Regulation of oestrogen-induced LH release in male and female marmoset monkeys (*Callithrix jacchus*)

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Summary. The positive-feedback action of oestradiol-17 β on LH release was studied in gonadectomized male and female and intact male marmoset monkeys. Positive feedback was observed in normal intact males in response to a single subcutaneous injection of 35 μ g oestradiol benzoate. The sustained elevation in oestradiol-17 β levels achieved by the injections resulted in a marked suppression of circulating testosterone concentrations. Subcutaneous injections (0.5 mg/injection) of testosterone or dihydrotestosterone to gonadectomized males and females immediately following and 8 h after oestradiol benzoate failed to inhibit positive feedback. Similar treatment with progesterone (1.0 mg/injection) tended to standardize both positive and negative components of the feedback response. In contrast, progesterone implants (achieving progesterone concentrations similar to those obtained with the injections), maintained during and for 8 days before the oestradiol benzoate treatment, effectively inhibited positive feedback. LH responses to the various treatments were similar in gonadectomized males and females. These data suggest a relative unimportance of testicular secretions in suppressing oestrogen-induced LH release in the adult marmoset and indicate similarities between the control of positive feedback in male and female primates.

Introduction

In rodents (Barraclough, 1966; Brown-Grant & Sherwood, 1971) and sheep (Short, 1974; Clarke, Scaramuzzi & Short, 1976) the mechanism which regulates cyclic gonadotrophin function is rendered permanently inoperative in males by testicular hormones secreted during a critical period of development. This sexual differentiation of the cyclic control centre in males abolishes the capacity to respond to an oestrogen stimulus with a gonadotrophin surge (i.e. positive feedback) (Neill, 1972; Karsch & Foster, 1975; Buhl, Norman & Resko, 1978). In primates, however, it has been shown that the mechanism controlling the positive feedback response is not sexually differentiated to the same extent (Goy & Resko, 1972; Karsch, Dierschke & Knobil, 1973; Knobil, 1974). Castrated (but not intact) male rhesus and pig-tailed macaques chronically treated with low doses of oestrogen will release LH in response to an additional oestrogen stimulus (Karsch *et al.*, 1973; Steiner, Clifton, Spies & Resko, 1976; Steiner, Schiller, Barber & Gale, 1978). Oestrogen-induced LH release has also been reported in man by some workers (Dörner, Rohde, Stahl, Krell & Masius, 1975; Kulin & Reiter, 1976) but not by others (Van Look, Hunter, Corker & Baird, 1977). More recently, a positive discharge of LH in response to a single oestrogen injection was clearly described in intact as well as castrated male marmoset monkeys (Hodges & Hearn, 1978).

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A more detailed study in the marmoset, designed to examine the positive feedback response in males and females under different hormonal conditions, is now described. LH responses to a single injection of oestradiol benzoate were examined in normal intact males. The effects of physiological levels of testosterone and dihydrotestosterone, and different regimens of progesterone treatment, on oestrogen-induced LH release in gonadectomized male and female marmosets are also described.

Materials and Methods

Sexually mature male and female marmoset monkeys from the M.R.C. Primate Colony, Edinburgh, were used. The animals weighed 0.22–0.32 kg and full details of their management have been published elsewhere (Hearn, Lunn, Burden & Pilcher, 1975).

Procedure

Six intact males were given 35 µg oestradiol benzoate (Organon Laboratories, Ltd.) as a single subcutaneous injection in 0.1 ml arachis oil. Three groups of marmosets (gonadectomized at least 3 months before the study), each comprising 4 males and 4 females, received similar oestrogen treatment followed immediately and after 8 h by single subcutaneous injections of 0.5 mg testosterone (Group 1), 0.5 mg dihydrotestosterone (Group 2) or 1.0 mg progesterone (Group 3) in 0.1 ml arachis oil. (Progesterone in oil was supplied by Organon Laboratories, Ltd Morden, Surrey; testosterone and dihydrotestosterone were obtained as crystalline preparations from Sigma Chemical Co. (Poole, Dorset) and dissolved in arachis oil as required.) The 4 gonadectomized males and 4 gonadectomized females in Group 4 received progesterone implants (1 × 50 mg plus 1 × 25 mg; Organon) 8 days before a single injection of 35 µg oestradiol benzoate in arachis oil. Progesterone implants were placed subcutaneously through an incision made ventrolaterally in the abdominal area. To serve as controls, 12 intact males and 12 gonadectomized animals (6 males, 6 females) were given an injection of 0.1 ml arachis oil only.

