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Blockade of Menstrual Cycle by Thyroidectomy in Japanese Monkeys (*Macaca fuscata fuscata*)

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To examine the role of thyroid hormones in the seasonal breeding cycle in Japanese monkeys (*Macaca fuscata fuscata*), sexually mature females were thyroidectomized (*n* = 6) in early December, during the midbreeding season, or they received sham operations (*n* = 4). They were housed indoors individually, and blood samples were collected two to three times a week to monitor gonadotropin and gonadal steroid hormone secretions. Control monkeys exhibited ovulatory cycles during the breeding season. The mean dates of onset and end of the ovulatory cycles were October 22 ± 13 d and February 25 ± 14 d, respectively. These dates coincided well with those of our colonies under captivity. By contrast, three of the six thyroidectomized monkeys terminated ovulatory cycles immediately after operations; the remaining three monkeys ovulated only once or twice after thyroid removal. The mean dates of onset and end of the ovulatory cycles of thyroidectomized monkeys were October 18 ± 4 d and December 31 ± 4 d, respectively. This was a significantly earlier termination of the ovulatory cycles than in controls. Mean concentrations of plasma thyroxine of control monkeys were maintained throughout the experimental period, whereas plasma thyroxine concentrations of thyroidectomized monkeys decreased abruptly to undetectable levels. Thyroidectomized monkeys exhibited significantly higher levels of plasma prolactin (PRL) than controls. Moreover, even in control monkeys, plasma PRL increased during the transition out of the breeding season. These results suggest that thyroid hormones play an important role in the regulation of ovulatory cycles in Japanese monkeys, directory or indirectly, possibly by mediating the changes of PRL secretion.

Key Words: Macaque monkey; menstrual cycle; thyroidectomy; seasonal breeding; prolactin.

Introduction

Gonadal function of most avian species living in the temperate zones increases under long-day exposures. Prolonged exposures to long days, however, result in a decrease in gonadal functions. This phenomenon is well known as photorefractoriness in birds *(1)*. The adaptive significance of this phenomenon is considered to be the simultaneous reactivation of the gonads of all members of the group in the subsequent breeding season. It is further reported that thyroidectomy in European starlings and in some other temperate-zone birds abolishes the onset and development of photorefractoriness, and the gonads continue to develop regardless of photoperiod or seasons *(1)*.

In 1988, Nicholls et al. *(1)* reported the first evidence that thyroidectomized ewes continue estrous cycles beyond the end of the normal breeding season, and further throughout the entire period of the following nonbreeding season. They suggested that the thyroid hormones play an important role in the termination of seasonal reproductive activity in both birds and sheep. Subsequent studies in ewes have suggested that thyroid hormones are necessary only during a limited interval late in the breeding season in order to promote seasonal reproductive suppression *(2–4)*.

Among macaques, some species such as the rhesus monkey and the Japanese monkey display a distinct seasonal pattern of breeding activity in outdoor environments. In both species, mating takes place in fall and winter and births occur during spring and summer, when sexual behavior is virtually absent *(5–10)*. Thus, these monkeys are so-called short-day breeders like sheep. Furthermore, seasonal breeding of the Japanese monkey seems to be governed by biannual changes in the response of the hypothalamo-hypophysial axis to the negative feedback action of estradiol, as is seen in sheep *(11)*. However, although it is believed that seasonal breeding of these monkeys is governed by the integration of environmental and internal factors as in other seasonally breeding mammals, little is known about environmental factors regulating the seasonal breeding of these monkeys *(12)*. For example, photoperiod may not be a critical environmental factor influencing the seasonal breeding of macaque monkeys, since artificial manipulation of photoperiod had no effect on the annual reproductive cyclicity of these animals *(13)*. Accordingly, the problem of photorefractoriness

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is even more challenging in view of the absence of photoperiodic influence in regulating the seasonal breeding of macaque monkeys.

Although it is reported in humans that both hyper- and hypothyroidism may result in menstrual disturbances *(14, 15)*, the literature is almost completely lacking on the involvement of thyroid hormones in reproduction in macaque monkeys. The present study was designed to investigate the possible role of thyroid hormones in the seasonal breeding of Japanese monkeys.

