Inhibition of ovarian function in subordinate female marmoset monkeys (*Callithrix jacchus jacchus*)*

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Summary. Plasma concentrations of progesterone, cortisol, LH and prolactin were measured in dominant and subordinate female marmosets in 10 well-established peer groups. Subordinate females never ovulated, had a reduced LH response to LH-RH and showed no positive feedback LH surge after oestrogen administration. There was no evidence of elevated plasma cortisol levels or hyperprolactinaemia in subordinates and all showed a similar prolactin response to TRH in comparison with dominants. However, subordinates showed a reduced prolactin response to metoclopramide. These results clearly indicate that high circulating levels of cortisol or prolactin are not responsible for the inhibition of ovulation in female marmosets.

Introduction

Prolactin release in response to specific or non-specific stress has been reported for many mammalian species (Raud, Kiddy & Odell, 1971; Horrobin, 1973; Lamming, Moseley & McNeilly, 1975; McNeilly, 1980). Elevated prolactin levels (hyperprolactinaemia) have also been linked with the inhibition of reproductive function (high-prolactinamenorrhoea) in women (Besser, Parke, Edwards, Forsyth & McNeilly, 1972; Tyson, Friesen & Anderson, 1972; Van Look, McNeilly, Hunter & Baird, 1977; Baird, McNeilly, Sawers & Sharpe, 1979) and in captive subordinate female talapoin monkeys, *Miopithecus talapoin* (Bowman, Dilley & Keverne, 1978; Keverne, 1979). In both cases, high prolactin concentrations were clearly associated with the inhibition of the ovulatory surge of pituitary LH in response to oestrogen. It was proposed by Bowman *et al.* (1978) and Keverne (1979) that in talapoin monkeys, increased prolactin values were due to the stress of attacks from dominant females. However, in these studies only two captive groups of talapoin monkeys were used, “only the most dominant and subordinate individuals” were compared, and it was not established how frequently high prolactin levels were associated with behavioural subordination and infertility in females. As acknowledged by Bowman *et al.* (1978), not all subordinate female talapoin monkeys are normally infertile because these animals are polygamous (Rowell & Dixon, 1975). Furthermore, all the females were ovariectomized and blood oestrogen levels were maintained by implants of oestradiol-17 β .

In the present study, 10 groups of captive marmoset monkeys (*Callithrix jacchus jacchus*) were used and the females were left intact. Marmosets are monogamous (Epple, 1967; Rothe, 1975; Stevenson, 1978) and only the single dominant female in any group reproduces because the subordinates do not ovulate (Abbott & Hearn, 1978). We investigated whether (1) the

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ovarian inhibition of subordinate female marmosets was associated with increased peripheral prolactin concentrations, and (2) a pituitary LH surge in these infertile animals could be elicited similar in magnitude and duration to that occurring at ovulation in normal cyclic females (Hodges & Hearn, 1977).

Materials and Methods

The animals and their management have been described elsewhere (Hearn, Lunn, Burden & Pilcher, 1975).

Peer groups and behavioural status

Ten peer groups were formed, each consisting of 3 male and 3 female post-pubertal marmosets (Abbott & Hearn, 1978). Dominant or subordinate status was assigned on the basis of an animal's aggressive and submissive interactions with others in the group (Abbott & Hearn, 1978; Abbott, 1979). In each group, the dominant male (Male 1) and the dominant female (Female 1) rapidly established a pair bond (Abbott & Hearn, 1978; Abbott, 1979). The subordinate or second and third ranking males (Males 2 and 3, respectively) and subordinate females (Females 2 and 3) formed no such social or sexual relationship with any animal in the group (Abbott, 1978, 1979). In 7 out of the 10 groups, one of the subordinate females had to be removed after the first 2–3 days because of persistent attacks from an animal of higher rank, as previously found (Abbott, 1978, 1979).