Blood samples were collected from experimental and control animals immediately before and 8, 20, 24, 28 and 36 h after the oestradiol benzoate or arachis oil injection and an additional sample was collected after 48 h from all intact males. Blood (0.4 ml) was withdrawn from the femoral vein with a heparinized 1.0 ml syringe and placed immediately on ice. The blood was centrifuged at 500 g for 10 min at 4°C and the plasma was stored at –20°C. Animals were not sedated before venepuncture.

Hormone assays

Plasma LH and oestradiol-17β were measured in all samples from experimental animals. Testosterone was measured in oestrogen-treated intact males and in gonadectomized animals receiving testosterone injections (Group 1). Progesterone levels were determined in animals given progesterone injections and implants (Groups 3 and 4). LH was measured in control animals.

Plasma LH levels were determined using a heterologous double-antibody radioimmunoassay previously described in detail and validated for marmoset LH (Hodges, 1978). The assay system utilizes a rat standard preparation (NIAMDD-LH-RP1) and an anti-ovine LH antiserum (610, V, Uilenbroek). The assay has a detection limit of 20 ng LH-RP1/ml, and an interassay coefficient of variation of 7.4% ($n = 15$).

Progesterone, testosterone and oestradiol-17β were measured using specific radioimmunoassays fully described by Scaramuzzi, Corker, Young & Baird (1975), Corker & Davidson (1978) and Baird, Swanson & Scaramuzzi (1976), respectively. Progesterone antiserum was raised in a rabbit against progesterone-11α-hemisuccinate-bovine serum albumin (BSA).

conjugate. The specificity has been described by Dighe & Hunter (1974). Antibody to testosterone was raised in a goat immunized with testosterone-3-carboxymethyl oxime conjugated to BSA. Cross-reactions of other steroids tested included 5 α -dihydrotestosterone (25%), oestradiol-17 β (0.2%) and androstenedione (0.08%). Antiserum to oestradiol-17 β (raised against oestradiol-6-carboxymethyl oxime-BSA in a rabbit) cross-reacted with oestrone (3.0%), oestradiol-17 α (2.8%) and other steroids (<1%). Cross-reaction of oestradiol benzoate in the assay was 0.38%.

Validation of these assays for marmoset plasma has been described in detail by Chambers & Hearn (1979). The assay sensitivities for progesterone, testosterone and oestradiol-17 β are 1700, 500 and 350 pg/ml, respectively. Interassay coefficients of variation were 12% for progesterone, 16% for oestradiol-17 β and 13% for testosterone.

Analysis of results

In this study, positive feedback (negative feedback) has been defined as an increase (decrease) in circulating LH to levels exceeding two standard deviations from mean control values. While lesser responses, both positive and negative, may reflect genuine feedback effects, it was felt necessary to impose such a definition in order to avoid confusion between feedback effects and episodic LH secretion. Statistical analysis of the data was performed using an unpaired Student's *t* test and two-way analysis of variance.

Results

Intact males

LH responses to a single injection of oestradiol benzoate in 6 individual males are shown in Text-fig. 1. In the presence of increasing plasma levels of oestradiol-17 β , there was an initial suppression of circulating LH in all animals although in 5 levels remained within the defined control limits. Plasma LH concentrations then rose abruptly to exceed control limits in 4 males, maximum LH levels occurring 28 h after the oestrogen injection. A slight rise in LH in the remaining 2 males was insufficient to be classified as positive feedback. Despite this, mean LH concentrations were significantly different from corresponding mean control values at each sampling time after the injection of oestrogen (Table 1). The increase in plasma oestradiol-17 β concentrations was associated with a marked suppression of circulating testosterone concentrations, mean ($\pm$ s.e.m.) levels falling from 27.0 ± 3.0 ng/ml before the oestrogen injection to 7.5 ± 1.0 ng/ml after 8 h. The duration of testosterone suppression is not clear because of the bleeding schedule, but would appear to be less than 24 h.

