Hormone Levels of Anterior Pituitary Gland and Serum in Nagase Analbuminemia Rats

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Abstract

The hormone levels in the anterior pituitary gland and serum in Nagase analbuminemia rats (NAR), a mutant strain established from Sprague-Dawley rats with hyperlipidemia, were examined. For the anterior pituitary gland, the prolactin, TSH, GH, LH and FSH contents in male NAR were significantly lower than those of normal rats. In female NAR, prolactin, TSH and LH levels were also lower than those in normal rats, whereas FSH and GH were normal. For the serum, the concentrations of TSH, total T3, total and free T4, estradiol-17β and testosterone were examined. The serum testosterone concentration in NAR was lower than that of normal rats. Histochemical examination of the hydroxysteroid dehydrogenase (HSD) activity of testes was made in relation to the serum testosterone level. NAR testes, which are rather small compared with those of normal rats, have lower HSD activity. A higher level of serum TSH was seen in NAR. Total and free T4 concentrations were low in the male NAR only. Estradiol-17β and T3 concentrations in NAR were unchanged. Changes in serum LH and FSH levels during the estrous cycle in NAR were also studied. Their patterns of change are normal.

Serum albumin is a major protein in plasma, and is known to be a carrier protein for many substances including bilirubin, ions, drugs and hormones.

Recently, we established a mutant strain of analbuminemic rats (NAR; Nagase Analbuminemia Rats), from a stock of Sprague-Dawley rats (Nagase et al., 1979). This animal inherits an autosomal recessive trait (Nagase et al., 1979, 1980). NAR have scarcely any albumin in their serum or tissues (Sugiyama et al., 1980), and the deficiency of serum albumin is associated with the absence of albumin mRNA in the liver of NAR (Esumi et al., 1980). NAR also had hyperlipidemia (Nagase et al., 1979, 1980; Ando et al., 1980) and slight anemia (Sugiyama et al., In press). Age-related increases in serum lipids, transferrin and ceruloplasmin concentrations were more marked in adult female NAR (Takahashi et al., 1983; Emori et al., 1983). These phenomena may be due to abnormalities in hormone metabolism in NAR. This paper reports studies on the several hormones in NAR.

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Materials and Methods

Animals

NAR and animals from the stock of normal Sprague-Dawley rats from which the NAR were obtained, were given CE-2 (CLEA JAPAN Inc., Tokyo, Japan) and water *ad libitum* and kept in an animal room under conventional conditions at 23 ± 2°C with lighting from 0600 to 1800 hr. Vaginal smears of all female rats except those at 5 weeks of age were taken every morning (0800–0830 hr) before experiments and only rats that exhibited at least three consecutive 4-day vaginal cycles were employed.

Blood samples

Blood samples were obtained from the abdominal aorta at 1000 hr. In the case of adult females, the day of sampling was the proestrous day. After one hour, serum was separated by centrifugation at 1500 × g for 10 min.

Anterior pituitary gland

Hormone levels in the anterior pituitary gland were examined at 5 and 9 weeks of age. Anterior pituitary glands were obtained at 1000 hr. Adult females were sacrificed on the diestrous day. Each anterior pituitary gland was homogenized with 2 ml of 0.25 M ammonium sulfate solution (pH 5.5) in a glass homogenizer. The homogenate was frozen and thawed and then centrifuged at 10000 × g for 20 min. The supernatant fluid was stored at −40°C until assay.

Blood sampling during estrous cycle

Female NAR at 9 weeks of age were divided into two groups. The first group were bled by cutting the tip of the tail at 1000, 1600, 1800 and 2100 hr on the proestrous day, and at 1000 hr on the estrous day. The second group were bled at 1400, 1700, 1900 and 2400 hr on the proestrous day, and at 0700 hr on the estrous day.

Assay methods

The rat prolactin, TSH, GH, LH and FSH were assayed with NIADDK radioimmunoassay kits kindly supplied by Dr. A. F. Parlow of Pituitary and Antisera Center, Harbor-UCLA Medical Center, Torrance, Calif, and National Hormone and Pituitary Program, Baltimore, Md., U.S.A. These kits were NIAMDD rat LH I-5, RP-1, anti-rat LH S-5, rat FSH I-4, RP-1, anti-rat FSH S-10, rat TSH I-4, RP-1, anti-rat TSH S-5, rat PRL I-3, RP-1, anti-rat PRL S-6, rat GH I-2, RP-1 and anti-rat GH S-4.

Total T3 and T4 were measured by radioimmunoassay using a Triiodothyronine Radioimmunoassay kit and Thyroxine Radioimmunoassay kit (EIKEN ICL Co., Ltd., Japan), respectively. Free T4 was determined with a Free Thyroxine Radioimmunoassay kit (DAMON DIAGNOSTICS Co., Ltd., France).