Blood sampling and hormone assays

Blood samples of 0.3 ml were withdrawn from the femoral vein into a heparinized syringe through a 0.41 mm diameter needle (27 gauge). Sampling was carried out between 09:30 and 12:30 h. The females were held in a restraining device and were not anaesthetized (Hearn, 1977). All samples were taken 2–4 min after removal of the animals from their cages and then immediately placed on ice. The blood was centrifuged at 500 *g* for 20 min at 4°C and the plasma stored at –20°C until assayed. Females were bled at 2–3-day intervals for 6 months, starting 5–12 months after the peer groups had been established. The frequency of sampling was sufficient to cover the approximately 10–12-day period of plasma progesterone elevation during the luteal phase of the 16–17-day ovarian cycle (Hearn & Lunn, 1975). Plasma prolactin and cortisol concentrations were estimated from samples 2–20 days apart.

Hormone assays

Plasma concentrations of progesterone (Chambers & Hearn, 1979), LH (Hodges, 1978), chorionic gonadotrophin (Chambers & Hearn, 1979) and prolactin (McNeilly, Abbott, Lunn, Chambers & Hearn, 1981) were determined by specific radioimmunoassays validated for use in the marmoset monkey. All samples were assayed in duplicate. Plasma cortisol was measured using a competitive protein-binding kit (Cortipac; The Radiochemical Centre, Amersham, U.K.) and cross-reactions with other steroids and corticosteroids have been described (Technical Bulletin 75/10). No significant departure from parallelism between the human standards (2–47.8 µg/100 ml) and serial dilutions of marmoset plasma (1:8–1:128) was demonstrated ($P < 0.10$) by a two-factor analysis of variance with replication. All samples were diluted to read within this range. Two pools of female marmoset plasma (diluted to 1:20 (v/v) with distilled water) were used to determine the inter- and intra-assay coefficients of variation (CV). One pool was repeatedly measured in duplicate in 5 assays and gave a mean value ($\pm$ s.e.m.) of 100 ± 3.5

$\mu\text{g}/100\text{ ml}$ with a CV between assays of 10.4%. The other pool was repeatedly measured in one assay and gave a value of $99.6 \pm 3.5\text{ }\mu\text{g}/100\text{ ml}$ with a CV of 7.8% ($n = 8$).

Ovarian cycle and detection of pregnancy

The luteal phase of each ovarian cycle was defined as lasting from the rise of plasma progesterone concentrations above 20 ng/ml until levels decreased again below that value. When plasma progesterone rises above 20 ng/ml in cyclic female marmosets, progesterone values quickly reach 50 ng/ml and more for at least 10–12 days, typical of the previously described luteal phase of the cycle (Hearn & Lunn, 1975).

Since females were excluded from the study while pregnant, and so as not to miss any early spontaneous abortion (Abbott, 1979), pregnancies were determined by radioimmunoassay of serial plasma samples for progesterone and chorionic gonadotrophin and by monthly transabdominal uterine palpation (Hearn & Lunn, 1975; Chambers & Hearn, 1979).

LH stimulation with LH-RH and oestradiol benzoate

At the end of the 6-month period of serial blood sampling, dominant and subordinate females were each given 2 μg LH-RH (Hoechst U.K. Ltd, Hounslow, Middlesex, U.K.) intravenously (Hodges, 1979), following an initial blood sample (0.4 ml). The animals were bled at 30, 45, 60 and 90 min thereafter. As controls, 6 dominant and 4 subordinate females received a similar volume of saline (9 g NaCl/l) 3 weeks later, and were bled after 0, 30, 45, 60 and 90 min.

One month after receiving LH-RH, each dominant and subordinate female was given 35 μg oestradiol benzoate (Organon Laboratories Ltd, Morden, Surrey, U.K.) in a single subcutaneous injection of arachis oil (0.2 ml), and then bled after 0, 8, 24, 28, 36, 48 and 60 h (Hodges & Hearn, 1978): 6 dominant and 4 subordinate females received a single injection of arachis oil 2 weeks later, as controls, and were bled similarly. A positive feedback response to an oestrogen challenge was considered to be an increase in circulating LH to values exceeding two standard deviations of the mean control values (Hodges & Hearn, 1978).