Results

Incidence of Menstrual Cycles

Controls

Plasma progesterone profiles of each of the four control monkeys are shown in Fig. 1. All monkeys exhibited regular cyclic elevations in plasma progesterone during the breeding season (autumn and winter months), indicating that cyclic ovulations occurred during that period. These monkeys received sham operations in early December, during the midbreeding season. This procedure had no effect on ovulatory cyclicity of these monkeys (Fig. 1). The mean dates of onset and end of the ovulatory cycles were October 22 ± 13 d ($n = 4$; range: October 1–November 26) and February 25 ± 14 d ($n = 4$; range: January 29–April 3), respectively (Fig. 1), which coincided well with the onset and end of the breeding season of our colonies *(11)*.

Experimental Animals

Plasma progesterone profiles of each of the six experimental monkeys are also shown in Fig. 1. These six monkeys exhibited regular cyclic elevations in plasma progesterone before thyroidectomy as well (Fig. 1). The mean date of onset of the ovulatory cycles in these monkeys was October 18 ± 4 d ($n = 6$: range: October 3–30), and, thus, there was no significant difference in mean dates of the onset of the ovulatory cycles between the two groups. These monkeys were thyroidectomized in early December, during the midbreeding season. In contrast to sham controls, three thyroidectomized monkeys (nos. 942, 1056, and 573) terminated ovulatory cycles immediately after surgery, and the remaining three monkeys (nos. 757, 758, and 769) ovulated only once or twice after thyroidectomy. The mean date of the end of the ovulatory cycles of these thyroidectomized monkeys was December 31 ± 4 d ($n = 6$; range: December 4–February 1), which was significantly earlier than termination of the ovulatory cycles in controls.

Changes in Plasma Thyroxine

Changes in mean concentrations of plasma thyroxine in thyroidectomized and in control monkeys are shown in Fig. 2. In control monkeys, plasma thyroxine levels were maintained throughout the experimental period. By contrast, in thyroidectomized monkeys, plasma thyroxine decreased abruptly to undetectable levels after thyroidectomy. In three of the six monkeys (nos. 757, 758, and 942), plasma thyroxine remained undetectable throughout the experimental period (until the end of April). In the remaining three animals (nos. 573, 769, and 1056), however, plasma thyroxine began to increase gradually after having remained undetectable for about 1 mo, and reached about two-thirds of the preoperative levels by the end of April. There was no relation between the dates of cessation of ovulatory cycles and postoperated plasma thyroxine levels in thyroidectomized monkeys.

Changes in Plasma Luteinizing Hormone and Estradiol

Changes in mean concentrations of plasma luteinizing hormone (LH) and estradiol in both control and thyroidectomized monkeys are shown in Fig. 3. No difference was found in mean concentrations of plasma LH between pre- and postoperations in either control or thyroidectomized monkeys. Mean concentrations of plasma estradiol of controls increased slightly during the autumn and winter months, but plasma estradiol of the thyroidectomized monkeys decreased significantly following thyroidectomy.

Changes in Plasma Prolactin

Changes in mean concentrations of plasma prolactin (PRL) in both control and thyroidectomized monkeys are shown in Fig. 4. In control monkeys, plasma PRL increased gradually during late winter and early spring; it then gradually declined until the end of April. The timing of the maximum increase in plasma PRL in control monkeys apparently coincided with or occurred slightly after the termination of the ovulatory cycles (Fig. 4). On the other hand, in thyroidectomized monkeys, plasma PRL increased rapidly following thyroidectomy, and remained high throughout the experimental period (until the end of April).

Discussion

In the European starling and Japanese quail, both are long-day breeders, and the reproductive season ends spontaneously during summer, although day length is still long, an endogenously generated condition referred to as photorefractoriness *(16)*. Thyroidectomy prevents refractoriness from developing and the birds remain in breeding condition indefinitely under long days *(17,18)*. In mammals, although relatively little information is available concerning the involvement of the thyroid gland in the seasonal reproductive cycles, previous studies have suggested that the thyroid gland is necessary for seasonal reproductive suppression in the golden hamster *(19)*, red deer *(20,21)*, mink *(22)*, and sheep *(16,23)*. In all these seasonal breeders, thyroidectomy results in prolongation of breeding condition and activity.

