EFFECT OF THE POSTERIOR PITUITARY HORMONE ON THE FUNCTION OF THE THYROID GLAND

AKIRA ARIMURA, YOSHIO TAKAGI AND TAKASHI UENO

In 1908 Herring found striking changes in the posterior pituitary lobe and in the laminae forming the floor of the third ventricle after thyroidectomy, as well as an increased activity of cells in the pars intermedia (1). More recently, some investigators reported that polydipsia and polyuria in experimental diabetes insipidus were decreased or abolished after thyroidectomy and that the subsequent administration of desiccated thyroid reestablished the syndrome (2, 3, 4, 5).

Blotner (6) showed some evidences of hyperactivity of the thyroid gland in the patients with diabetes insipidus. There appeared to be an imbalance in the pituitary-thyroid axis in this disease. He suggested that the hyperactivity of the thyroid gland in diabetes insipidus might be caused by the increased activity of the anterior pituitary and that the diuretic effect of the anterior lobe was through its influence to the thyroid gland (7). From these evidences we can presume some interrelationships between the function of the thyroid gland and that of the posterior pituitary. In a previous paper (8) we reported an inhibitory effect of the posterior pituitary hormone, especially vasopressor fraction, on the release of ACTH. It is probable that this hormone affects the release of other anterior pituitary hormones. If this is true in case of TSH, the thyroid activity should be changed after the administration of posterior pituitary hormone. The present experiments have been designed to clarify this problem.

METHOD

Oxygen consumption and plasma protein-bound-iodine (PBI) were determined and histological observation of the thyroid gland was done to measure thyroid activity.

Animals: Single strain male rats (Wistar) weighing 170 g. to 240 g. were used. All rats were kept for a period of at least 8 weeks at a constant environmental temperature of 29.5 ± 1.0°C. before the experiment. By this way the level of the oxygen consumption was lowered and kept in constant within narrow limits (9, 10). When the level of oxygen consumption had remained constant within 2 per cent of the mean value of 3 successive determinations on alternate days, they were considered to be ready for the experiments. Animals were fed on a constant diet and provided with tap water. Artificial light was supplied from 8:30 a.m. to 6:00 p.m. every day.

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**Oxygen consumption:** Oxygen consumption was measured using the slightly modified apparatus described by Bargenton and Krumm-Heller (11). The diagram is shown in fig. 1. Two or three rats were put in this apparatus together and their oxygen consumption was determined at the same time. Animals were not supplied any food for 17 hours or more before the determination of oxygen consumption, but water was given ad libitum. The apparatus, in which rats had been put, was submerged in a water bath maintained at a temperature of 29.5 ± 0.1°C. A constant ventilation of 1.2-1.5 l. per min. was done by a submersible membrane pump. When the animals were quiet, successive readings of the oxygen consumption were done over 3 min. period. When the values were within a range of 0.4 ml. in 3 or more successive determinations and when the animals were quiet, the mean value was calculated as oxygen consumption rate.
for a 3 min. period.

After a fast of 17 hours, the respiratory quotient of rats is assumed to be 0.72 (12, 13), giving the caloric equivalent of oxygen 4.702 Cal/l. The mean oxygen consumption (corrected to B.T.P.) was converted to its heat equivalent per 24 hours and this in turn expressed as Cal/sq.m/24 hrs. The body surface area of rats was estimated from the formula (9) \( S = W^{2/3} \times 0.091 \) where \( S \) is the surface area in square meters, \( W \) the body weight in kg. and 0.091 the Meeh factor.

**Plasma protein-bound-iodine (PBI):** Blood sample was collected by heart puncture into an oxalated test tube prepared in advance by evaporating 0.051 ml. of 20 per cent potassium oxalate solution in each. If the volume of the plasma obtained from one rat was less than 2 ml., plasma of two rats in the same experimental group was mixed together and the value of PBI was determined by the alkaline incineration technique described by Barker, Humphrey and Soley (14).