Prolactin stimulation with TRH and metoclopramide

TRH (25 μg ; Roche Products Ltd, London, U.K.) was given intravenously in 0.2 ml saline to 7 dominant and 9 subordinate female monkeys at the time of LH-RH infusion, and blood samples were taken –30, 0, 15, 30, 45, 60 and 90 min. The same females were injected i.m. with 2 mg metoclopramide in 0.2 ml saline (Maxolon: Beecham Research Labs, Brentford, Middlesex, U.K.) 1 week later and blood samples were taken at –30, 0, 30, 60 and 90 min. Both TRH and metoclopramide are known to raise prolactin levels in female marmoset monkeys (McNeilly *et al.*, 1981). TRH was injected simultaneously with LH-RH since LH-RH has no significant effect on prolactin concentrations (McNeilly *et al.*, 1981) and TRH produces no marked change in LH concentration (Hodges, 1978). As controls, 5 dominant and 5 subordinate females were given saline 4 weeks later.

Analysis

Six females were excluded from the LH-RH experiment and 1 from the oestradiol benzoate experiment because they had high progesterone levels (i.e. luteal phase or pregnant). As in other species (Fink, 1979), high progesterone concentrations inhibit LH release (J. K. Hodges, personal communication). Animals in poor condition at the time of an experiment were also excluded (see Text-figure legends for numbers included in each experiment).

The plasma cortisol and prolactin concentrations in subordinate females obtained during the

6-month sampling period were each compared against those of the dominant female in their group by Student's *t* test. The plasma LH concentrations after LH-RH administration, and the plasma prolactin concentrations after TRH and metoclopramide injection were all analysed by a two-way analysis of variance for repeated measures. Comparisons of individual means were made using an *a posteriori* Newman-Keuls test (Winer, 1962). A one-way analysis of variance for repeated measures was performed separately on values from dominant and subordinate females if a significant behavioural status-by-time sampled interaction was achieved ($P < 0.05$).

Results

Progesterone and pregnancy

The dominant female in each of the 10 groups exhibited circulating concentrations of progesterone characteristic of the follicular and luteal phases of a normal ovarian cycle (Table 1). In contrast, subordinate females had only follicular-phase concentrations of progesterone, indicating that none had ovulated. All of the dominant females subsequently became pregnant, but pregnancy was not detected in any subordinate female.

Table 1. Mean $\pm$ s.e.m. plasma progesterone concentrations (ng/ml) in dominant and subordinate female marmosets in 10 peer groups sampled every 2–3 days for 6 months

Group	Dominant females			Subordinate females
	Follicular phase	Luteal phase	Peak luteal values	All values
1	4.9 $\pm$ 1.6	78.3 $\pm$ 11.0	109.1 $\pm$ 20.5	3.2 $\pm$ 0.4 4.4 $\pm$ 0.7
2	8.1 $\pm$ 2.0	91.8 $\pm$ 14.1	141.2 $\pm$ 11.3	7.8 $\pm$ 1.6
3	2.7 $\pm$ 0.6	50.7 $\pm$ 9.8	136.3 $\pm$ 30.8	2.7 $\pm$ 0.3
4	6.0 $\pm$ 0.9	65.8 $\pm$ 3.5	101.5 $\pm$ 13.9	3.0 $\pm$ 0.2
5	9.2 $\pm$ 2.9	93.2 $\pm$ 29.4	146.8 $\pm$ 22.2	6.2 $\pm$ 2.0 6.1 $\pm$ 2.0
6	3.6 $\pm$ 0.4	43.9 $\pm$ 4.2	75.3 $\pm$ 9.8	3.5 $\pm$ 0.4 4.9 $\pm$ 0.8
7	3.2 $\pm$ 0.4	59.2 $\pm$ 4.9	97.7 $\pm$ 16.6	2.9 $\pm$ 0.3
8	4.0 $\pm$ 0.5	47.6 $\pm$ 4.7	56.3 $\pm$ 5.8	3.0 $\pm$ 0.6
9	7.5 $\pm$ 1.5	82.2 $\pm$ 7.4	119.8 $\pm$ 23.4	3.9 $\pm$ 0.4
10	2.6 $\pm$ 0.3	72.6 $\pm$ 3.7	109.9 $\pm$ 9.1	3.0 $\pm$ 0.2

Cortisol

In 2 of the 5 groups sampled, there were similar plasma cortisol concentrations in the dominant and subordinate females (Groups 7 and 9; Table 2). In Group 6, the dominant female had cortisol levels similar to those of the second-ranking female, but higher than those of the third. In the remaining 2 groups, both dominant females had cortisol levels higher than those of their respective subordinates.