On the other hand, the present study clearly shows that thyroidectomy in Japanese monkeys results in significantly earlier termination of the breeding season than in controls.

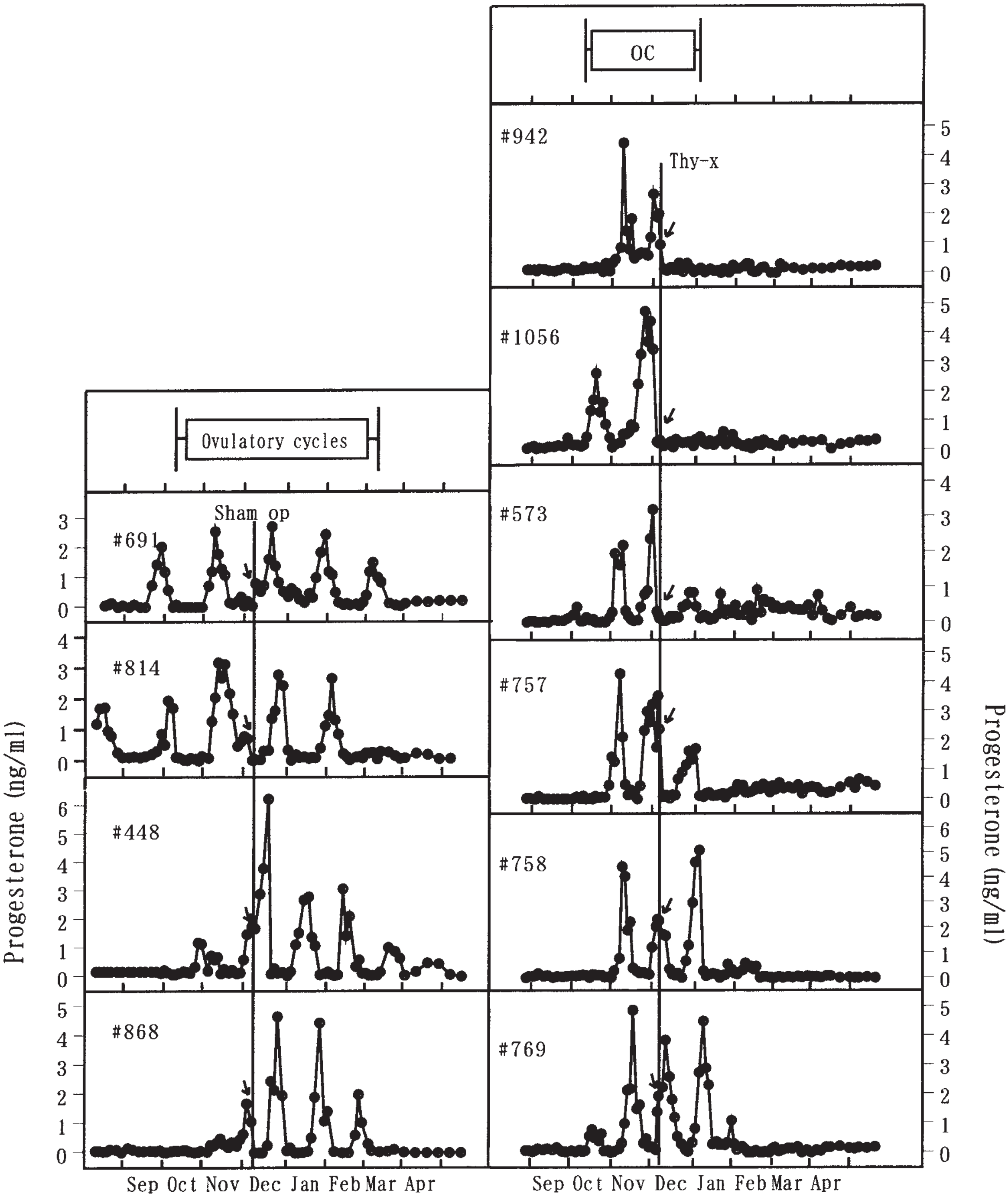

Fig. 1. Plasma concentrations of progesterone in individual monkeys. They were sham operated (**left; Sham Op**) or thyroidectomized (**right; Thy-x**) in early December (arrows), during the midbreeding season. (**Left**) Sham op and Thy-x indicate the time of operation. Note cyclic changes in plasma progesterone in all four monkeys during the breeding season, autumn through winter months. (**Right**) Note cyclic changes in plasma progesterone in all six monkeys during the breeding season before thyroidectomy. Also note an abrupt cessation of the cyclic elevations of plasma progesterone in nos. 942, 1056, and 573, following thyroidectomy. The horizontal bars in the uppermost panels represent the mean dates ± SEM of onset and end of the ovulatory cycles (OC) of each group of monkeys, which were designated as the day of estimated LH surge in the first ovulatory cycle of the season, and the first day of onset of the menses following the last ovulatory cycle of the same season, respectively.