The estradiol-17β and testosterone radioimmunoassays were performed using a double antibody technique. One ml of serum was extracted twice with 5 ml of ether, and then ether was evaporated under nitrogen. The dried extract was dissolved in 0.4 ml of BSA-borate buffer (1 % BSA and 0.1 M borate, pH 8.0), and measured for estradiol-17β and testosterone concentrations by radioimmunoassay. Radioimmunoassay of estradiol-17β was carried out using a specific rabbit antiserum to 17β-Estradiol-6-0-carboxymethylxime conjugated with bovine serum albumin (MAKOR CHEMICALS Ltd., Israel). Only minor cross-reaction was observed with the following phenolic steroids: estrone, 0.1%; and estriol, 0.1%. The serum testosterone level was measured with an extracted sample in the incubation media diluted 1:20 or 1:40 with 1 % BSA-0.1 M borate buffer pH 8.0. Antiserum to testosterone was raised in rabbit using Testosterone-3-0-carboxymethylxime-BSA as an antigen (MAKOR CHEMICALS Ltd., Israel). Cross-reaction was observed with the following steroids: Dihydrotestosterone, 20.7 %; androst-4-ene-3, 17-dion, 0.18 %; progesterone, 0.002 %; estrone; 0.002 %; estradiol, 0.002 %; and estriol, 0.002 %.

Body weight and testes weight

Related to the hormone level, the body weight and testes weight of NAR were compared with normal rats.

Histochemical study on HSD activity in testes

For the histochemical test for HSD, the testes isolated were quickly frozen and sliced into 12-μm thickness on a Microtome Cryostat (DAMON/IEC, U.S.A.) maintained at −18°C. Thin sections were attached to glass cover slips by momentary thawing, allowed to dry at room temperature, rinsed with acetone for 20 min at −20°C and air dried at room temperature for 5–10 min. Then the sections were incubated in
a medium prepared according to the method of Wiebe. (1976) for 30 min at 37°C, using dehydroepiandrosterone as substrate. Following incubation, the sections were fixed in neutral 10% formalin and stained with 0.2% methyl green. Finally the sections were dehydrated through ethanol series and mounted in balsam. For histological examinations, the sections (6-μm) were stained with hematoxylin and eosin.

Though NAR were derived from Sprague-Dawley rats, it would be rather difficult to say that NAR and "normal" rats belong to the same strain. For this reason, we used strict criteria in estimating the significance of differences; i.e. differences between values for NAR and normal rats at p < 0.01 by Student's t-test were regarded as significant.

Results

Hormone content in the anterior pituitary glands

Pituitary LH, FSH and GH contents in NAR and normal rats are shown in Fig. 1. Their contents in male NAR were significantly lower than those of normal rats (p<0.01). LH content in female NAR was also lower than that of normal rats, although this difference was not statistically significant (0.1<p<0.05). FSH and GH contents in female NAR were almost the same as those in normal rats. A sex difference in LH and FSH contents was found in both animals. NAR have lower prolactin and TSH contents than normal rats (Fig. 2).

Serum hormone levels

Age-related changes in serum testosterone and estradiol-17β concentrations in NAR and normal rats are shown in Fig. 3. Serum testosterone levels in male NAR were 63–82% less than those of normal rats. The testosterone concentrations in female NAR were also lower than those of normal rats, but the differences were rather small. The serum estradiol-17β level in NAR was normal, and a slight increase with age was found in both female animals (Fig. 3B).

Serum LH and FSH levels in male NAR and normal rats are shown in Fig. 4. The levels of them in male NAR were almost the same as those in normal rats. Fig. 5 shows serum TSH and total T3 and T4 levels.
levels in NAR and normal rats. NAR have a clearly higher TSH level than that of normal rats \((p<0.01)\), and a slight increase with age in both strains. The T3 level tended to increase in the NAR, although this increase was not statistically significant \((0.1<p<0.05)\). The total T4 concentration in male NAR was significantly lower than that of normal rats \((p<0.01)\) except at 5 weeks of age \((p<0.05)\), while the level in female NAR was unchanged. The serum free T4 level in NAR and normal rats at 9 weeks of age was also investigated (Table 1). The level in male NAR was significantly lower than that of normal rats, while the level in female NAR was comparable to that in normal rats.

Changes in serum LH and FSH concentrations during estrous cycle in NAR

Changes in serum LH and FSH levels during estrous cycle in NAR are shown in Fig. 6. Serum LH rose to its maximum level at 1700 hr on the proestrous day, depicting a typical surge. And then it rapidly decreased to the lowest level at 2400 hr on the proestrous day. The serum FSH concentration in NAR was increased at 1900 hr on the proestrous day, and a slight decrease in the level was seen at 1000 hr on the estrous day.

Body weight and testes weight

The body weight of male NAR was 20-30\% less than that of normal rats except at 4 weeks of age. However, differences in body weight of female from those of normal rats were not significant (Fig. 7A). The weight
Fig. 4. Age-related changes in serum LH and FSH concentrations in male NAR and normal rats. (A), LH concentration; (B), FSH concentration. The symbols are as for Fig. 1.

Fig. 5. Age-related changes in serum TSH, T3 and T4 concentrations in NAR and normal rats. (A), TSH concentration; (B), T3 concentration; (C), T4 concentration. The symbols are as for Fig. 1.