LH stimulation

LH-RH. There was a significant difference between dominant and subordinate females in their LH responses to LH-RH ($P < 0.01$) which, because of their identical basal LH concentrations, led to a significant behavioural status-by-time sampled interaction ($P < 0.001$; Text-fig. 1a). Following the LH-RH injection, plasma LH concentrations in dominant females were elevated by 30 min and rose to a peak after 45 min ($P < 0.001$; Text-fig. 1a). By 90 min, LH levels were still elevated above baseline. In contrast, there was no significant elevation of plasma

Table 2. Plasma cortisol concentrations (mean $\pm$ s.e.m. for no. of observations in parentheses) in female marmosets from 5 peer groups

Group	Plasma cortisol ($\mu\text{g}/100\text{ ml}$)		
	Female 1	Female 2	Female 3
3	414 $\pm$ 76 (6)	285 $\pm$ 42* (8)	
6	184 $\pm$ 12 (16)	238 $\pm$ 50 (7)	88 $\pm$ 12** (8)
7	189 $\pm$ 8 (14)	182 $\pm$ 17 (12)	
8	523 $\pm$ 69 (6)	320 $\pm$ 36* (8)	
9	190 $\pm$ 6 (10)	280 $\pm$ 55 (9)	

Values significantly different from those of Female 1, * $P < 0.05$, ** $P < 0.01$ (Student's t test).

LH concentrations in subordinate females. Nevertheless, 2 out of the 9 subordinates tested showed LH responses equal in magnitude and duration to those of the dominant females (Text-fig. 1b).

Text-fig. 1. Plasma LH concentrations (mean $\pm$ s.e.m.) in (a) 4 dominant (○—○) and 9 subordinate (●—●) female marmosets given 2 μg LH-RH i.v. at 0 min and (b) 2 of the 9 subordinates responding to 2 μg LH-RH (▲, ■) and 6 dominant (○—○) and 4 subordinate (●—●) females given saline (at 0 min). Values significantly different from those at 0 min: * $P < 0.05$, ** $P < 0.01$. The sensitivity limit of the assay (20 ng/ml) is shown by the broken horizontal line.

Oestradiol benzoate. The LH responses of dominant and subordinate females to a single injection of oestradiol benzoate are shown in Text-fig. 2. Plasma LH concentrations increased abruptly in 8 of the 9 dominant females between 8 and 24 h after the oestrogen injection and, in all 8 females, the criterion set for positive feedback was reached at the 24 h sample. The remaining animal showed a positive feedback response between 24 and 36 h.

None of the 13 subordinate females showed any positive feedback response of LH to the oestrogen challenge: plasma LH levels remained below the sensitivity limit of the assay throughout the 60 h (Text-fig. 2c). These sustained low LH concentrations were not observed when subordinates were given the oil vehicle (range of individual values: <20–43 ng/ml). Some females in both dominant and subordinate categories also showed an inexplicable drop in plasma LH concentrations 24–36 h after oil administration.

Text-fig. 2. Plasma LH concentrations in (a) 5 dominant, (b) 4 dominant, and (c) 13 subordinate female marmosets after the s.c. administration of 35 µg oestradiol benzoate at 0 h. The shaded area represents the area covered by two standard deviations from mean control values of 6 dominant females in (a) and (b) and 4 subordinate females in (c) given oil. The sensitivity limit of the assay (20 ng/ml) is shown by the broken horizontal line.

Prolactin

There were no differences in plasma prolactin levels between dominant and subordinate females in 5 out of the 10 groups (Table 3). In another 4 groups, a subordinate had circulating prolactin concentrations lower than those of the dominant animal. In the last group, the subordinate had higher prolactin levels than the dominant.