Moreover, since half of the thyroidectomized monkeys terminated ovulatory cycles immediately after the operations, the thyroid gland seems to play an important role in maintaining ovulatory cycles in the Japanese monkey, rather than involvement in the termination of the breeding season. Thus, the present results indicate a different role for thyroid hormones than they have in other seasonally breeding birds and mammals. Furthermore, since ovulatory cycles were

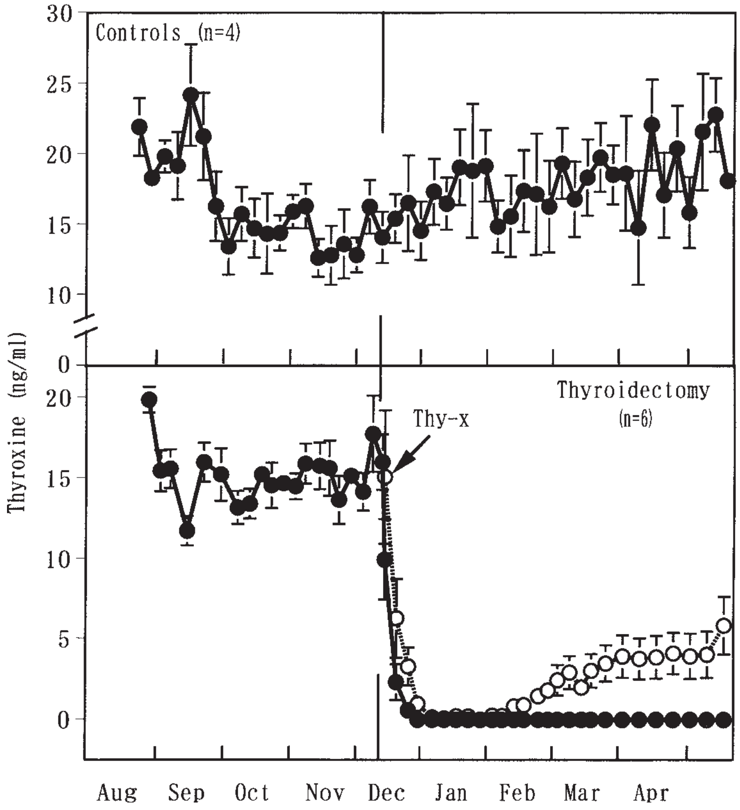

Fig. 2. Plasma concentrations of thyroxine (mean ± SEM) in control (**top**) and thyroidectomized (**bottom**) monkeys. Thy-x indicates the time of thyroidectomy. In three of the six thyroidectomized monkeys, plasma thyroxine remained undetectable throughout the experimental period (●), whereas in the remaining three thyroidectomized monkeys, plasma thyroxine began to increase gradually after having remained undetectable for about 1 mo (○).

