EFFECTS OF VITAMIN A DEFICIENCY ON THYROID FUNCTION AND SERUM THYROXINE LEVELS IN THE RAT

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(Received April 19, 1979)

Summary The effects of vitamin A deficiency, which results in a substantial decrease in the level of serum retinol binding protein, on the existent state of serum thyroxine and thyroid function were examined. In the vitamin A-deficient rats, the thyroid weight increased and the level of serum thyroxine decreased to one half that of the control rats. Normal thyroid weight and serum thyroxine levels were recovered by the replenishment of retinyl palmitate in the vitamin A-deficient rats. In addition, decreased hormone synthesis was observed in the thyroid glands of the vitamin A-deficient rats.

The determination of thyroxine distribution in rat serum proteins in vivo showed that thyroxine-binding prealbumin (TBPA) is a major thyroxine transport protein in the control rats, whereas in the deficient rats the amount of thyroxine bound to TBPA decreased and the thyroxine bound to thyroxine-binding globulin (TBG) increased significantly as compared with observations in control rats. These findings suggest that the existent state of serum thyroxine and thyroid function is affected by the serum level of vitamin A.

Keywords retinol-binding protein, prealbumin, thyroxine binding protein, thyroxine binding globulin, thyroid gland, hyperthyroidism, hypothyroidism

In plasma, vitamin A is bound to a specific protein, retinol-binding protein (RBP), which has a molecular weight of 21,000, and RBP also forms a protein-protein complex with a larger thyroxine-binding prealbumin in a 1:1 molar ratio (1). It has been suggested that the complex formation between RBP and prealbumin results in the stabilization of the binding of retinol with RBP and the complex serves to prevent the loss of RBP from the plasma by glomerular filtration of a relatively small protein molecule (2,3). However, the physiological significance of the fact that both RBP and thyroxine bind to the same prealbumin protein
molecule is not presently understood. In the rat, especially, it has been suggested from in vitro experiments that prealbumin is the major thyroxine transport protein in plasma (4).

From that point of view, it is conceivable that the level of serum thyroxine has some effect on the existent state of serum retinol and that the existent state of serum retinol has some effect on serum thyroxine and thyroid function in the opposite direction.

Thus, this study was undertaken primarily to examine the effects of vitamin A deficiency, which results in a substantial decrease in the level of serum RBP (5), on the existent state of serum thyroxine and thyroid function.

MATERIALS AND METHODS

Animals and experimental diets. Male weanling rats of the Wistar strain were used in this experiment. The composition of the vitamin A-free diet is shown in Table 1. For the control diet, 1,000 I. U. of vitamin A palmitate (Eisai Co., Ltd., Tokyo) was added to 100 g of vitamin A-deficient diet. All rats were housed in individual hanging cages in an air-conditioned room kept at 22 ± 1°C and with lighting automatically regulated to provide a 12-hr light period (08:00 to 20:00) and a 12-hr dark period (20:00 to 08:00). All rats had free access to food and drinking water. When the body weight of the deficient rats began to decline, the control and deficient animals were anesthetized with ether, and blood samples were then collected by cardiac puncture.

On the 55th day of feeding, about one half of the rats in the deficient group was orally replenished with 100 I. U. of vitamin A (as retinyl palmitate) per rat daily.

Determination of serum vitamin A and thyroxine levels. Vitamin A levels in the serum were measured by the trifluoroacetic acid method (6, 7). The thyroxine

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Vitamin-free casein(^a)</td>
<td>20</td>
</tr>
<tr>
<td>Potato starch</td>
<td>70</td>
</tr>
<tr>
<td>Cotton seed oil</td>
<td>3</td>
</tr>
<tr>
<td>Salt mixture(^b)</td>
<td>5</td>
</tr>
<tr>
<td>Water-soluble vitamin mixture(^b)</td>
<td>1</td>
</tr>
<tr>
<td>Fat-soluble vitamin mixture(^c)</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\) The vitamin-free casein was prepared by refluxing with 0.1% HCl in ethanol for 8 hr.
\(^b\) Compositions were described by Harper (6).
\(^c\) One gram of vitamin mixture contains vitamin D 200 I.U., vitamin E 10 mg, p-aminobenzoic acid 10 mg, choline chloride 200 mg.

level in serum was measured by radioimmunoassay using a T\textsubscript{4} RIA kit purchased from Dinabbot Radioisotope Laboratory Co., Ltd., Tokyo.