Table 3. Plasma prolactin concentrations (mean ± s.e.m. for no. of observations in parentheses) in female marmosets from 10 peer groups

Group	Plasma prolactin (ng/ml)		
	Female 1	Female 2	Female 3
1	19.6 ± 3.4 (10)	20.8 ± 3.4 (10)	8.3 ± 2.0** (9)
2	17.0 ± 4.6 (15)	7.8 ± 1.1** (13)	
3	22.0 ± 7.5 (9)	11.5 ± 1.1* (17)	
4	17.2 ± 1.6 (26)	7.5 ± 0.7** (25)	
5	13.7 ± 1.2 (18)	17.2 ± 2.1 (17)	15.2 ± 1.8 (18)
6	13.9 ± 1.4 (19)	11.8 ± 1.7 (18)	10.7 ± 2.0 (8)
7	15.6 ± 2.6 (15)	14.3 ± 0.6 (15)	
8	13.4 ± 1.7 (15)	11.5 ± 2.2 (8)	
9	13.9 ± 1.2 (16)	14.7 ± 1.1 (16)	
10	11.1 ± 1.1 (26)	15.5 ± 1.5* (28)	

Values significantly different from those of Female 1, * $P < 0.05$, ** $P < 0.01$ (Student's t test).

TRH. Circulating concentrations of prolactin in dominant and subordinate females were similar before and after TRH treatment, and both types of female showed a significant prolactin response to TRH ($P < 0.001$; Text-fig. 3). Prolactin concentrations were elevated after 15 min, but had significantly declined by 60 min and returned to baseline by 90 min. While the dominant females consistently showed a greater response than did the subordinates, this was not statistically significant, perhaps because of the large variability in the prolactin response of both types of female.

Text-fig. 3. Plasma prolactin concentrations (mean $\pm$ s.e.m.) in 7 dominant (○—○) and 9 subordinate (●—●) females given 25 μ g TRH i.v. at 0 min. As controls, 5 dominant (○----○) and 5 subordinate (●----●) females were given saline (at 0 min). Values significantly different from those at -30 or 0 min: * P < 0.05, ** P < 0.01. Values at 30–90 min significantly different from those of the peak response at 15 min: † P < 0.05, ‡ P < 0.01.

Metoclopramide. The pre-treatment prolactin levels of dominant and subordinate females were indistinguishable (-30 and 0 min; Text-fig. 4). Prolactin levels rose sharply in all animals between 0 and 30 min after administration of metoclopramide (P < 0.001) and mean ($\pm$ s.e.m.)

Text-fig. 4. Plasma prolactin concentrations (mean $\pm$ s.e.m.) in 7 dominant (○—○) and 9 subordinate (●—●) female marmosets given 2 mg metoclopramide i.v. at 0 min. As controls, 5 dominant (○----○) and 5 subordinate (●----●) females were given saline (at 0 min). Values significantly different from those at -30 and 0 min: ** P < 0.01. Values significantly different at 60–90 min from peak response at 30 min: ‡ P < 0.01.

peaks of 136.7 ± 13.1 ng/ml and 126.6 ± 11.1 ng/ml were reached in the dominant and subordinate females, respectively. However, by 60 and 90 min, prolactin levels had significantly declined only in the subordinate females (Text-fig. 4), producing a significant behaviour status-by-time sampled interaction ($P < 0.05$). Nevertheless, prolactin levels in the subordinate females were still well elevated above the pre-treatment values ($P < 0.001$).

Discussion

This is the first report describing changes in plasma levels of LH, cortisol and prolactin in subordinate female marmoset monkeys. The results clearly show that subordinate females suffer from complete ovarian failure apparently due to gonadotrophin insufficiency, although the circulating concentrations of cortisol and prolactin are not elevated.

