Thyroidectomy results in termination of photorefractoriness in starlings (*Sturnus vulgaris*) kept in long daylengths

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Summary. Four groups of 10 male starlings were transferred from short daylengths (8 h light/day) to long daylengths (18 h light/day), which caused the testes to develop rapidly to maximum size and then to decrease to minimal size as birds became photorefractory. Birds were surgically thyroidectomized at 8, 16 or 28 weeks. A fourth group was left intact. Testicular volume and plasma FSH and prolactin concentrations were measured. After 42 weeks all birds were castrated and plasma FSH was measured during the next 6 weeks. Testicular growth began in all thyroidectomized birds between 4 and 8 weeks after thyroidectomy. By 42 weeks, the testes of all thyroidectomized birds were large, whereas those of intact birds were still of minimal size. Plasma FSH concentrations remained low in all birds and plasma prolactin values, originally elevated by long daylengths, decreased at a similar rate in thyroidectomized and intact birds. After castration at 42 weeks, plasma FSH values increased rapidly in all thyroidectomized birds but remained low in non-thyroidectomized birds. The results demonstrate that thyroidectomy of photorefractory starlings does not induce immediate testicular growth but may initiate a process which eventually terminates photorefractoriness in a way similar to that caused by return to short daylengths.

Introduction

In most species of birds from mid- and high latitudes the breeding season is terminated by the onset of photorefractoriness. Although gonadal growth is stimulated by increasing daylength during spring, the gonads are maintained in a functional state only for a limited period after which they spontaneously regress. This occurs during long, and often increasing, daylengths. After the onset of photorefractoriness, exposure to a period of short daylengths is necessary for most species (including starlings) before they again become photosensitive.

The onset of photorefractoriness in starlings is known to be dependent on the thyroid gland because thyroidectomized birds do not become photorefractory when transferred to long daylengths (Wieselthier & van Tienhoven, 1972; Goldsmith & Nicholls, 1984a; Dawson, Follett, Goldsmith & Nicholls, 1985) and because photorefractoriness can be induced by treatment with thyroxine (Goldsmith & Nicholls, 1984b; Nicholls, Goldsmith, Dawson, Chakraborty & Follett, 1984). The effect of thyroidectomy on birds that have already become photorefractory is unclear, although the results of Wieselthier & van Tienhoven (1972) suggested that it might result in gonadal growth.

In many previous studies on starlings we have found that the onset of photorefractoriness is associated with increased plasma prolactin concentrations and that this increase appears to be caused by increased daylength (Dawson & Goldsmith, 1982, 1983, 1984; Elbing, Goldsmith &

Follett, 1982; Goldsmith & Nicholls, 1984c). However, in thyroidectomized starlings transferred to long daylengths plasma prolactin (Goldsmith & Nicholls, 1984a) and pituitary prolactin (Dawson *et al.*, 1985) concentrations remain very low. Plasma prolactin concentrations were measured in this study because the effect of thyroidectomy on plasma prolactin, which had already been increased as a result of transfer to long daylengths, was unknown. The occurrence of feather moulting was also recorded. In intact birds, a full moult begins after they become photorefractory as the testes reach minimal size. In birds thyroidectomized before transfer to long daylengths, moult is prevented (Woitekewitsch, 1940; Dawson *et al.*, 1985). The effect of thyroidectomy once moult has begun was not known.

Materials and Methods

Experimental design

Juvenile male starlings (*Sturnus vulgaris*) were trapped during autumn and maintained in outdoor aviaries. In November, 40 birds were transferred indoors and kept in cages (5 birds per cage) in a photoperiod of 8 h light (09:00–17:00 h) and 16 h darkness per day (8L:16D). Food (chick starter crumbs, BOCM Silcock) and water were freely available.

After 5 weeks, 10 control birds (Group C) were laparotomized; they were anaesthetized with an intramuscular injection of 60 µl metomidate (Hypnodil: Crown Chemical Co., Lamberhurst, Kent, U.K.), a small incision was made on the left side and the dimensions of the left testis were measured. Photoperiod was then increased to 18L:6D (08:00–02:00 h). The same 10 birds were laparotomized again 3 weeks later. After 8 weeks the 10 control birds and 10 experimental birds were laparotomized and a blood sample (800 µl) was collected from the wing vein. Blood samples were always collected between 10:00 and 12:00 h. The blood was centrifuged and plasma stored at –20°C. Moult was recorded by quantifying the progressive loss and renewal of the 9 primary flight feathers (Newton, 1966). A few days later the 10 experimental birds were anaesthetized and surgically thyroidectomized (Group T8). Blood samples were collected from these thyroidectomized birds and from control birds after 1 and 2 weeks. Further blood samples were collected, and testicular size and moult were recorded, every 4 weeks thereafter. At 16 weeks after transfer to 18L:6D, 10 more birds (Group T16) were blood sampled, laparotomized and thyroidectomized, and then blood sampled and laparotomized every 4 weeks. After 28 weeks, the final group of 10 birds (Group T28) was treated in the same way.

