Pineal Gland Prothyroid Stimulating Substance in White Leghorn Cockerels

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Abstract

Pinealectomy in 2 day old White Leghorn cockerels resulted at 50 days of age in a significant decrease in thyroid weight, which was independent of body weight. The effect was first noted at 40 days but disappeared by 70 days. Analysis of anterior pituitary glands from experimental animals showed that at 50 days of age there was a significant increase in thyroid stimulating hormone (TSH) content of pinealectomized animals as compared to sham and control animals. By 70 and 80 days of age a significant decrease in anterior pituitary TSH content of pinealectomized animals was noted. Propylthiouracil fed birds showed a significant increase in pineal weight as compared to control fed animals. Subsequent treatment with thyroxine reversed the effect of propylthiouracil treatment returning pineal weights back to control levels.

Results indicate that there is a prothyroid stimulating substance produced by the pineal gland between 40 and 60 days that causes an increase in thyroid weight. This effect, however, is only transitory in nature as shown by the return of thyroid weight to normal without additional treatment to pinealectomized birds. The pineal gland was also shown to be sensitive to the levels of thyroxine produced by the thyroid, indicating a possible feedback relationship between the thyroid and pineal gland.

Introduction

Through the years, a number of endocrine functions have been attributed to the pineal gland; investigations of the avian pineal gland have been contradictory at best. Recently it has been shown by numerous investigators that environmental lighting plays a major role in pineal function (1, 2, 5, 6, 7, 11, 12, 18, 17, 18, 19). These studies have shown the extreme sensitivity of the pineal gland to varying environmental lighting which may be held responsible for some of the early contradictory results obtained when working with the pineal gland.

The mammalian pineal gland has been intensely investigated as to its role in reproduction and its effects on other endocrine glands. It has recently been shown (3, 8, 10) to be a thyroid inhibitor, which may be due to the release of melatonin in the rat, but opinions differ as to how this effect is mediated. The gonadal and extra gonadal effects of the avian pineal gland have not been as extensively investigated as the mammalian pineal gland and most investigations have not taken into consideration environmental lighting (13, 14).

This investigation was undertaken to determine the effects of pinealectomy on immature White Leghorn cockerels, with special care taken to control the lighting conditions to which animals were exposed.

Materials and Methods

Animals. The single comb White Leghorn cockerels used in these experiments were obtained when 1 day old from the State Farm Bureau Co-op., Indianapolis, Indiana. All birds not autopsied within the first 48 hours were maintained in cages in controlled temperature (72°F) and light (14L:10D) rooms and supplied with food and water *ad libitum*.

Pinealectomy. Chicks when 2 days old were anesthetized with ether and a small piece of skin at the apex of the head was removed. A median V-shaped incision was made through the skull at the fusion of the frontal and parietal bones. The pineal gland was then removed with a pair of fine pointed forceps placed under the incision. The skull flap was then replaced, and a wound clip was applied to the skin. Sham operations were performed in the same manner with the exception that the pineal gland was not removed. At autopsy birds showing residual pineal tissue were discarded.

Three groups of animals (intact controls, sham operated, and pinealectomized) were maintained for 40, 50, 60, 70, and 80 days. Special care (cage rotation) was taken to maintain equal numbers of each group at specific cage levels, to avoid differences in lighting to each group of birds. At autopsy, body, comb, testes, adrenal, thyroid, pineal, and pituitary weights were taken. The comb factor (length in mm $\times$ height in mm/2) was also determined at this time. The anterior pituitaries were placed in acetone and refrigerated for later use in assays of pituitary TSH content.

TSH Assay. (4) The animals used in these assays were 2-day-old cockerels maintained without food or water. The animals were divided randomly into groups of ten birds for later treatment. Group 1 acted as control in each experiment receiving water injections. Groups 2, 3, and 4 received varying dosages of Bovine B-5 NIH-TSH (0.003U, 0.006U, and 0.012U/bird). The remainder of the treated groups received 1.5 mg. equivalent fresh anterior pituitary gland obtained from pooled pituitaries of intact, sham, or pinealectomized birds. Injections were made subcutaneously in the neck region, 0.2cc/bird, five hours before autopsy.

In addition each bird received 3.0 uc ^{32}P in 0.2cc distilled water one hour before autopsy. At autopsy chicks were sacrificed by cervical dislocation and the thyroids were removed, cleaned, weighed, and placed on individual planchets. The thyroids were then dried under a heat lamp for one hour and their radioactivity counted on a Nuclear Chicago automatic gas-flow counter. Results were expressed as counts per minute minus background per milligram of wet tissue (cpm-BG/mg). The TSH was a gift from the NIH Endocrinology Study Section.

