 3, p < 0.05 by Student’s t-test) in 13-wk-old rats (255 ± 39 μmol/l) than in 5-wk-old animals (406 ± 49 μmol/l). This result suggests that SDH (or threonine dehydratase) was physiologically active. Since

![Fig. 3. Determination of the Half-life of SDH mRNA.](image)

- (a) Twelve rats at 4 wk old or 12 wk old were maintained on the 25% casein diet for 1 week. When the rats had reached at 5 wk (● or ○) or 13 wk (▲ or △) old, 9 rats from the respective group were injected with actinomycin D (● or ▲) and the remaining three were injected with vehicle only (○ or △) and killed at the indicated time (n = 3). The amount of liver SDH mRNA was determined as described in the “Experimental procedures” section. (b) Twelve rats at 4 wk old were maintained on the 25% casein diet for 4 days, and then starved for 3 days. Nine rats were injected with actinomycin D (●) and the remaining three were injected with the vehicle only (○), before being killed at the indicated time (n = 3).

![Table 2. Comparison of the Half-life of Liver SDH, TAT and PEPCK mRNA](table)

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*Table 2. Comparison of the Half-life of Liver SDH, TAT and PEPCK mRNA*

1Data were taken from Fig. 3.
2Samples and methods used for determining the half-life of TAT and PEPCK mRNA were the same as those described in Fig. 3.
Thr is an indispensable amino acid, the plasma concentration largely depends on the degradation rate, and therefore, the increased activity of SDH may cause the decreased level of plasma Thr. On the other hand, Ser is a dispensable amino acid, and the plasma concentration could thus be maintained despite the increase in SDH activity, if the synthesis rate of Ser increased concomitantly. In the catabolism of amino acids, the amino group of most amino acids is once fixed to Gln or Glu by transamination, then ammonia is liberated for urea synthesis by the action of glutaminase (EC 3.5.1.2) or glutamic acid dehydrogenase (EC 1.4.1.3). If Ser shares the similar role to that of Gln or Glu, it is conceivable that SDH induction is one of the mechanisms for maintaining nitrogen homeostasis when a rat takes dietary protein in excess of its requirement. In addition to this possibility, the physiological meaning as well as the factor(s) regulating the “protein requirement-dependent” induction of SDH remain to be clarified.

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References