After 42 weeks all surviving birds were blood sampled and then castrated. They were kept in 18L:6D and further blood samples were collected after 1, 2, 4 and 6 weeks.

Hormone assays

Concentrations of FSH and prolactin in plasma samples were measured using heterologous radioimmunoassays (Follett, 1976; McNeilly, Etches & Friesen, 1978). These assays have been validated for use with starling plasma (Dawson & Goldsmith, 1982; Ebling *et al.*, 1982). Plasma samples were shown to give dilution curves parallel to those of the assay standards (NIADDK ovine prolactin S-12 and rat FSH RP1 respectively). Plasma samples were assayed in duplicate 20 µl volumes. The inter-assay coefficients of variation were 17.9% ($n = 9$) and 20.7% ($n = 10$) for the prolactin and FSH assays respectively and sensitivities were 2 µg/l and 20 µg/l respectively. Plasma LH concentrations were not measured because our avian LH assay cross-reacts with TSH. The degree of cross-reaction is acceptable in intact birds but not in thyroidectomized birds. There is no significant cross-reaction of TSH in the FSH assay. Thyroxine concentrations were measured in a plasma sample from each bird to verify the success of the thyroidectomies. The assay used has been validated for starling plasma (Dawson, 1984). Inter-assay variance was 14% ($n = 7$), sensitivity was 1 nmol/l and cross-reaction with triiodothyronine was 3%.

The results were assessed by analysis of variance with repeated measures followed by Newman-Keuls tests to determine within treatment differences (Winer, 1971). Between treatment differences were measured using Student's *t* tests.

Results

Transfer of birds from 8L:16D to 18L:6D caused an increase in testicular volume from about 1 mm³ to about 150 mm³ after 3 weeks. By 8 weeks, birds had become photorefractory and the testes had decreased to their initial volume.

Not all of the subsequent thyroidectomies were successful in that in some birds functional thyroid glands developed later, presumably from small pieces of tissue left after the operation. Such birds could be easily distinguished from successfully thyroidectomized birds which had undetectable plasma thyroxine concentrations and abnormal feathers. Thyroidectomy before transfer to long daylengths prevents subsequent moult but, in the present experiment, in which birds were thyroidectomized at the beginning of moult, or during it, moult continued. The feathers grown after successful thyroidectomy failed to mesh together and were longer and paler than normal. By these two criteria, it was determined that only 5/10 birds in Group T8 were successfully thyroidectomized, and that 7 in each of Groups T16 and T28 were successful. Other birds were eliminated from the experiment.

In Group T8 birds, there was no testicular growth during the 4 weeks after thyroidectomy and only slight growth after 8 weeks (Text-fig. 1). By 12 weeks testicular size had increased significantly

Text-fig. 1. Volume of the left testis in intact (Group C) and thyroidectomized (Groups T8, T16, T28) male starlings after transfer from a photoperiod of 8 h light:16 h darkness per day (8L:16D) to 18L:6D. Arrows denote times at which thyroidectomies were carried out. Testicular volume was calculated as $4 \cdot 2(a^2b)$ where *a* is the radius of the testis at its widest point and *b* is half of its length. Note that volume is plotted on a log scale. Each point represents the mean $\pm$ s.e.m.

($P < 0.01$), was significantly higher ($P < 0.001$) than in Group C, and continued to increase thereafter. In the birds in Groups T16 and T28, there was no testicular growth during the 4 weeks after thyroidectomy, but a substantial increase in testicular size ($P < 0.01$) by 8 weeks gave values significantly higher ($P < 0.001$) than in Group C. In all three groups, therefore, testicular growth began between 4 and 8 weeks after thyroidectomy, although growth rate appeared to be faster in Groups T16 and T28. At 42 weeks after transfer to 18L:6D all thyroidectomized birds had large testes, whereas the testes of control birds were still of minimal size.

Plasma FSH remained below or just above the detection limit of the assay (20 $\mu\text{g/l}$) in all samples taken before 42 weeks after transfer to 18L:6D, in intact and thyroidectomized birds. Castration of thyroidectomized birds at 42 weeks resulted in a significant ($P < 0.01$) increase in plasma FSH concentration 1 week later, significantly higher than in Group C ($P < 0.001$), in all three thyroidectomized groups, and these high levels were maintained over the next 6 weeks (Text-fig. 2). Castration of the non-thyroidectomized birds at the same time did not cause an increase in plasma FSH and values remained below 35 $\mu\text{g/l}$.

Text-fig. 2. Plasma FSH concentrations after castration in thyroidectomized (Groups T8, T16, T28) and non-thyroidectomized (Group C) male starlings kept in 18L:6D. All birds were castrated at Week 0, which was 42 weeks after transfer to 18L:6D. Each point represents the mean $\pm$ s.e.m.