**Histological examination:** Immediately after the blood of rats was withdrawn for determination of PBI, rats were sacrificed in chloroform gas as quickly as possible. Excised thyroid glands were fixed in Susa’s solution. Sections were cut at a thickness of 5 µ and stained in iron hematoxyline or hematoxyline and eosin.

**Material:** Vasopressin and oxytocin (15) were diluted with polyethyleneglycol to the concentration of 0.8 u. per ml.

**EXPERIMENT**

Rats were divided into 6 groups (A, B, C, D, E and F). (A) group was control to which 0.05 ml. of polyethyleneglycol per 100 g. body weight was subcutaneously injected twice a day during a period of experiment. Rats of (B) group were exposed to cold environment ranging from 10°C to 15°C and kept in this condition for 10 days. During this period, they were injected with 0.05 ml. polyethyleneglycol per 100 g. body weight twice a day. Rats of (C) group were given 40 mu. of vasopressin in 0.05 ml. polyethyleneglycol per 100 g. body weight twice a day, namely 80 mu. of vasopressin daily. (D) group was exposed to cold for 10 days and administered with vasopressin in the same manner as in (C) group during a period of exposure to cold. Rats of (E) group were injected with 40 mu. of oxytocin in 0.05 ml. of polyethyleneglycol per 100 g. body weight twice a day for 10 days. Group (F) was exposed to cold for 10 days and administered with 40 mu. of oxytocin twice a day during the period of cold exposure.

Oxygen consumption of rats in each group was measured several times on alternate days before the experiment and on the last day of the treatment. On the day following the last determination of oxygen consumption, blood PBI was determined and thyroid glands were excised for the histological examination.
RESULT

**Oxygen Consumption:** The levels of oxygen consumption and BMR of each group are summarized in table 1. In the control group (A) the value of BMR was not changed by the successive injection of polyethylenglycol, while in group (B) it increased by 9.0 per cent after exposure to cold. Though no significant change was found in group (C), which was treated with vasopressin, the value of BMR increased by 5.9 per cent in rats administered with vasopressin and exposed to cold (group (D)). This extent of increase in BMR in group (D) was less than that in group (B). These experiments were done in winter.

Another series of the experiments were carried out in the warm season, from May to July. Though the level of BMR in the warm season was 5.1 per cent less than that in the cold season, the results were essentially same as those in winter. After exposure to cold the value increased by 7.4 per cent in group (B), while it increased scarcely in group (D) injected with vasopressin daily during the period of cold exposure. This fact may indicate that the increase of BMR of rats due to exposure to cold is inhibited by exogenous vasopressin.

In group (E), which was injected with oxytocin for 10 days, the value of BMR was not changed, while in group (F), which was given oxytocin and exposed to cold, the value increased by 6.9 per cent, indicating that exogenous oxytocin has no inhibitory effect on the increase of BMR in cold environment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Difference between before and after (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>O₂ cons.</td>
<td>BMR</td>
<td>O₂ cons.</td>
</tr>
<tr>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Control</td>
<td>5</td>
<td>157.38±1.28</td>
<td>740±6</td>
<td>159.93</td>
</tr>
<tr>
<td>B</td>
<td>Cold</td>
<td>5</td>
<td>156.53±0.85</td>
<td>738±4</td>
<td>170.87</td>
</tr>
<tr>
<td>C</td>
<td>Vasopressin</td>
<td>5</td>
<td>155.29±1.49</td>
<td>730±7</td>
<td>154.40</td>
</tr>
<tr>
<td>D</td>
<td>Vasopressin cold</td>
<td>5</td>
<td>153.98±1.49</td>
<td>724±7</td>
<td>163.12</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>155.76±1.28</td>
<td>733±4</td>
<td>(S.D. 0.5-1.0%)</td>
</tr>
</tbody>
</table>

