THE INFLUENCE OF THYROXINE ON THYROTROPHIC ACTION OF PREGNANT MARES' SERUM GONADOTROPHIN IN RATS

By

Amiya B. Kar, J. N. Karkun and S. N. Datta

The inhibitory influence of thyroxine on the response of the ovary of immature rats to gonadotrophic hormone has been indicated by a number of workers (Smith & Engle, 1930; Fluhmann, 1934; Leonard, 1936; Tyndale & Levin, 1937; Tolksdorf & Jensen, 1939; and Warner & Meyer, 1949). Recently, Kar et al. (1954) studied the nature of such inhibition and showed that thyroxine only diminishes the accelerated rate of follicular maturation caused by persistent gonadotrophin administration, but does not interfere with the hormonal activities of the ovary. They have further demonstrated that some of the pathological side-effects of precocious ovarian stimulation with gonadotrophic hormone like cyst formation and follicular hemorrhage, could be blocked by simultaneous administration of thyroxine. These findings led them to postulate that thyroxine either slows down the rate of utilization of gonadotrophin by the ovarian tissue or causes greater inactivation of gonadotrophin at some unknown site.

It is evident from the above that thyroxine tends to antagonize the action of gonadotrophic hormone on the ovary. Such an antagonism, however, appears to be mutual as it has been shown that the metamorphosis of tadpoles of *Rana temporaria*, induced by thyroxine, can be inhibited by the addition of blood from a pregnant woman containing chorionic gonadotrophin (Sax, 1940). Besides, the reports on thyroid-stimulating effects of gonadotrophin (Riddle et al., 1933; Fluhmann, 1934) make the issue a complicated one and raise the question as to whether thyroxine exerts a similar inhibitory influence on the thyrotrophic action of gonadotrophic hormone. In the present paper an attempt has been made to provide an answer to this question.
EXPERIMENTAL PROCEDURE

Female albino rats weighing 29 ± 6 gm., were used in this study. A total of 24 animals were used of which 6 were treated with gonadotrophic hormone, 6 with gonadotrophin plus thyroxine, 6 with thyroxine and the remaining 6 served as the controls. All the animals were maintained in cages under uniform husbandry conditions throughout the experimental period.

Pregnant mares' serum gonadotrophin (PMS) ('Antex' Dumex Products, Copenhagen) was administered in a dosage of 7.5 I. U. daily for a period of 7 days and thyroxine ('Thyroxine' Hoffman-La Roche Ltd., Basle) at the rate of 0.08 mg. daily for the same period. The combined-treated group was injected with 7.5 I. U. of gonadotrophin plus 0.08 mg. of thyroxine for 7 days. The control animals received sterile distilled water alone in a similar manner. All the injections were given by the intramuscular route.

Autopsy followed 24 hours after the final treatments. The thyroids were carefully dissected out, weighed accurately and finally fixed in 10 per cent formol saline for histological studies. Serial paraffin sections of the gland were stained with Ehrlich's hematoxylin followed by alcoholic eosin. Thyroid response was evaluated by microhistometric measurement of acinar cell height according to the method of Rawson & Salter (1940).

RESULTS

The mean weight of the thyroid of control animals is presented in Table 1. Histologically, the follicles are filled with colloid and the acinar epithelium is of the cuboidal nature (Fig. 1). The mean height of the acinar cells is 3.96 ± 0.03 μ and they contain a few eosinophilic colloid droplets. The basement membrane supporting each follicle and the interfollicular connective tissue are inconspicuous. Occasional macrophages and anastomotic capillaries are discernible in the interfollicular tissue.

Gonadotrophin treatment causes a significant increase in thyroid weight (Table 1). Histological examination also reveals considerable stimulation of the gland. The follicular epithelium shows pronounced hypertrophy and hyperplasia and tends to be columnar in shape (Fig. 2). The epithelial cells are filled with strongly eosinophilic colloid droplets and their mean height is significantly greater than that of the controls. The amount of colloid is considerably reduced in all the follicles. In some, the colloid is practically absent and the lumen is invaded by the hyperplastic epithelial cells. The colloid appears to be dilute in nature and shows marked vacuolization. The basement membrane supporting each follicle is well developed and the hypertrophied interfollicular connective tissue contains many macrophages. Apart from these, the vascularity of the organ is very conspicuous. The histological features of the gland appear to resemble those of colloid goitre.