Inhibition of ovarian function in subordinate female marmosets was maintained during the complete 6-month sampling period (animals had been in their peer groups between 5 and 18 months; Table 1) and plasma progesterone concentrations never reached the luteal-phase values of the dominant females. The low progesterone values from subordinate females are consistent with previous findings of low total urinary oestrogen excretion in subordinate female marmosets. Urinary oestrogen concentrations were suppressed 10-fold in comparison with those of dominant females, and resembled the excretion pattern from bilaterally ovariectomized animals (Lunn, 1978). Furthermore, histological examination of the ovaries from 9 subordinate females revealed many primary and atretic follicles, but no preovulatory follicles, corpora lutea or corpora albicantia (D. H. Abbott, T. G. Baker & S. F. Lunn, unpublished). Failure to observe appropriate elevations in circulating LH concentrations (Text-figs 1b and 2c; D. H. Abbott, unpublished) in the face of this evidence of chronically low ovarian hormone secretion, suggests that a deficiency in hypothalamic LH-RH and/or pituitary LH had accompanied the behavioural subordination of female marmosets, and that the impairment of ovarian function was probably due to insufficient gonadotrophic stimulation.

When challenged with a standard dose of LH-RH (Hodges, 1979), 7 out of 9 subordinate females showed a negligible increase in plasma LH values, unlike the large LH response from dominant females (Text-fig. 1). The pituitary of subordinate female marmosets therefore appears to be less responsive to LH-RH. Nevertheless, the poor pituitary LH response of subordinate animals to exogenous LH-RH might just as well reflect insufficient endogenous LH-RH stimulation from the hypothalamus, leading to a reduced pituitary LH content and a reduced pituitary capacity to synthesize LH. The LH responses of subordinate females to different doses of LH-RH and to multiple injections of a given dose of LH-RH require evaluation before these possibilities can be resolved. However, as 2 of the 9 subordinate females showed a normal LH response to LH-RH administration, the lack of a pituitary response to LH-RH may only be present in some subordinates or may only occur intermittently in each individual. Unfortunately, there is no reliable information concerning basal plasma LH levels in dominant and subordinate animals, because LH values for intact marmosets frequently fall below the sensitivity limit of the available assay (Hodges & Hearn, 1977) and there is at present no suitable assay for marmoset FSH.

Each subordinate female marmoset was also insensitive to a standard oestrogen challenge, unlike the response found in dominant females, and hence the positive feedback surge of LH required for ovulation did not occur (Text-fig. 2). In fact, plasma LH levels in each of the 13 subordinates given oestrogen were suppressed below the sensitivity limit of the assay, and below the LH values of subordinates given oil, as if an intense negative feedback system was in operation. This would imply that if there was a preovulatory follicle present in the ovary of a subordinate female marmoset, the increased circulating oestrogen might fail to induce an ovulatory surge of LH from the pituitary by one of two ways. If the female marmoset is similar to the female rhesus monkey (Knobil, Plant, Wildt, Belchetz & Marshall, 1980) and the pituitary

can respond to the increased circulating oestrogen with an LH surge provided that a regular LH-RH release is maintained, then the lack of a pituitary LH response to oestrogen in subordinate females might arise from either a lack of normal hypothalamic LH-RH release or pituitary insensitivity to LH-RH (see above) which would produce the anovulatory condition. On the other hand, if the female marmoset is similar to many other mammalian species (see Fink, 1979, for references) and an increase in the release of hypothalamic LH-RH is apparently required to release an LH surge from the pituitary, then hypothalamic insensitivity to increased circulating oestrogens would lead to sterility, regardless of whether or not the pituitary was responsive to LH-RH. The evidence from this study favours the latter possibility because exogenous LH-RH elicited a normal LH response from two of the subordinate female marmosets although they were acyclic (Text-fig. 1b). However, an oestrogen challenge would have to be administered to LH-RH-treated subordinates before pituitary insensitivity could be ruled out.