| Early summer|                    |                |          |     |          |     |                                      |
| A     | Control            | 4              | 149.30±1.49 | 702±7 | 148.45  | 698 | -0.6                                  |
| B     | Cold               | 4              | 146.96±2.76 | 691±13 | 157.81  | 742 | +7.4                                  |
| D     | Vasopressin cold   | 4              | 150.79±1.92 | 709±9 | 153.34  | 721 | +1.7                                  |
| E     | Oxytocin           | 5              | 148.02±1.70 | 696±8 | 148.45  | 698 | +0.3                                  |
| F     | Oxytocin cold      | 5              | 151.64±7.02 | 713±33 | 162.06  | 762 | +6.9                                  |
|       | Mean               |                | 149.34±2.98 | 702±4 | (S.D. 1.0-4.6%) |                                      |

**Plasma Protein Iodine:** As shown in table 2, the value of PBI of the control group (A) was higher in the cold season than in the warm season. A similar tendency was also found in the animals exposed to cold for 10 days (B), while the PBI values were generally higher in the latter (12.2 per cent higher
in the cold season and 22.8 per cent higher in the warm season). On the other hand, in group (C), which was administered with 80 mu. of vasopressin daily for 10 days, the value of PBI was considerably less than that in the control group (A), indicating that exogenous vasopressin decreases the level of PBI in the circulating blood. The animals in group (D), which were exposed to cold and given vasopressin during a period of cold exposure, gave a mean value of 1.01 gamma per cent in winter and just a trace in summer. This shows that the PBI of the animals treated with vasopressin further falls when they are exposed to cold at the same time. In group (E), which was given oxytocin, and in group (F), which was exposed to cold and injected with oxytocin, no marked difference in the values of PBI was found compared with those in control groups (A and B). Exogenous oxytocin may, therefore, be considered not to change the level of PBI of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
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<th>Early summer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of animals</td>
<td>PBI</td>
<td>No. of animals</td>
</tr>
<tr>
<td>A</td>
<td>Control</td>
<td>5</td>
<td>2.78</td>
</tr>
<tr>
<td>B</td>
<td>Cold</td>
<td>5</td>
<td>3.12</td>
</tr>
<tr>
<td>C</td>
<td>Vasopressin</td>
<td>5</td>
<td>1.52</td>
</tr>
<tr>
<td>D</td>
<td>Vasopressin cold</td>
<td>5</td>
<td>1.01</td>
</tr>
<tr>
<td>E</td>
<td>Oxytocin</td>
<td>5</td>
<td>2.51</td>
</tr>
<tr>
<td>F</td>
<td>Oxytocin cold</td>
<td>5</td>
<td>2.51</td>
</tr>
</tbody>
</table>

**Histological Examination:** Normal control rats (A): Thyroid follicles were in general large and contained concentrated colloid, in which only few absorption vacuoles were found. The shape of most cells was cubic. Nuclei with thin nuclear membranes contained fine nests of chromatin and small nucleolus. Cold exposed rats (B): Follicles were generally constricted and occupied by poorly stained colloid. Some of the follicles became so small that the storage of colloid completely disappeared. Absorption vacuoles were abundantly found in the periphery of the colloid, into which free edges of the epithelial cells juted out. Remarkable hypertrophy of the epithelium was observed and the protoplasma of the epithelium cells became rough. Relatively strong hyperemia was found between follicles. Most nuclei of the epithelium cells showed an irregular round shape and light appearance due to roughly structured nest of chromatin. These findings indicate hyperactivity of the thyroid, which may be caused by the increased secretion of TSH from the anterior pituitary gland. Vasopressin administered rats (C): Follicles with plane epithelial cells were larger than those of control rats and contained strongly stained colloid. Absorption vacuoles were scarcely found. Small darkly stained nuclei were situated in the center of each epithelium-cell. This picture is considered to indicate hypoactivity of the thyroid gland. Vasopressin administered rats during a period of cold exposure (D): Follicles were somewhat larger than those of cold exposed rats (B), which were not given any vasopressin. Strongly stained colloid was richly stored in the follicles,
1: The thyroid of a rat in control group.

