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New hyperprolactinemia and anovulation model in common marmoset (*Callithrix jacchus*) and effect of cabergoline

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Abstract

We aimed to develop an anovulation model, using sulpiride-induced hyperprolactinemia in common marmosets. The serum prolactin level gradually increased during the twice-daily administration of sulpiride and reached a plateau after 4 days. Sulpiride produced as big a response at 10 mg kg^{-1} as at 50 mg kg^{-1}. In this study, the length of the ovarian cycle was approximately 30 days in normal common marmosets. Serum progesterone and estradiol levels showed no consistent change during the first 2 months of treatment with sulpiride. When treatment with sulpiride had been continued for more than 2 months, serum progesterone and estradiol levels fell to within the range seen in the follicular phase of the normal cycle and absence of ovulation was recognized by laparoscopy. A single oral administration of cabergoline (at doses between 0.01 and 0.1 mg kg^{-1}) dose dependently reduced the elevated serum prolactin level. Bromocriptine (at an oral dose of 10 mg kg^{-1}) also reduced the serum prolactin level at 4 and 8 h after its administration. With bromocriptine, the prolactin level had recovered at 24 h, but with cabergoline at doses of 0.05 mg kg^{-1} or more, it had still not recovered at 48 h. In anovulatory common marmosets, oral administration of cabergoline at a daily dose of 0.05 mg kg^{-1} restored ovarian function and resulted in ovulation in 100% of the group (following a reduction in the serum prolactin level). Bromocriptine at a daily oral dose of 10 mg kg^{-1} resulted in ovulation in 67% of the group, but this dose was about 200 times higher than the dose of cabergoline. We could produce an anovulatory model induced by sulpiride repeatedly administered over a long time period. It is suggested that, in this anovulatory model in common marmosets, cabergoline has a potent and long-lasting action as a dopamine D_2 receptor agonist, and thus could be a useful drug for the treatment of galactorrhea and hyperprolactinemic amenorrhea and/or anovulation.

Keywords: Cabergoline; Hyperprolactinemia; Anovulation; Laparoscopy; (Common marmoset); Dopamine D_2 receptor agonist

1. Introduction

The lactotroph is unique among anterior pituitary cells in that both its secretory, and proliferative, activity are under tonic inhibitory control by hypothalamic dopamine acting via cell surface dopamine D_2 receptors. Hyperprolactinemia may be the cause of childlessness in as many as one-third of infertile women (Robert et al., 1996). The ability of dopamine agonists to suppress both prolactin secretion and mitogenic activity in lactotrophs is well documented, and this ability is almost invariably maintained in prolactinomas (Lloyd et al., 1975; Barrow et al., 1984). Since their introduction, dopamine receptor agonists have become the treatment of choice for the majority of patients with hyperprolactinemic disorders (Ho and Thorner, 1988; Molitch, 1989; Crosignani and Ferrari, 1990; Bevan et al., 1992). Bromocriptine, an ergot derivative that is now the dopamine D_2 receptor agonist most widely used in clinical practice, has a potent prolactin-lowering effect (Tallo and Malarkey, 1981; Cooper, 1991; Philosophe and Seibel, 1991). However, bromocriptine needs to be administered several times per day, and is not always well tolerated. Recent work in this field has been directed towards a search for drugs with a more sustained action which would need to be given less frequently to patients and might therefore lead to improved compliance.

Cabergoline (1-[(6-allylergolin-8β-yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethylurea), a new ergot alkaloid

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derivative, has been reported to have a long-lasting dopamine D_2 receptor agonistic action (Ferrari et al., 1986; Mattei et al., 1988; Ferrari et al., 1989; Ciccarelli et al., 1989). However, there is only limited clinical experience with this drug. Moreover, there are only a few animal models sensitive to dopamine receptor agonists, such as spontaneous and drug-induced hyperprolactinemic models (Di Salle et al., 1982, 1983, 1984; Kinukawa et al., 1996), although a few studies on the effects of cabergoline have been done using estrogen-induced hyperprolactinemia and pituitary enlargement (Dall'Ara et al., 1988; Eguchi et al., 1995). Furthermore, there is as yet no report of the effects of cabergoline in a hyperprolactinemic anovulation model. Interestingly, sulpiride has been found clinically to cause amenorrhea after a certain period of administration, and it has been shown in endocrinological studies to induce hyperprolactinemia in humans (Mancini et al., 1976; Aono et al., 1978; Oseko et al., 1988), baboon (Aso et al., 1982), and rats (MacLeod and Lobyn, 1977).

In the present study, we used sulpiride to induce hyperprolactinemia and also to produce a long-term hyperprolactinemic anovulation model in a non-human primate, the common marmoset. We examined (i) the effects of single oral administration of cabergoline and bromocriptine on the serum prolactin level during sulpiride-induced hyperprolactinemia, and (ii) the effects of their repeated administration on serum prolactin, progesterone, and estradiol levels and the condition of the ovaries throughout the ovarian cycle in our hyperprolactinemic anovulation model.