Table 1. Mean $\pm$ s.e.m. circulating LH concentrations (ng/ml) in intact male marmosets at various times after injection of oestradiol benzoate or oil (controls)

Treatment	No. of animals	Time after injection (h)				
		0	8	24	28	36
Oil	12	44.2 ± 4.7	45.9 ± 3.1	40.3 ± 3.4	40.5 ± 3.8	45.2 ± 3.5
Oestradiol	6	49.6 ± 6.2	$31.2 \pm 4.9^\dagger$	$108.0 \pm 21.2^*$	$132.3 \pm 28.4^*$	$111.0 \pm 18.1^*$

Values significantly different from corresponding control value; * $P < 0.001$, $^\dagger P < 0.01$.

Gonadectomized males and females

The LH response to injections of oestradiol benzoate and testosterone (Group 1) is shown in Text-fig. 2. Testosterone concentrations rose to about 20 ng/ml (normal range for males, 5–50

ng/ml) after 8 h and remained at or above this level until 20 h after the initial injection. The increment in circulating testosterone concentrations after the second injection of testosterone at 8 h was not detected with the bleeding schedule used. As oestradiol-17 β and testosterone concentrations rose, LH levels fell and negative feedback was seen in 3 animals. All males subsequently showed an increase in circulating LH to exceed control limits, with maximum levels occurring after 24 h.

Text-fig. 1. Plasma LH (individual values), oestradiol-17 β and testosterone (mean $\pm$ s.e.m.) concentrations in 6 intact male marmosets after a single s.c. injection of 35 μ g oestradiol benzoate in oil (arrow). The broken lines represent the limits of 2 standard deviations above and below the mean control LH levels in 12 intact males (oil injection).

The effect of dihydrotestosterone on oestrogen-induced LH release (Group 2) is shown in Text-fig. 3. Mean circulating oestradiol-17 β levels achieved by the injections were similar to those obtained previously. Dihydrotestosterone concentrations were not measured, although the dose administered and the times of injections relative to oestradiol benzoate were the same as for Group 1. Circulating LH concentrations indicate that negative and positive feedback occurred in all animals, and that maximum levels were attained after 24 h.

The effect of progesterone by injections or implants on the LH response to oestrogen is shown in Text-fig. 4. Mean circulating progesterone concentrations achieved by the injections rose to approximately 35 ng/ml by 8 h and remained above this level until 20 h after the initial injection (normal luteal-phase range, 20–60 ng/ml). Maximum progesterone levels achieved by the second injection are not known, but probably exceeded 40 ng/ml. LH responses were very consistent between individuals, negative followed by positive feedback being observed in all animals. The progesterone implants maintained circulating progesterone concentrations similar to those obtained with the injections and LH levels were clearly suppressed in all animals after 8 h. However, LH values rose above control limits in only 1 animal and a large variation in LH responses occurred in the others.

Text-fig. 2. Plasma LH (individual values), oestradiol-17 β and testosterone (mean $\pm$ s.e.m.) concentrations in 4 gonadectomized male marmosets after a single s.c. injection of 35 μ g oestradiol benzoate (arrow) and s.c. injections of 0.5 mg testosterone (double arrow) after 0 and 8 h. The broken lines represent the limits of 2 standard deviations above and below mean control LH levels in 6 gonadectomized males (oil injection).

Mean ($\pm$ s.e.m.) LH responses in gonadectomized males and females in Groups 1–4 are shown in Table 2. In all groups, mean LH levels in males and females 8 h after the oestrogen injection were significantly lower than respective mean control values (not shown) ($0.001 < P <$

Table 2. Summary table of the mean $\pm$ s.e.m. circulating LH concentrations (ng/ml) in gonadectomized male (N = 4) and female (N = 4) marmosets in response to hormone treatments after treatment with oestradiol benzoate (see Text-figs 2, 3, 4a and 4b)