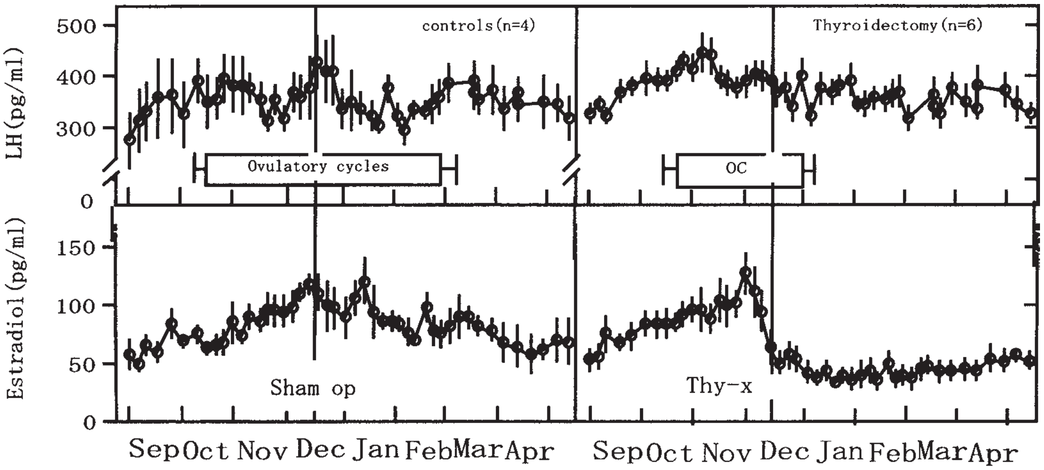

Fig. 3. Plasma concentrations of LH and estradiol (mean ± SEM) in control (**left**) and thyroidectomized (**right**) monkeys. Sham op and Thy-x indicate the time of operation. The horizontal bars in upper panels represent the mean dates ± SEM of onset and end of the ovulatory cycles (OC) of the respective groups of monkeys.

terminated soon after thyroidectomy, no evidence of induced refractoriness was suggested in the present study.

It has been reported in humans and rats that both hypo- and hyperthyroidism can produce menstrual disorders, amenorrhea and/or sterility *(15,24–26)*, and alteration of testicular function *(27)*. It has been reported also that in rats after thyroidectomy, cyclic changes in LH-releasing hormone (LHRH) in the hypothalamus were disrupted and serum concentrations of LH and estradiol failed to increase during the proestrous day *(28)*. Administration of thyroxine restored normal concentrations of LHRH in the hypothalamus, and LH and estradiol in the blood circulation *(28)*. Thus, it seems likely that interference with the normal balance of thyroid hormones provokes a variety of physiologic