Thyroid-Pineal Feedback. In an effort to clarify the possible pineal-thyroid relationship an experiment was performed to determine if there was a feedback relationship between these two glands. Animals used in this experiment were 50 days of age at sacrifice. The animals were divided into 5 groups at random, one group acting as controls received normal feed while groups 2-5 received 0.1% propylthiouracil (PTU), by weight, in the feed for 7 days. Groups 1 and 2 were injected for 4 days before sacrifice with 0.2 cc distilled water per bird subcutaneously in the neck region, while groups 3-5 received varying dosages of thyroxine (T_4), (5 ug to 20 ug/day). Animals were sacrified by cervical dislocation, and the pineals, thyroids, and anterior pituitary glands were removed and weighed. L-Thyroxine was purchased from Calbiochemical, La Jolla, California.

Statistical Analysis. In all statistical comparisons, analysis of var-

TABLE 1. *The Effects of Pinealectomy at 2 days of age on 40, 50, 60, 70, and 80 Day Old Cockerel Organ Weights.*

	N	Body g±SE	Comb g±SE	Comb Factor	Testes mg±SE	Pituitary mg±SE	Thyroid mg±SE	Thyroid % Body Weight
40 day C	8	323.0± 6.0	1.7±0.3	428.0± 22.0	74.8± 5.6	4.5±0.3	17.1±0.6	0.0053
40 day S	8	319.0± 8.0	1.9±0.2	471.0± 34.0	76.0± 5.4	4.1±0.5	15.0±1.2	0.0047
40 day P	8	284.0±12.0**	1.0±0.1	318.0± 25.0*	64.4± 5.3	4.0±0.2	13.1±0.5**	0.0047
50 day C	8	410.0± 6.0	1.7±0.2	579.0±151.0	121.9± 7.3	5.0±0.1	21.6±0.8	0.0053
50 day S	8	443.0±11.0	1.9±0.3	602.0±187.0	120.1±11.5	5.1±0.3	27.4±6.1	0.0051
50 day P	8	338.0±12.0**	1.1±0.2*	352.0±101.0*	89.5± 6.1*	4.7±0.5	13.8±0.6**	0.0041*
60 day C	10	542.0±11.0	3.0±0.3	657.0± 67.0	133.6± 8.5	5.7±0.3	26.1±1.6	0.0050
60 day S	9	490.0± 7.0	4.1±0.4	949.0± 87.0	168.6±13.5	5.7±0.2	26.9±1.1	0.0055
60 day P	7	404.0±28.0**	1.9±0.2*	468.0± 48.0**	96.1± 9.1*	5.3±0.9	22.8±2.0	0.0057
70 day C	13	605.0±18.0	4.5±1.3	1069.0± 68.0	181.3±14.0	5.9±0.3	28.1±1.0	0.0047
70 day S	8	565.0±25.0	4.2±0.8	1000.0±148.0	211.6±11.7	5.8±1.0	27.4±2.1	0.0048
70 day P	6	542.0±11.0	4.1±0.8	927.0± 39.0	190.3±51.5	6.0±0.4	24.0±2.5	0.0045
80 day C	9	784.0±42.0	6.9±1.5	1294.0±157.0	278.3±28.0	6.2±1.2	44.0±6.1	0.0057
80 day S	8	812.0±35.0	9.1±1.4	1698.0±161.0	369.8±54.4	6.9±0.2	39.6±2.8	0.0049
80 day P	7	765.0±38.0	7.6±1.7	1492.0±194.0	367.0±53.6	6.4±0.2	52.2±4.2*	0.0069*

* significant at 5% level.
** significant at 1% level.
C = Control.
S = Sham.
P = Pinealectomized.

iance was employed. First comparing for any signification variation between the groups then by partitioning and comparing with control animals.

Results

The results of pinealectomy are shown in Table 1. At 40 days of age pinealectomized cockerels showed a significant decrease in body, comb, and thyroid weight, as well as a decrease in comb factor. These weights continued to be significantly lower in 50- and 60-day-old cockerels with an additional significant decrease in testes weight of pinealectomized birds. By 70 days of age the body weights of pinealectomized birds returned within normal range of control and sham weights. On the basis of percent body weight, the thyroid weight of 50-day-old pinealectomized birds was still significantly decreased. In an additional study of 50-, 70-, and 80-day-old birds the results parallel those already described and only the data for 80-day-old birds is noted where a significant increase in thyroid weight was observed. There was no change detected in adrenal or pituitary weights.

In a further effort to verify the results obtained following pinealectomy, anterior pituitary TSH content for the different groups was also determined (Table 2) to see if there was a correlation between observed decreases in thyroid weight and pituitary TSH content. Anterior pituitaries from 50-day-old pinealectomized animals showed a significant increase in TSH content while 40- and 60-day-old animals were shown to have the same amounts of TSH present in their pituitaries. At 70 and 80 days of age there was a reversal in TSH content found in which anterior pituitaries from pinealectomized birds showed a significant decrease in TSH content.