Treatment	Sex	Time after oestradiol injection (h)				
		0	8	24	28	36
Testosterone	♂	67.5 $\pm$ 9.5	37.0 $\pm$ 5.8	124.0 $\pm$ 16.0	134.5 $\pm$ 21.9	104.5 $\pm$ 16.6
	♀	74.0 $\pm$ 6.3	35.7 $\pm$ 3.8	161.0 $\pm$ 21.9	152.0 $\pm$ 17.2	124.8 $\pm$ 15.2
DHT	♂	66.0 $\pm$ 6.4	40.2 $\pm$ 3.3	141.0 $\pm$ 14.0	123.7 $\pm$ 11.0	104.2 $\pm$ 4.7
	♀	60.0 $\pm$ 7.9	32.8 $\pm$ 4.1	120.8 $\pm$ 29.3	109.5 $\pm$ 17.7	79.0 $\pm$ 16.7
Progesterone, injected	♂	56.7 $\pm$ 4.1	21.0 $\pm$ 0.4	119.8 $\pm$ 7.7	121.3 $\pm$ 5.1	100.5 $\pm$ 5.6
	♀	80.3 $\pm$ 5.4	24.0 $\pm$ 3.0	140.5 $\pm$ 9.8	125.5 $\pm$ 6.3	107.3 $\pm$ 8.3
Progesterone, implanted	♂	51.5 $\pm$ 6.4	21.5 $\pm$ 1.5	49.0 $\pm$ 9.3	53.7 $\pm$ 17.4	52.5 $\pm$ 7.2
	♀	52.8 $\pm$ 6.1	<20	45.5 $\pm$ 12.1	54.0 $\pm$ 12.1	49.3 $\pm$ 7.5

0.01, Student's *t* test). In Groups 1, 2 and 3, but not in Group 4, mean LH concentrations in males and females were higher than mean control values after 24 and 28 h ($0.001 < P < 0.01$, Student's *t* test). Two-way analysis of variance showed that there was no significant difference between the pattern of response in males and females for any of the treatment groups.

Text-fig. 3. Plasma LH (individual values) and oestradiol-17 β (mean $\pm$ s.e.m.) concentrations in 4 gonadectomized male marmosets after a single s.c. injection of 35 μ g oestradiol benzoate (arrow) and s.c. injections of 0.5 mg dihydrotestosterone (double arrow) after 0 and 8 h. The broken lines represent the limits of 2 standard deviations above and below mean control LH levels in 6 gonadectomized males (oil injection).

Discussion

The results of this study confirm the previous report (Hodges & Hearn, 1978) of a positive-feedback response to oestrogen in intact as well as gonadectomized male marmoset monkeys. Although oestrogen-induced LH release has also been described in gonadectomized male rhesus monkeys (Karsch *et al.*, 1973) and pig-tailed macaques (Steiner *et al.*, 1978), no such response could be demonstrated in intact males of these species. Van Look *et al.* (1977) were unable to induce positive feedback in normal men or in subjects with testicular feminization, but a female type of LH release in response to oestrogen treatment was demonstrated in one patient with XY pure gonadal dysgenesis. When positive feedback has been

reported in normal (Kulin & Reiter, 1976) and homosexual men (Dörner *et al.*, 1975), the data are inconclusive and the LH rises described were not comparable either in magnitude or duration to those seen in normal women. The existence of a clear positive-feedback response to oestrogen in an intact male primate at present therefore appears unique to the marmoset.

Text-fig. 4. Plasma LH (individual values), oestradiol-17 β and progesterone (mean $\pm$ s.e.m.) concentrations in 4 gonadectomized male marmosets after (a) a single s.c. injection of 35 μ g oestradiol benzoate (arrow) and s.c. injections of 1.0 mg progesterone (double arrow) after 0 and 8 h, and (b) implants of progesterone (horizontal bar) inserted 8 days before the oestrogen treatment. The broken lines represent the limits of 2 standard deviations above and below mean control LH levels in 6 gonadectomized males (oil injection).

Maximum oestradiol-17 β concentrations achieved by the oestrogen injections were similar to those (0.8–2.0 ng/ml) found in intact females immediately before the spontaneous LH surge (Hearn & Lunn, 1975). Although oestradiol-17 β concentrations fell progressively after approximately 8 h, it is unlikely that the observed positive discharges of LH were due to a 'rebound' from negative feedback, because in general oestradiol-17 β concentrations remained >1 ng/ml until after maximum LH concentrations were attained, and suppression of LH levels (below basal control values) recurred 36 h after the LH discharge (Hodges, 1977).