2. Material and methods

2.1. Experimental animals

Twenty-four healthy and sexually mature female common marmosets (*Callithrix jacchus*) weighing between 250 to 450 g were used in this study. The marmosets were housed individually in separate cages (W460 × D570 × H750 mm) in an air-conditioned room (temperature 28 ± 2°C, humidity 50 ± 10%), with artificial lighting for 12 h day^{-1} (0800 to 2000 h). They were fed a commercial pellet diet (New World Monkey-SPS, Oriental Yeast, Tokyo, Japan) with fruit (apples and bananas) and water supplied ad libitum. This study was approved by the Animal Experimentation Committee of Kissei Pharmaceutical.

2.2. Drugs

Cabergoline (1-[6-allylergolin-8β-yl)carbonyl]-1-[3-(dimethylamino) propyl]-3-ethylurea) (provided by Pharmacia-Milan, Milan, Italy) and bromocriptine (Sigma, St. Louis, MO, USA) were suspended in 0.5% methylcellulose. They were administered orally. Sulpiride was obtained from Fujisawa Pharmaceutical (Dogmatyl®; Osaka, Japan). It was administered intramuscularly.

2.3. Experimental procedures

2.3.1. Influence of sulpiride treatment for 5 days on serum prolactin level

Six female common marmosets were given sulpiride at a dose of 10 or 50 mg kg^{-1} intramuscularly at 1000 and 1600 h daily for 5 days. Blood samples were taken twice daily just before the sulpiride injection from the femoral vein without anesthesia. The blood was centrifuged at $2500 \times g$ for 15 min, and serum samples were stored at −20°C until assay.

2.3.2. Hyperprolactinemia

In 24 female common marmosets, hyperprolactinemia was induced by daily administration of sulpiride (10 mg kg^{-1}, 2 × day^{-1}, i.m. between 0900–1000 and 1700–1800 h) for 6 days. At 5 days after the start of this course of injections, cabergoline was administered orally as a single dose of 0.01, 0.025, 0.05, or 0.1 mg kg^{-1}, or bromocriptine was administered as a single dose of 10 mg kg^{-1}. Blood samples were taken from the femoral vein without anesthesia at 0, 1, 2, 4, 8, 24, and 48 h after the administration of the dopamine D_2 receptor agonist. The blood was centrifuged at $2500 \times g$ for 15 min, and serum samples were stored at −20°C until assay.

2.3.3. Hyperprolactinemic anovulation

A hyperprolactinemic anovulation model was produced by repeatedly administering sulpiride (10 mg kg^{-1} day^{-1} or 10 mg kg^{-1}, 2 × day^{-1}, i.m.) over a long period of time (2–3 months). After two normal ovulatory cycles had been confirmed, the marmosets were started on a course of repeated intramuscular injections of sulpiride that lasted throughout the experimental period. When a given marmoset had shown hyperprolactinemic anovulation for at least 1 month, courses (each lasting more than a month) of cabergoline (oral doses of 0.01 and 0.05 mg kg^{-1} day^{-1}) and bromocriptine (oral doses of 1 and 10 mg kg^{-1} day^{-1}) were given. To assess the changes in the serum levels of prolactin, progesterone, and estradiol during the ovarian cycle, 5 marmosets were bled (0.6 ml) between 0900 and 1000 h every 3 to 4 days just before drug administration throughout the experimental period. The blood was centrifuged at $2500 \times g$ for 15 min, and serum samples were stored at −20°C until assay. Normal ovulatory ovarian cyclicity was identified by measurement of the serum progesterone and estradiol levels. Ovulation was identified by the increase in the serum estradiol and progesterone levels and by the presence of the corpus luteum on laparoscopic ovarian examination.

2.4. Measurement of prolactin, progesterone, and estradiol

The serum prolactin concentration was measured by the double-antibody radioimmunoassay method described else-

where (Moro et al., 1995). The assay used human prolactin as a standard (equivalent to the int. st. MRC 81/541) and anti-human prolactin antiserum raised in the rabbit (UCB-Bioproducts, Brussels, Belgium), with ^{125}I-labeled human prolactin (Dupont New England Nuclear, Boston, MA, USA) as a tracer. A sheep antirabbit γ-globulin second antibody (UCB-Bioproducts) was also used. The immunotitration curve for common marmoset serum prolactin was found to be parallel to the human prolactin standard. The inter- and intra-assay variabilities were 10.8% and 8.0%, respectively.