**Preparative disc electrophoresis of rat serum protein.** Disc electrophoresis was performed using 7.5% acrylamide gel without a concentrating gel(8). The upper electrode buffer used was 0.05 M Tris-glycine, pH 8.9 and the lower was 0.1 M Tris-HCl, pH 8.1. The elution buffer was the same as the lower buffer (9). The prepared gel column, about 8.0 cm high, was prerun for 3 hr at 40 mA before the sample was applied. In the usual procedure, 3–5 ml of serum were chromatographed by Sephadex G-25 with the upper buffer for desaltation of the sample and the sample was then applied to the top of the gel. Electrophoresis was begun at a current of 40 mA at 5°C. After 30 min, the current was adjusted to 45 mA and was maintained at this level. The elution rate was 35 ml/hr. The eluates were analyzed for protein concentration and for \textsuperscript{131}I radioactivity.

**Determination of iodoamino acid pattern in thyroid gland.** The incorporation of \textsuperscript{131}I into the metabolites related to thyroid hormone synthesis was studied. Each rat was intraperitoneally given a single dose of 20 \textmu Ci carrier-free \textsuperscript{131}I, and killed 24 hr later by exanguination under anesthesia with ether. The thyroid was then removed. Chromatographic analysis of the labeled metabolites in thyroid was conducted essentially in the same way as described by Inoue and Taurog (10).

**RESULTS**

*Growth rates of vitamin A-deficient rats*

The growth rates of the rats in each of the experimental groups are shown in Fig. 1. The body weights of the vitamin A-deficient and control rats gradually increased, but the weights of vitamin A-deficient rats began to decline after 50 days of feeding. After the 55th day, the serum vitamin A concentrations of the control and deficient rats were 135 I.U./100 ml and less than 5 I.U./100 ml, respectively.

![Graph](image)

Fig. 1. Mean growth rates of the rats in each of the two experimental groups. Each growth rate was exhibited as the average for 9 rats.
Effects of vitamin A deficiency on thyroid weight and serum thyroxine level

The average weight of thyroid gland in vitamin A-deficient rats was 5.5 mg/100 g body weight and was statistically different from that observed for control rats (Table 2). The mean concentration of thyroxine in serum of the deficient rats was 2.5 μg/100 ml serum, which was one half that of the control rats.

Table 2. The effects of vitamin A deficiency on thyroid weight and serum thyroxine concentration.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Thyroid weight (mg/100 g)</th>
<th>Serum thyroxine (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>285 ± 11.8</td>
<td>3.1 ± 0.26</td>
<td>5.0 ± 0.16</td>
</tr>
<tr>
<td>VA-deficient</td>
<td>210 ± 6.08</td>
<td>*5.5 ± 0.12</td>
<td>**2.5 ± 0.31</td>
</tr>
</tbody>
</table>

All values listed are mean ± S.E.M. for 5 rats. * p<0.05 compared with control, ** p<0.01 compared with control.

Effects of vitamin A deficiency on T₄-distribution in rat serum proteins

The distribution of thyroxine in rat serum proteins was determined by the method of preparative disc electrophoresis. Each rat of the control and deficient groups intraperitoneally received a single dose of 20 μCi carrier-free ¹³¹I, and plasma from these treated rats was collected at 48 hr after injection. The obtained plasma samples were analyzed by preparative disc electrophoresis under the conditions described in MATERIALS AND METHODS, three distinct proteins being obtained (Fig. 2). In both deficient and control groups, the radioactivity of prealbumin was higher than that of the other two protein fractions. Notably, about 70% of the total radioactivity in the control group was recovered in the prealbumin.

Fig. 2. Thyroxine distribution in rat serum protein. Experimental procedure was noted in MATERIALS AND METHODS. Pooled serum of 3 rats was used in each group. The number underlined represents the percentage of radioactivity in each collected fraction.
fraction. This result supports the findings of a recent study which showed that prealbumin might be a major thyroid hormone transport protein in rat plasma. No difference was observed between the radioactivity of the albumin fraction of deficient rats and that of control rats. However, about 35% of the total radioactivity was found in the post-albumin fraction of the deficient rats, although a trace of radioactivity was detected in the control rats.