A comparison of the thyroid weights of gonadotrophin and the gonadotrophin plus thyroxine treated animals indicates that the thyroid weight is significantly less in the latter group (Table 1). Evidently, thyroxine depresses the weight of the gland in the gonadotrophin treated rats. However, the thyroid weight of the control animals is practically the same as in the combined group. The histological picture of the thyroid of the combined treated animals too is indicative of an inhibitory influence of thyroxine on the gland. In contrast to the hypertrophied columnar epithelium of the follicles of the gonadotrophin administered group, the acinar cells of the combined treated animals are of the squamous type (Fig. 3). The mean height of the cells is also significantly
Effects of pregnant mares' serum gonadotrophin (PMS) and thyroxine on the thyroid of rats. × 100.

**Fig. 1.** Section through the thyroid of a normal rat.

**Fig. 2.** Section through the thyroid of a gonadotrophin treated rat. Note the changes in the acinar cells and the loss of colloid from the follicles. Compare with Fig. 1.

**Fig. 3.** Section through the thyroid of a gonadotrophin plus thyroxine treated rat. Note the squamous type of acinar epithelium and the distension of follicles with colloid. The presence of interfollicular connective tissue could be recognized at points indicated by arrow. Compare with Fig. 2.

**Fig. 4.** Section through the thyroid of a thyroxine treated rat. Compare with Fig. 3. Interfollicular connective tissue is absent.
Table 1.
The thyroid weight and acinar cell height in normal and experimental rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean weight of the thyroid with S. E. (mg.)</th>
<th>Mean height of the acinar cells with S. E. (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>3.41 ± 0.13</td>
<td>3.96 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>Gonadotrophin</td>
<td>5.51 ± 0.33</td>
<td>9.06 ± 0.12</td>
</tr>
<tr>
<td>3</td>
<td>Gonadotrophin plus thyroxine</td>
<td>3.81 ± 0.22</td>
<td>1.36 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>Thyroxine</td>
<td>2.23 ± 0.11</td>
<td>1.39 ± 0.02</td>
</tr>
</tbody>
</table>

Analysis:

Thyroid weight
Group 1 vs. Group 2 – t = 6.44; P < .001 (significant)
Group 2 vs. Group 3 – t = 4.93; P < .001 (significant)
Group 1 vs. Group 3 – t = 1.50; P < .2 > .1 (insignificant)
Group 3 vs. Group 4 – t = 6.60; P < .001 (significant)
Group 1 vs. Group 4 – t = 5.89; P < .001 (significant)

Acinar cell height
Group 1 vs. Group 2 – t = 41.97; P < .001 (significant)
Group 2 vs. Group 3 – t = 64.11; P < .001 (significant)
Group 1 vs. Group 3 – t = 68.78; P < .001 (significant)
Group 3 vs. Group 4 – t = 2.00; P < .10 > .05 (insignificant)
Group 1 vs. Group 4 – t = 73.21; P < .001 (significant)

less than either the controls or the gonadotrophin treated animals. The follicles are distended with colloid and the acinar cells are devoid of colloid droplets. The basement membrane is ill-defined but the presence of interfollicular connective tissue is discernible in some locations. The vascularity of the organ appears to be poor.

Thyroxine treatment causes a significant loss of thyroid weight as compared to the controls. But the thyroid weight of the combined-treated group is significantly greater than that of the animals which received thyroxine alone (Table 1). Histologically, the picture is closely akin to that of the combined treated animals. The follicles are distended with colloid and are lined by a layer of squamous epithelium (Fig. 4). The mean height of the ocinar cells is significantly less than that of controls but is practically the same as in the combined treated group (Table 1; Fig. 5). In contrast to the latter group, the interfollicular connective tissue is almost absent. The vascularity of the gland is inconspicuous.