2: The thyroid of a vasopressin administered rat.

3: The thyroid of a oxytocin administered rat.

4: The thyroid of a cold exposed rat.

5: The thyroid of a rat injected with vasopressin during a period of cold exposure.

6: The thyroid of a rat injected with oxytocin during a period of cold exposure.

Fig. 2. Histological picture of thyroid of rats.
but absorption vacuoles were rarely found. Epithelial cells were plane or cubic and their free edges were smooth. Nuclei of the epithelial cells were small and located in the center of the cell. They were stained dark due to concentrated chromatin nest. Hyperemia did not appear. This picture is different from that of the thyroid of cold exposed rats, which were not given vasopressin. The finding of the former indicates lower activity of the thyroid gland than the latter. **Oxytocin administered rats** (**E**): Follicles were as those of vasopressin administered rats and contained strongly stained colloid, in which absorption vacuoles were rarely observed. Nuclei of the epithelial cells were small and pyknotic. This picture is almost the same as that found in the rats given vasopressin. **Oxytocin administered rats exposed to cold** (**F**): Follicles were generally small. Hypertrophy of the epithelial cells was remarkable. Nuclei were poorly stained and considerably large. They were located near the basal membrane. Somewhat strong hyperemia was noticed. This picture shows active stage of the gland. However, the changes were less distinct than those in group (**B**).

**DISCUSSION**

As can be seen from table 1, the mean value of heat production of the rats adapted to an ambient temperature of 29.5°C. was 733 Cal/sq.m/24 hrs. (S.D. 0.5 or 1.0 per cent) in the winter. In early summer (June and July) the mean value was 702 Cal/sq.m/24 hrs. with low standard deviation (S.D. 1.0 or 4.6 per cent). The fact that the mean value of the heat production of animals in each season lay within a narrow range with low standard deviation would indicate that adaptation of these animals to environmental temperature was complete. Bargeton, Krumm-Heller and de Fombelle (16) found in rats adapted to temperature of 29.5°C. that the mean value of heat production was 767 Cal/sq.m/24 hrs. (S.D. 17.03 or 2.2 per cent) at the fourth week of adaptation and 756 Cal/sq.m/24 hrs. (S.D. 10.0 or 1.5 per cent) at the sixth week. According to Beattie and Chambers (11), the mean heat production ranged from 690 to 712 Cal/sq.m/24 hrs. in the months between August and May. About the end of May it fell and remained low (657-679 Cal/sq.m/24 hrs.) until sometime in August, when it returned to the winter level. The values in the present experiment are lower than those given by the French workers but higher than those by the British ones. The difference may be due to difference in the strains of rat, because the apparatus used were almost the same with each other. We found that the values of heat production of heat adapted rats in winter were higher than those in summer. This result coincides with that given by Beattie and Chambers (10). They described that the change from “winter” to “summer” levels of heat production appeared to occur towards the end of May and was complete with in 2 weeks and the reverse change occurred in early August.

When animals were transferred to a cold environment for a period of 10 days, their heat production level rose by 7.4 per cent in early summer and 9.0 per cent in winter. The level of PBI of heat adapted rats was 2.78 r% in winter and 2.32 r% in early summer. On the 10th day of cold expose, the mean value
of PBI was 3.12% in winter and 2.85% in early summer. Though an increase in the level of PBI was found on cold exposure in the two seasons, its extent was so small as was statistically of no significance. Rand, Riggs and Talbot (17) exposed rats to cold long enough to cause stimulation of the thyroid. Then they determined the PBI of the serum and compared it with that of rats which had been kept at room temperature. There was no difference. However, histological picture of the thyroid gland of cold exposed rats indicated the hyperactivity of the thyroid. It is generally known that cold induces an increase in the output of TSH from the pituitary gland. Considering this together with the histological picture of the thyroid gland and the increase in heat production in cold exposed rats, it seems certain that the thyroid is so activated by cold exposure that the output of thyroid hormone is augmented. But when the destruction of this hormone is accelerated by increased activity of the tissues, no rise in the level of this hormone in the blood, as indicated by PBI, may resulted.