It is possible that an insensitive positive feedback mechanism in subordinate female marmosets might reflect an extremely sensitive negative feedback response of the hypothalamus to the administered oestrogen. In other words, the negative feedback system has been 'set' at too sensitive a level in subordinates so that even very low circulating concentrations of oestrogen inhibit the secretion of LH-RH and hence LH. This could explain why the injected oestradiol benzoate completely suppressed plasma LH levels in subordinates below the sensitivity limit of the assay for 60 h (Text-fig. 2) while LH-RH administration elicited at least some plasma LH response (Text-fig. 1). Consequently, the pituitary of subordinate females would also be starved of hypothalamic LH-RH stimulation. In these respects, subordinate adult females may well resemble pre-pubertal female marmosets in which the LH response to LH-RH is similarly poor (J. K. Hodges & D. H. Abbott, unpublished). The subordinate female marmoset may be useful in examining the factors controlling ovarian inactivity, since 6 out of the 13 subordinates had had cycles before inclusion in a peer group (Abbott, 1979) and all the subordinates exhibited ovarian cyclicity 10–30 days after removal from the peer group (Abbott & Hearn, 1978).

The poor LH responses of subordinate female marmosets cannot be attributed to increased levels of cortisol (Table 2) or prolactin (Table 3). The adrenal glands from subordinates were the same size and weight as those from dominant females (S. F. Lunn & D. H. Abbott, unpublished). The stress of behavioural subordination in this female monkey therefore does not induce hyperprolactinaemia or elevated cortisol levels, a result in marked contrast to that reported by Bowman *et al.* (1978) and Keverne (1979), who found that behavioural subordination in female talapoin monkeys was associated with elevated prolactin and cortisol concentrations, suggesting that the suppression of oestrogen-induced LH surges in subordinate females was causally related to the elevated prolactin. The different prolactin and cortisol findings between talapoin and marmoset monkeys may partly arise because subordinate female talapoins continue to receive aggression long after captive groups are established (Bowman *et al.*, 1978; Keverne, 1979), whereas aggressive interactions virtually cease 2–3 days after captive marmoset groups are set up (Abbott & Hearn, 1978; Abbott, 1979), and this difference in aggression may, in turn, be related to the oestrogen treatment of the former. Nevertheless, since subordinate females in both species fail to show oestrogen-induced LH surges, a common mechanism(s) inhibiting positive feedback may be operating independently of circulating prolactin concentrations. In subordinate female marmosets, prolactin responses to TRH were consistently, but not significantly, lower than those of dominant females (Text-fig. 3). Metoclopramide, a pituitary cell-receptor antagonist of dopamine (a neurotransmitter which acts to inhibit prolactin release from the pituitary; Hökfelt & Fuxe, 1972; Olson, Fuxe & Hökfelt, 1972) produced a more striking difference (Text-fig. 4): at 30 min after injection of metoclopramide, prolactin levels of subordinates were similar to those of dominant females, but had fallen by 60 and 90 min. It is therefore possible that, because of increased dopamine release or turn-over, pituitary prolactin secretion is inhibited in subordinates, hence the reduced

response to pituitary stimulation. This explanation would be in accordance with the lower prolactin levels found in subordinates in 4 out of the 10 groups when compared with the dominant female (Table 3). Increased dopamine turn-over is also known to suppress LH secretion (LeBlanc, Lachelin, Abu-Fadil & Yen, 1976; Lachelin, LeBlanc & Yen, 1977; Fuxe *et al.*, 1976; Judd, Rakoff & Yen, 1978; McNeilly, 1980), presumably by reducing the hypothalamic output of LH-RH (de Cotte, De Menzes, Bennett & Edwardson, 1980). This information would fit with the finding of reduced LH secretion in subordinate female marmoset and talapoin monkeys. Changes in plasma prolactin levels under such conditions would therefore be only a secondary effect, as indeed acknowledged by Bowman *et al.* (1978). Since oestrogen blocks dopamine at its receptor (Labrie, Baulieu, Caron & Raymond, 1978), ovariectomy followed by oestrogen treatment of the subordinate talapoins and reduced oestrogen levels of the subordinate marmosets might contribute directly to the dissimilar prolactin findings in these two species of monkey.

The mechanisms by which the dominant female marmoset inhibits ovulation in her subordinates are unknown but they probably involve a combination of pheromones from her frequent scent marking (Epple, 1973; Epple, Golob & Smith, 1979) and physical and visual contact (Abbott, 1979). Such a system would help to enforce the marmoset's monogamous mating system and encourage movement of subordinate females away from a group to pair with a male or become dominant in another group where the suppression of fertility would be removed.

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