**Iodoamino acid pattern in thyroid digests**

In order to estimate the degree of thyroid activity, the radioiodinated compounds as hormone precursors and thyroxines were assayed. Twenty-four hours after the intraperitoneal injection of 20 μCi carrier-free $^{131}$I of each rat, the radioiodine uptake in the thyroid in the deficient rats was about 84% compared with that in the control rats. The percentages of radioactivity in monoiodotyrosine (MIT) and diiodotyrosine (DIT) in the control and deficient rats are shown in Table 3. The ratio of MIT to DIT was calculated, since this is known to be affected by the degree of iodine deficiency and also by the presence of an antithyroid hormone in vivo (11). However, no difference between the deficient and control rats was observed. In contrast, the radioactivities of the $T_4$ and $T_3$ fractions in the deficient rats decreased significantly, whereas the ratio of inorganic iodine to hormonal iodine increased.

<table>
<thead>
<tr>
<th>Groups</th>
<th>% of total radioactivity</th>
<th>MIT/DIT</th>
<th>I/H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>MIT</td>
<td>DIT</td>
</tr>
<tr>
<td>Control</td>
<td>3.5</td>
<td>36.0</td>
<td>47.8</td>
</tr>
<tr>
<td>VA-deficient</td>
<td>2.7</td>
<td>37.0</td>
<td>*54.4</td>
</tr>
</tbody>
</table>

Abbreviations: I, inorganic iodine; MIT, monoiodotyrosine; DIT, diiodotyrosine; $T_3$, triiodothyronine; $T_4$, tetraiodothyronine; H, hormone. MIT/DIT and I/H ratio are represented as mean±S.E.M. for 4 rats. * $p<0.05$ compared with control, ** $p<0.01$ compared with control.

**Effect of vitamin A replenishment on serum thyroxine level**

After the daily administration of an oral dose of 100 I.U. vitamin A palmitate to the vitamin A-deficient rats, the body weights increased steadily and reached the control level within 10 days of replenishment. Neither thyroid weight nor serum thyroxine level changed significantly from 4 days replenishment of vitamin A palmitate. However, these levels were close to those of the control rats after 10 days replenishment (Fig. 3).
DISCUSSION

The existence of RBP-PA complex in plasma has been well established by Goodman et al. (1). Muto et al. reported that the complex plays an important role in vitamin A transport in vertebrates of classes higher than fish (12). The apo-RBP synthesized binds with retinol in liver and then forms holo-RBP which is immediately released into the bloodstream (13). Vitamin A deficiency interferes with the secretion of holo-RBP from the liver and results in a decrease in the level of serum RBP (5), although the detailed mechanism had not been yet elucidated.

There have been many reports on the biological effects of vitamin A on thyroid activity (14, 15), but the concept thereof is not well understood. Our evidence shows that thyroid weight increases and serum thyroxine decrease to half the level of the control rats in vitamin A deficiency. These facts suggest that the decrease of thyroxine levels in vitamin A-deficient rats may be due to reduced hormone synthesis in the thyroid gland.

Recently, however, Morley et al. reported an increased serum thyroxine level in vitamin A-deficient rats. These data are inconsistent with our present results (16, 17). This discrepancy might be explained by the extent of the deficiency, because in Morley’s experiments, weanling 30-day-old rats were fed with a vitamin A-deficient diet and it was observed that the weights of such rats were not significantly different from those of the pair-fed control rats. In contrast, we used 20-day-old rats which were fed a vitamin A-deficient diet, and the apparent weight loss was shown in the vitamin A-deficient rats as compared with the control rats. Therefore, it appears that the rats in our study were more markedly deficient. At an early stage of nutritional deficiency the function of the organs involved sometimes may show hypersensitivity.

It is well known that in man thyroxine circulates in plasma, associating with three plasma proteins: thyroxine-binding globulin (TBG), prealbumin (TBPA) and albumin (TBA) (18). Thyroxine-binding globulin is the main carrier of this
hormone (19). In the rat, however, recent reports suggest that TBPA is a major protein which can transport thyroid hormone whereas TBG lacks hormone binding activity (20, 21). Although those experiments were carried out in vitro, similar results were obtained from the control rats in our study. With regard to the deficient rats, however, the percentage of hormones bound to prealbumin decreased, whereas that of TBG significantly increased. These data indicate that in the vitamin A-deficient rats the level of serum prealbumin declines or that the normal binding ability of prealbumin with thyroxine seems to be lowered. Furthermore, since it has been found that the binding capacity of TBG increases in hypothyroidism (22), it appears that the enhancement of TBG function in our vitamin A-deficient rats is related to the decreased thyroid function. Detailed mechanisms which control TBG function in vitamin A deficiency are not presently known.

REFERENCES