Since oestrogen will induce LH release in orchidectomized (Karsch *et al.*, 1973) but not in intact macaque monkeys (Yamaji *et al.*, 1971; Steiner *et al.*, 1978), it has been suggested (Knobil, 1974) that testosterone may be responsible for abolishing the positive-feedback response in intact male primates. The present data suggest that this does not pertain to the

marmoset monkey. Although testosterone concentrations in intact males were suppressed by the oestrogen treatment before the LH surge, administration of exogenous testosterone to maintain circulating levels in the normal range for intact males throughout the experiment failed to effect a blockade of the LH discharge in gonadectomized males or females. However, testosterone can be aromatized to oestradiol-17 β in the peripheral circulation (Longcope, Kato & Horton, 1969) or by neural tissue (Naftolin *et al.*, 1975), and other testicular androgens may be more effective in inhibiting oestrogen-induced LH release. For this reason, dihydrotestosterone, a potent reduced A-ring metabolite of testosterone which is not convertible to oestradiol-17 β or other known oestrogens (Ito & Horton, 1971), was also tested for its ability to abolish positive feedback. However, under the experimental conditions used in this study, no inhibition was achieved. Since circulating levels of dihydrotestosterone were not measured, the possibility that higher doses or more prolonged treatment could effect a blockade cannot be discounted.

It would be tempting to suggest that the difference between the marmoset and macaque species with respect to positive feedback in the intact male is due to a differential sensitivity to the inhibitory effects of testicular androgens. However, Steiner *et al.* (1978) were also unable to block oestrogen-induced LH release with various regimens of testosterone or dihydrotestosterone treatment in orchidectomized rhesus and pig-tailed macaque monkeys. These authors interpreted their data as challenging the hypothesis that testosterone or its direct metabolites mediate the observed testicular blockade in macaque species and suggested that other substances, such as inhibin, may be involved. Whatever the exact nature of the testicular substance(s) responsible for abolishing oestrogen-induced LH release in intact male macaques, it does not appear to block the response in the marmoset. Although the reason for this is not obvious, there are marked species differences in embryonic development, possibly resulting in differing organizational effects on neural components involved in the expression of positive feedback, which may in turn affect hypothalamic and/or pituitary responsiveness to steroid action in adult life. The distinctive embryology of the marmoset (e.g. high incidence of fraternal twinning and the presence of placental vascular anastomoses between twin fetuses) (Benirschke finding in women (March, Goebelsmann, Nakamura & Mishell, 1979) and is consistent with the of oestrogens during gestation in the marmoset (Chambers & Hearn, 1979) compared with macaque species (Challis, Davies, Benirschke, Hendrickx & Ryan, 1974) are both factors which may be important in this regard.

The role of progesterone in the expression of the spontaneous preovulatory LH surge in primates is not fully understood. Administration of progesterone has been shown to inhibit oestrogen-induced LH release in women (Netter, Gorius, Thomas, Cohen & Joubinaux, 1973) and intact female rhesus monkeys (Dierschke *et al.*, 1973; Clifton, Steiner, Resko & Spies, 1975) and this inhibitory effect of progesterone on oestrogen-induced LH release may account for the failure to demonstrate oestrogen-induced positive feedback during the luteal phase of the rhesus menstrual cycle (Dierschke *et al.*, 1973). Since, however, simultaneous administration of progesterone and oestrogen to ovariectomized rhesus monkeys tended to advance rather than inhibit the onset of LH release, it has been suggested that the ovary is necessary, either directly or indirectly, for the blocking effect of progesterone (Clifton *et al.*, 1975). The present results are inconsistent with this, but suggest instead that the length of exposure of the hypothalamic-pituitary system to progesterone may be of importance in determining its influence (inhibitory or stimulatory) on oestrogen-induced positive feedback. This interpretation is supported by a recent finding in women (March, Goebelsmann, Nakamura & Mishell, 1979) and is consistent with the idea that luteal-phase progesterone blocks positive feedback (Dierschke *et al.*, 1973) whereas periovulatory progesterone may actually facilitate this response (Johansson & Wide, 1969; Leyendecker, Wardlaw & Nocke, 1972). A dual action of progesterone on oestrogen-induced LH release in gonadectomized males, although devoid of physiological significance, is an interesting observation which further exemplifies the unusual status of the male marmoset with respect to positive feedback.

The results of the present study indicate superficial similarities between the characteristics of oestrogen-induced LH release in male and female marmoset monkeys. The effect of testicular secretions on the positive feedback mechanism in primates is not yet understood, although a positive response to oestrogen can still be obtained in the male marmoset despite the presence of active testes. The persistence of a positive feedback response in the male is unlikely to have any physiological significance in the control of LH secretion, but does lend support to the idea that the ability to release LH in response to oestrogen may be an intrinsic characteristic of the primate hypothalamic–pituitary unit, regardless of sex. Why positive feedback in normal intact males can be demonstrated in the marmoset, but apparently not in man or macaques is an interesting question which remains to be answered.

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