### Table 1. Serum free and total T4 concentrations in NAR and normal rats at 9 weeks old

<table>
<thead>
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<th>Hormonesa</th>
<th>Male</th>
<th>Female</th>
<th>Significance of difference</th>
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<tr>
<td></td>
<td>NAR</td>
<td>Normal rats</td>
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<tr>
<td>Total T4</td>
<td>20.1 ± 1.8b</td>
<td>47.6 ± 4.2</td>
<td>p &lt; 0.005</td>
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<td>(ng/ml)</td>
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<tr>
<td>Free T4</td>
<td>17.5 ± 1.4</td>
<td>26.8 ± 2.2</td>
<td>p &lt; 0.01</td>
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<td>(pg/ml)</td>
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<td></td>
<td>NAR</td>
<td>Normal rats</td>
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<tr>
<td>35.1 ± 1.9</td>
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<td>34.5 ± 4.2</td>
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<td></td>
<td>24.4 ± 1.3</td>
<td>23.0 ± 1.1</td>
<td>NS</td>
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a, Hormone concentrations were determined by the method described in the text.
b, Values are given as means ± S.E. (n = 5).
c, Statistical significance was determined by Student’s t-test. NS; Not significant.
of the testes in NAR were significantly lower than those of normal rats (Fig. 7B).

Histochemical study of HSD activity in testes
A decrease in serum testosterone concentrations and testes weight were found in male NAR. However, no abnormality was found in the morphology of NAR testes (Fig. 8A, B). The activities of HSD were examined in interstitial tissue from testes of NAR and normal rats at 5 weeks of age (Fig. 8C-F). The HSD activity in NAR testes was lower than that of normal rats.

Discussion
The present study shows that the hormone contents of the pituitary gland in NAR tend to be lower than those in the controls, the difference being more marked in males.

Prolactin is the primary hormone for mammary tumorigenesis (Nagasawa et al., 1976). Nagase reported that mammary tumorigenesis induced by 7, 12-dimethylbenz[a]anthracene and the incidence of spontaneous mammary tumors were much lower in NAR than in normal rats. Associated with this lower mammary tumorigenic response, female NAR have significantly lower serum prolactin levels than controls during

Fig. 6. Changes in serum LH and FSH levels during estrous cycle in NAR. Closed and open symbols indicate values for LH and FSH, respectively. Each bar represents mean and S.E. for 5 animals.

Fig. 7. Body weight and testes weight in NAR and normal rats. (A), Body weight; (B), Relative testes weight. Closed and open symbols indicate values for NAR and normal rats, respectively. Squares and circles indicate the values for male and female, respectively. Each bar represents mean and S.E. for 10 animals.
Fig. 8. Histochemical study of HSD activity in NAR and normal rats testes. (A), (C) and (E), NAR; (B), (D) and (F), normal rats. (A) and (B), Microscopic appearance of frozen section (6-μm) of 5-week rat testes. The sections were stained with Hematoxylin Eosin. (×100) (C)–(F), HSD activity in frozen section (12-μm) from the same testes used for (A) and (B), respectively. (×100) (C) and (D), The tissue was lightly counterstained with methyl green.
proestrus at 7–8 weeks of age (Nagase et al., 1984). The low level of pituitary gland prolactin in NAR seems to be contribute to a low circulating prolactin level.

Though NAR show no significant pathological abnormalities, they have small testes. Moreover, the serum testosterone level in NAR was only one third (or fourth) of that in normal rats in spite of the normal level in serum LH. The lower HSD activity in NAR testes was found by histochemical examination. The low level of HSD might be responsible for the low serum testosterone level. The body weight of male NAR is less than that of normal rats, and the lower body weight level may be partly due to the low levels of GH and testosterone. Biochemical studies related to testosterone metabolism in NAR are in progress.

Unexpectedly, female NAR showed only slight abnormalities in the hormone level. Changes in serum LH and FSH levels during the estrous cycle in them are similar to those of normal rats (Neill., 1972; Buther et al., 1984). The serum estradiol-17β level was also unchanged. Thus, NAR can be maintained in a normal way. They reproduce well under the usual conditions for animal care and their only abnormality is hyperlipidemia. From the viewpoint of other hormone metabolism we are now studying why female rats have a much higher lipid level.

The TSH level in the pituitary gland in NAR was lower than that of normal rats, whereas the level of the former serum was high. This fact may indicate that the TSH secretion from the pituitary gland is more active in NAR. The higher serum TSH level may be related to the higher lipid level and the extrathyroidal action of TSH, such as the lipolytic action of TSH in adipose tissue (Freinkel, 1961; Burns et al., 1967).

Serum albumin has been considered to be an essential component in serum protein, and it participates in the transport of hormones. The total serum globulin level in NAR is increased (Emori et al., 1983). High levels of serum globulins may compensate in part for the functions of albumin. It would be interesting to investigate the transport of hormones in NAR serum. NAR should be a useful model to use in endeavouring to clarify the action of albumin on hormone metabolism. Further studies on hormones in NAR are under way.

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References