Discussion

The results of the present study clearly indicate that thyroxine tends to inhibit the thyroid-stimulating action of pregnant mares' serum gonadotrophin. The extent of such inhibition, however, appears to be quite marked as the
Fig. 5.


The histological picture of the thyroid and the microhistometric data on acinar cell height in the combined-treated animals agree with those of the thyroxine-treated group. Besides, as judged by the microscopical features and the acinar cell height response it would appear that thyroxine imprints its characteristic depressive effects on the thyroid irrespective of the presence of gonadotrophin. This signified that considerable inhibitory influence is exerted on the mechanism responsible for the thyrotrophic action of gonadotrophic hormone. An attempt to interpret the data on thyroid weight, however, leads to a somewhat anomalous situation. It is evident that thyroxine depresses the weight of the thyroid whether given alone or in combination with gonadotrophin. Nevertheless, as pointed out before, the thyroid weight of the combined group is significantly greater than that of the animals which received thyroxine alone (Table 1). This would imply that either the thyroid stimulating action of gonadotrophin is only partially blocked by thyroxine or the latter fails to exert its own characteristic effects on the thyroid and simply antagonizes the action of gonadotrophin.

The above interpretation, however, becomes untenable when histometric responses are taken into consideration. As these are strikingly similar in the two thyroxine-treated groups it would seem that the inhibition of thyrotrophic
effect of gonadotrophin is almost total. Probably, the presence of interfollicular connective tissue in the combined treated animals is a factor responsible for the greater weight of the thyroid in this group. Incidentally, a lack of correlation between thyroid weight and histometric data after hormonal treatments has been pointed out by a number of investigators (see Collip, 1942; Kar & Ghosh, 1950) and Adams & Beeman (1942) even questioned the feasibility of using weight as a criterion for evaluation of thyroid response. These facts, therefore, tend to emphasize the importance of histometric index for the measurement of thyroid change and the present findings also appear to support this.

The mechanism responsible for the thyroid-stimulating action of gonadotrophic hormone merits a detailed comment. Riddle et al. (1933) and Fluhmann (1934) observed a frequent association of gonadotrophic and thyrotrophic effects. Jensen & Tolksdorf (1940) considered this association to be due to the identity of TSH with gonadotrophin. This view, of course, would furnish a simple explanation for the thyrotrophic influence of gonadotrophic hormone. However, Rawson (1949) has recently demonstrated that TSH is readily inactivated by explanated thyroid cells cultured in vitro, whereas gonadotrophin is not inactivated by similar cells grown in tissue culture. This is indeed a strong evidence against the identity of the two hormones. In view of this, it seems more plausible that gonadotrophin exerts its trophic effect on the thyroid through a stimulation of TSH production by the adenohypophysis. This concept would readily account for the inhibitory influence of thyroxine on the thyrotrophic action of gonadotrophic hormone as it is now well established that the former blocks the elaboration of hypophyseal TSH (Salter, 1950). However, it remains to be seen whether thyroxine antagonizes gonadotrophin action prior to stimulation of hypophyseal TSH production or opposes the effect of gonadotrophin at the level of the hypophysis proper.

SUMMARY

Intramuscular injection of pregnant mares' serum gonadotrophin causes a marked stimulation of the thyroid in rats. Simultaneous administration of thyroxine blocks the thyrotrophic action of gonadotrophin. It is suggested that the latter stimulates he thyroid through an augmentation of hypophyseal TSH output and thyroxine only exerts its typical inhibitory effects on thyrotrophic activity of the hypophysis.

ACKNOWLEDGMENTS

The authors wish to express their indebtedness to Dr. B. Mukerji for the keen interest he has taken in this study. Thanks are due to Mr. A. K. Banerjee for technical assistance and Mr. S. Banerjee for the illustrations.

6
REFERENCES

Jensen, H. & Tolksdorf, S.: J. Biol. Chem. 133, 1, 1940.