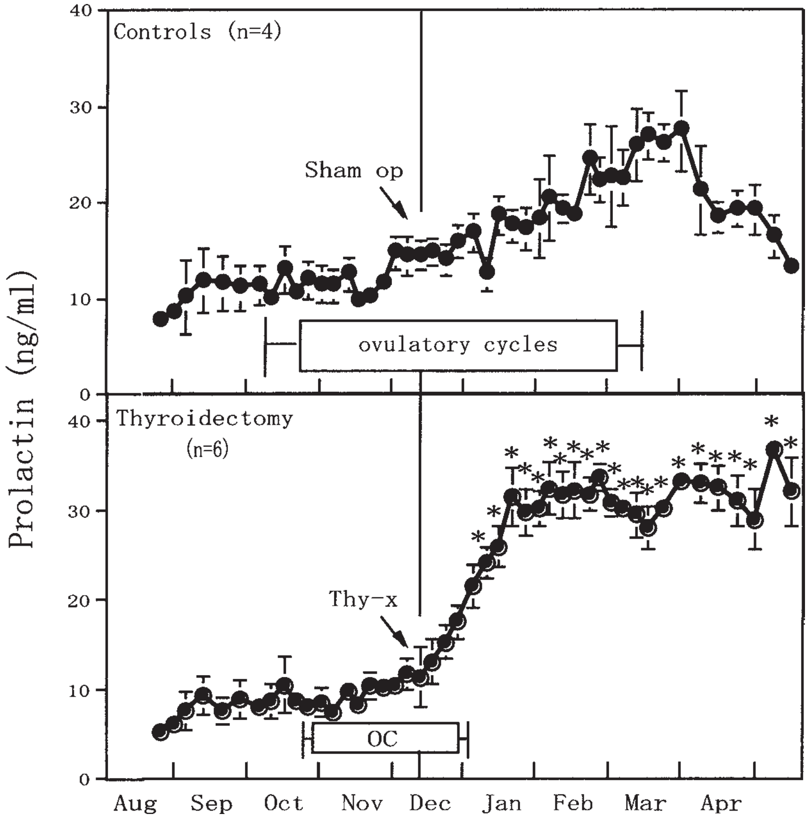

Fig. 4. Plasma concentrations of PRL (mean ± SEM) in control (**top**) and thyroidectomized (**bottom**) monkeys. Sham op and Thy-x indicate the time of operation. Note an abrupt increase in plasma PRL in thyroidectomized monkeys following thyroidectomy. The horizontal bars in each panel represent the mean dates ± SEM of onset and end of the ovulatory cycles (OC) of the respective groups of monkeys. The asterisk in the lower panel shows significant differences between corresponding dates of the two groups ($p < 0.05$).

and pathologic effects in the reproductive system in these animals.

In the present study, plasma PRL increased rapidly after thyroidectomy. However, previous results on PRL secretion following thyroidectomy or hypothyroidism have been contradictory among mammals. In primary hypothyroidism of humans, baseline plasma PRL concentrations are usually within the normal range, although a highly variable incidence of hyperprolactinemia (ranging from 0 to 39%) has also been well established *(29,30)*. Plasma levels of PRL do not change significantly in thyroidectomized ewes *(31,32)*, whereas they are lowered in thyroidectomized lactating rats *(33)*. Again, the present results in Japanese monkeys differ markedly from those previously reported in other mammals.

Mechanisms involved in PRL secretion following thyroidectomy are not clear in Japanese monkeys. However, the marked increase in plasma PRL seen in thyroidectomized monkeys may be attributed to the increased secretion of hypothalamic thyrotropin-releasing hormone (TRH) owing to the elimination of the negative feedback action of thyroid hormones, which stimulates secretion of both thyrotropin and PRL. Hypothalamic dopamine may also be involved in hyperprolactinemia of thyroidectomized Japanese monkeys, since dopamine has been identified as a major inhibiting factor of PRL release in mammals *(34,35)*. In support of the latter possibility, it has been suggested in humans that hyperprolactinemia in primary hypothyroidism might result from decreased hypothalamic dopamine secretion rather than from an increase in TRH secretion *(30,36)*. In contrast to humans, it has been reported in rats that hypothyroidism increases dopamine release into hypophysial stalk blood, which results in the decrease in plasma PRL levels *(33)*. Thus, differences in circulating levels of PRL accompanying hypothyroidism among species seem to reflect, at least in part, the difference of response of hypothalamic dopamine to thyroid hormone levels.

In the present study, plasma PRL levels of control monkeys were unchanged during the transition into the breeding season, but increased during the transition out of the breeding season. In partial agreement with the present results, Walker et al. *(37)* also reported in outdoor-housed rhesus monkeys that serum PRL levels remained elevated in lactating females until 50 d before the first ovulation of the breeding season, but were low throughout the ovulatory, anovulatory and ensuing ovulatory period. In neither humans nor monkeys PRL secretion is affected by physiologic changes in estradiol throughout an ovulatory cycle *(38,39)*. Furthermore, consistent changes in plasma PRL concentrations have not been found after ovariectomy and subsequent replacement therapy with estradiol in monkeys *(39)*. Accordingly, such an increase in plasma PRL during the transition out of the breeding season may be attributed to the decrease in hypothalamic inhibition of PRL secretion. Although seasonal breeding of the Japanese monkey is possibly governed by biannual changes in the response of the hypothalamo-hypophysial axis to the negative feedback action of estradiol *(11)*, it is not clear whether the same mechanisms are involved in the seasonal increase in PRL secretion. In either case, the present study showed that even in control monkeys termination of the ovulatory cycles is accompanied by the increased secretion of PRL.

In the present study, only three of the six thyroidectomized monkeys were successfully thyroidectomized, as judged from postoperated plasma thyroxine levels. The recovery of plasma thyroxine in several thyroidectomized monkeys may be attributed to the compensatory hypertrophy of the remaining thyroid tissues, which were left therein together with the parathyroid gland.

Finally, the present study suggests that thyroid hormones are involved in the regulation of the ovulatory cycles of Japanese monkeys, directly or indirectly, possibly by mediating the modification of PRL secretion.