There was no significant change in oxygen consumption and in histological picture of the thyroid glands of heat adapted rats after the administration of vasopressin or oxytocin. On the other hand, PBI was remarkably decreased after vasopressin, but not after oxytocin, administration. As the metabolism of tissues may not be changed by the administration of vasopressin, so far as indicated by heat production of the animals, the amount of thyroid hormone spent by tissues may be not changed. The fact that the thyroid glands of rats injected with vasopressin were histologically similar with those of rats injected with oxytocin, indicates that vasopressin does not have any marked influence on the gland in this condition. Therefore, the decrease of PBI due to vasopressin may suggest a specific effect of this hormone on the iodine metabolism.

When vasopressin was successively administered into rats during the period of cold exposure, the mean value of heat production of the rats was 767 Cal/sq.m/24 hrs. and the rate of rise in heat production was 5.9 per cent in winter. In early summer the former was 721 Cal/sq.m/24 hrs. and the latter was 1.7 per cent. The rate of rise in heat production was smaller than that of rats which were exposed to cold but not given any vasopressin, namely 9.0 per cent in winter and 7.4 per cent in early summer. The above facts indicate that exogenous vasopressin exerts an inhibitory effect on the rise in heat production of rats due to cold exposure. After vasopressin, hypofunction of the thyroid glands was also observed histologically. However, such an effect was not found in the case of oxytocin. On the other hand the level of PBI decreased extremely with vasopressin, but not with oxytocin. This fact may be explained as follows: Cold exposure may accentuate the metabolism in tissues and in turn increase removal of thyroid hormone from the circulating blood. On the other hand the output of the thyroid hormone from the gland is inhibited by the exogenous vasopressin, as indicated by the histological pictures. Therefore, the level of PBI may decrease in larger extent in vasopressin injected and cold exposed animals than in those not exposed to cold. Though cold exposure let animals' tissues spend more amount of thyroid hormone in order to pursue an increased metabolism, exogenous vasopressin may inhibit its supply. As this may be fol-
lowed by disturbing the increase in metabolism of the tissues, the heat production of these animals may remain in low level. In the case of oxytocin, such a phenomenon as with vasopressin may not occur.

The mechanism of the inhibitory action of vasopressin on the thyroid is still remained in question. Vasopressin may affect the anterior pituitary to reduce the secretion of TSH, or directly the thyroid to depress its activity. It is also possible that the release of the thyroid hormone from the gland is reduced due to the vasoconstriction in the gland resulted by the direct action of vasopressin. Further study must be performed to come to the conclusion.

SUMMARY

1. In male albino rats, adapted to live at 29.5°C for a period of more than 8 weeks, and then exposed to cold environmental temperature between 10° and 15°C for 10 days, a rise in oxygen consumption occurred. The histological picture of the thyroid indicated hyperactivity of the gland. However, change in the level of PBI was small.

2. In the heat adapted rats, which were successively given vasopressin or oxytocin, there was no significant change in oxygen consumption. However, the level of PBI became lower in the case of vasopressin but not in the case of oxytocin. Histological picture indicated a slightly depressed activity of the thyroid after vasopressin and also after oxytocin.

3. Rats were adapted to live at 29.5°C for the same time and then exposed to cold temperature between 10° and 15°C for 10 days and they were successively administered with the posterior pituitary hormone during a period of cold exposure. In the case of vasopressin the rate of rise in oxygen consumption and the level of PBI were remarkably lower than that of control ones which were exposed to cold but not given any vasopressin. Histological examination also showed an evidence of inhibitory effect on increasing of thyroid activity due to cold exposure. In the case of oxytocin these effects were hardly found.

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