Propylthiouracil (PTU) and thyroxine (T_4) treatment (Table 3) showed that birds that received PTU treated feed had a significantly increased pineal weight, as well as, pituitary and thyroid weights at 50 days of age, when compared to control fed animals. Subsequent treatment with 10 and 20 ug. of thyroxine resulted in weights that showed no statistical difference when compared to control animals.

TABLE 2. *Assay of Anterior Pituitary TSH from 40, 50, 60, 70, and 80 Day Old Intact Sham Operated and Pinealectomized Cockerels. Each group contained 10 assay birds.*

Treatment	Thyroids cpm—BG/mg±SE				
	40 Day	50 Day	60 Day	70 day	80 Day
Water Controls	45.6± 2.7	30.1±2.9	31.0±2.2	40.9±2.9	44.9±1.7
0.003U NIH TSH	84.9± 7.4	64.7±3.4	72.5±2.5	77.1±2.6	88.5±3.4
0.006U NIH TSH	134.1±10.5	93.5±4.2	92.0±4.3	106.5±5.5	124.4±6.5
0.012U NIH TSH	184.8± 6.7	112.1±6.2	120.8±3.5	147.0±7.1	147.7±7.2
1.5mg AP from intact donors	111.1±10.5	43.1±2.5	69.0±3.1	71.8±4.8	73.9±3.5
1.5mg AP from sham donors	111.0± 6.7	42.4±2.4			
1.5mg AP from pineal-ectomized donors	130.0± 7.4	52.6±2.4**	66.5±5.3 65.4±2.9	70.3±2.2 60.5±3.4*	71.7±2.5 56.5±2.8**

* significant at 5% level.
** significant at 1% level.

TABLE 3. *The Effects of Propylthiouracil (PTU) and Thyroxine (T_4) Treatment on Pineal, Pituitary, and Thyroid Weights of 50 Day Old Cockerels*

Treatment	N	Thyroid mg±SE	Pituitary mg±SE	Pineal mg±SE
Control	10	27.0±1.5	5.3±0.1	3.5±0.1
PTU	10	48.7±3.7**	6.5±0.2**	4.1±0.1**
PTU+T_4 (5ug)	10	42.1±2.0**	6.2±0.2**	4.1±0.2**
PTU+T_4 (10ug)	10	32.5±2.0	5.7±0.1	3.7±0.1
PTU+T_4 (20ug)	10	23.0±1.8	5.3±0.2	3.4±0.1

** significant at 1% level.

Discussion

Pinealectomy resulted in a significant decrease in thyroid weight in 50-day-old cockerels indicating a prothyroid stimulating substance. This effect was no longer observed at 70 days of age, indicating that the animals are capable of overcoming the absence of this pineal substance. This result is in contradiction to those found when working with rodents, in which it has been found the pineal is antithyrotropic (3, 8, 10). This difference could be accounted for by differences in species, as there is other evidence to indicate that avian and mammalian pineal glands differ in response to the same experimental conditions. An example of this is the response of the pineal gland to continuous light, the avian pineal shows increased hydroxyindole - 0 - methyl transferase (HIOMT) activity while under the same conditions the rat's pineal shows the opposite effect (2). A recent study by Oishi (9) showed that photoperiod changes could effect thyroid weights in Japanese quail and the observed effect could be reversed by pinealectomy, indicating a pineal-thyroid relationship in avian species. It was also noted that the effect decreased with increasing age.

Of even greater significance is a report by White, *et al.* (16), that showed the presence of significant amounts of gonadotropin-releasing hormone (Gn-RH) and thyrotropin-releasing hormone (TRH) in ovine, bovine, and porcine pineal glands. This presents the possibility that the prothyroid stimulating substance proposed by this investigation might in fact be TRH from the avian pineal gland.

That there is a prothyroid stimulating substance being released from the pineal gland of the White Leghorn cockerel was further supported by results of TSH analysis of the anterior pituitaries from experimental birds. Ablation of the pineal gland would remove the prothyroid stimulating substance from the animal which could prevent or at least not stimulate the release of TSH from the anterior pituitary. If this were the case, elevated levels of TSH would be expected in 50-day-old pinealectomized animals and decreased levels in 70- and 80-day-old animals. The data from the experiments presented here support this hypothesis.

If the relationship between the pineal and thyroid gland is hormonal, a feedback mechanism should be expected. The data presented here supports this mechanism as shown by the sensitivity of the pineal gland to the levels of thyroxine while the thyroid gland in turn is influenced by a pineal substance.

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