Materials and Methods

Animals

Ten sexually mature female Japanese monkeys (*Macaca fuscata fuscata*), 6–15 yr old and weighing 6.4–9.5 kg, with normal reproductive histories were used. Before the experiment, animals had been housed in outdoor quarters in social groups consisting of one male and several females. They were transferred to individual cages in indoor quarters in early August 1991, during the nonbreeding season, where a 12-h light/12-h dark regimen was employed throughout the year, but ambient temperature was modified only during severe seasons. The animals were fed a standard monkey pellet food supplemented with sweet potatoes or fruit daily and allowed free access to water. Olfactory, visual, and auditory access to each other was not restricted. All animal husbandry except for blood collections was performed between 9:30 and 11:30 AM. A check for vaginal bleeding was also made daily during animal husbandry. Blood samples (2 to 3 mL) were collected between 2:30 and 4:30 PM from the cubital vein without anesthesia two to three times a week throughout the experimental period. Monkeys were well acclimated to blood collections, so that they appeared minimally stressed when they occurred. The plasma was separated and stored at –30ºC until the assay.

Thyroidectomy

Thyroidectomy was performed in six monkeys under deep anesthesia with halothane and nitrous oxide gas in early December 1991, during the midbreeding season. The parathyroid gland is located within or adjacent to the thyroid gland and care was taken to remove only thyroid tissues. The remaining four monkeys received a sham operation and served as controls. The experimental protocol was approved in accordance with the Guide for the Care and Use of Laboratory Primates prepared by the Primate Research Institute, Kyoto University in 1986.

Radioimmunoassays

Immunoreactive LH was measured by a heterologous radioimmunoassay (RIA) described previously *(11)*. In brief, the radioiodinated preparation was National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) rat LH-I-5, and the antiserum was antiovine LH (YM #18), which was provided by Dr. Y. Mori, University of Tokyo, Tokyo, Japan. Results are expressed in terms of NIDDK rat LH-RP-2. Pituitary homogenates and peripheral plasma of Japanese monkeys gave displacement curves parallel to those of rat LH RP-2 standard. The lower limit of sensitivity of the assay was 50 pg/mL. The intra- and interassay coefficients of variation (CVs) were 7.8 and 10.3%, respectively. The concentrations of progesterone and estradiol-17β were determined by specific RIAs using progesterone antiserum (lot no. HAC-AA63-01RBP79) and estradiol-17β antiserum (lot no. HAC-AA64-01RBP79) *(11)*. These antisera were provided by Dr. K. Wakabayashi (Gunma University, Gunma, Japan). The lower limits of sensitivity of the assay for progesterone and estradiol-17β were 100 and 10 pg/mL, respectively. The intra- and interassay CVs were 2.8 and 5.7% for progesterone and 2.7 and 7.9% for estradiol-17β, respectively. Plasma thyroxine was measured by a double-antibody RIA. The standard buffer was 0.2 *M* glycine (pH 8.6) containing 0.128 *M* sodium acetate, 0.4% (w/v) gelatin, and 2% (w/v) sodium salicylate. Antiserum to thyroxine was kindly provided by Dr. M. Suzuki (Gunma University, Gunma, Japan). ^{125}I-1abeled thyroxine was purchased from ICN. The intra- and interassay CVs were 9.4% and 10.9%, respectively.

Statistical Analyses

Ovulations were inferred on the basis of increases in plasma estradiol coinciding with peak levels of LH secretion, followed by sustained elevations of progesterone (>1 ng/mL). If an LH surge was not detected, presumably owing to infrequent sampling, the day of the LH surge was estimated from the profiles of plasma estradiol and progesterone during the preovulatory period *(24,25)*. The day of ovulation was tentatively assigned as that of the preovulatory LH surge. The days of onset and end of the ovulatory cycles were designated as the day of estimated LH surge in the first ovulatory menstrual cycle of the season, and the first day of onset of the menses following the last ovulatory menstrual cycle of the same season, respectively. All data obtained were expressed as the group mean ± SEM. Differences between two means were evaluated with student's *t*-test or Cochran-Cox test. All statistical tests with $p < 0.05$ were considered significant.

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