Plasma prolactin concentrations were measured in Group T8 birds for 12 weeks after thyroidectomy, and in control birds over the same period. In control birds plasma prolactin decreased significantly ($P < 0.01$) from 38.2 ± 2.6 to 9.1 ± 0.7 $\mu\text{g/l}$ (Text-fig. 3). In thyroidectomized birds there was also a decrease in plasma prolactin. The decrease appeared to be more rapid than in the controls, but those birds that were to be thyroidectomized had, by chance, higher prolactin concentrations than did the controls immediately before the operation. At no time during the following 12 weeks were plasma prolactin values in the thyroidectomized birds significantly different from those in control birds.

Discussion

The results clearly demonstrate that removal of the thyroid glands from photorefractory starlings kept in long daylengths resulted in testicular growth, whereas in intact starlings the testes remained small. Testes of intact photorefractory starlings kept in long daylengths remain small indefinitely (Hamner, 1971).

Text-fig. 3. Plasma prolactin concentrations in thyroidectomized (Group T8) and intact (Group C) male starlings. Birds were thyroidectomized at Week 0, 8 weeks after transfer from 8L:16D to 18L:6D. Each point represents the mean $\pm$ s.e.m.

Although it has been known for some time that photosensitive starlings, thyroidectomized before exposure to long daylengths, grow their testes normally after transfer to long daylengths, but that they do not become photorefractory and their testes remain large (Wieselthier & van Tienhoven, 1972; Goldsmith & Nicholls, 1984a), it was not known what would be the effect of thyroidectomy of photorefractory birds. Wieselthier & van Tienhoven (1972) have suggested that there may be gonadal growth, but it was unclear from their data whether this occurred immediately after thyroidectomy, or some months later. The present results indicate that thyroidectomy is not followed by immediate gonadal redevelopment: 4–8 weeks elapse before there is any testicular growth and this delay is not markedly affected by how long birds have been photorefractory before thyroidectomy. Thyroidectomy does not immediately abolish photorefractoriness during long daylengths, but apparently permits the mechanism by which photosensitivity is regained to begin. After transfer of intact photorefractory birds from long to short daylengths, photosensitivity is regained after approximately the same period (Goldsmith & Nicholls, 1984d; A. Dawson, unpublished results). Thyroidectomy may therefore initiate a process similar to that caused by return to short daylengths.

Despite the fact that testes grew in all thyroidectomized birds, plasma FSH concentrations remained low. This was not unexpected. In intact starlings transferred from short to long daylengths there is a rapid increase in plasma FSH concentrations (Dawson & Goldsmith, 1983; Goldsmith & Nicholls, 1984b) but by 6 weeks after transfer levels decrease to initial values as birds become photorefractory. Castration of such birds does not cause an increase in plasma values (Goldsmith & Nicholls, 1984b) and pituitary FSH content is low (Dawson *et al.*, 1985); the low plasma gonadotrophin concentrations characteristic of photorefractory birds are independent of gonadal steroid feedback. When thyroidectomized starlings are transferred from short to long daylengths there is also a rapid increase in plasma FSH values during the period of testicular growth, but again, by 6 weeks, plasma levels decrease to initial values. In this case, however, low plasma concentrations are due only to feedback by gonadal steroids: pituitary content is still high (Dawson *et al.*, 1985) and castration of these birds results in an immediate increase in plasma FSH

values (Goldsmith & Nicholls, 1984a). In the present study, the change from a photorefractory state to a photosensitive state some weeks after thyroidectomy was probably gradual, and the acute increase in plasma FSH concentrations seen in birds transferred to long daylengths did not occur. However, it was demonstrated by castration that these birds were photosensitive, and that low plasma levels were due to gonadal steroid feedback. Gonadectomy resulted in a rapid increase in plasma FSH values in thyroidectomized birds, but no increase in the intact birds.

Transfer of intact starlings from short to long daylengths causes increased plasma prolactin concentrations which peak at about the time birds become photorefractory and then slowly decline (Ebling *et al.*, 1982; Dawson & Goldsmith, 1983; Goldsmith & Nicholls, 1984b). However, in thyroidectomized starlings transferred to long daylengths, both plasma prolactin (Goldsmith & Nicholls, 1984a) and pituitary prolactin (Dawson *et al.*, 1985) values remain very low. The effect of thyroidectomy on birds with already high plasma prolactin concentrations was unknown, and the present results are ambiguous. Plasma prolactin levels decreased in thyroidectomized birds but it is impossible to say whether they decreased faster than in control birds. Clearly though, thyroidectomy did not result in an immediate decrease in plasma prolactin concentrations to values as low as those in birds thyroidectomized before transfer to long daylengths (Goldsmith & Nicholls, 1984a).

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