The serum concentrations of progesterone and estradiol were measured using commercially available kits (Progesterone I-125 kit and Estradiol I-125 kit, Sorin Biomedica, Saluggia, Italy). Inter- and intra-assay coefficients of variation were, respectively, 9.5% and 7.9% for progesterone, and 10.9% and 5.5% for estradiol.

2.5. Laparoscopic procedure

The laparoscopic procedure used in this study was the same as that reported by Torii et al. (1996). Briefly, the procedure was as follows. Common marmosets were anesthetized with ketamine hydrochloride in combination with xylazine hydrochloride at intramuscular doses of 10 and 2 mg kg^{-1}, respectively. Each animal was placed on its back with its head down on a 30° slope laparoscopy stage. Both ovaries were carefully examined using a laparoscope (ART-30, Machida, Tokyo, Japan, with a light source (RH-150 II, Machida, Tokyo, Japan)) which was inserted into the abdominal cavity through a 2–3 mm incision made in the mid-ventral skin. A second incision was made 10 mm caudal to the umbilicus in the mid-ventral line, and a polyethylene catheter for manipulating the abdominal organs was inserted. A camera was used to take color photographs. The observations took 15–30 min. Each animal was injected i.m. with 30,000 units of penicillin after the incisions had been sutured.

2.6. Statistical analysis

The data showing the serum levels of prolactin were analyzed by means of Student's *t*-test and by Dunnett's or Scheffe's multiple comparison test, preceded by an analysis of variance (ANOVA). The ovulation data were analyzed using Fisher's exact probability test. A probability of $P < 0.05$ was taken as statistically significant.

3. Results

3.1. Sulpiride-induced hyperprolactinemia

The serum prolactin level gradually increased during the twice-daily administration of sulpiride, and reached a plateau after 4 days (Fig. 1). Sulpiride produced as big a response at 10 mg kg^{-1} as at 50 mg kg^{-1}. As shown in Fig. 2 (see 0 h time point), repeated administration for 5 consecutive days (10 mg kg^{-1}, 2× day^{-1}, i.m.) significantly ($P < 0.01$) raised serum prolactin levels (12.1 ± 1.47 ng ml^{-1}) above those seen in normal common marmosets (0.5 ± 0.07 ng ml^{-1}).

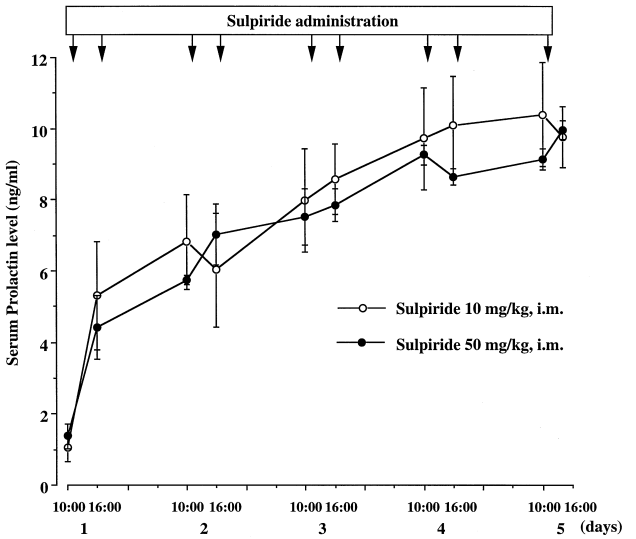


Fig. 1. Effect on serum prolactin levels of repeated treatment with sulpiride in female common marmosets. Each point indicates the mean ± S.E.M. of data from three animals. ↓: Sulpiride was administered intramuscularly.

3.2. Influence of prolonged administration of sulpiride on the ovarian cycle

The changes in the serum estradiol, progesterone, and prolactin levels following daily sulpiride treatment for more than 3 months are shown in Fig. 3A–C. It is known that ovulation occurs following a rise in serum progesterone levels (> 10 ng ml^{-1}) in animals (Kholkute et al., 1988). During the luteal phase, the serum progesterone and estradiol revealed synchronous dynamics. In the three common marmosets studied, the length of the ovarian cycle, shown by the interval between successive elevated progesterone levels, was approximately 30 days. Before each ovulation, serum estradiol levels increased transiently. The serum progesterone and estradiol levels rose after ovulation, reaching maximum levels of 35–70 ng ml^{-1} and 150–2000 pg ml^{-1}, respectively and these levels showed no consistent change during the first 2 months of treatment with sulpiride. However, during this period the serum prolactin level was raised by sulpiride and was significantly higher than the pretreatment level (23.8 ± 1.53 ng ml^{-1} vs. 1.6 ± 0.23 ng ml^{-1}; $P < 0.01$). This higher prolactin level was maintained throughout the period of medication. When treatment with sulpiride had been continued for more than 2 months, serum progesterone and estradiol levels fell to within the range seen in the follicular phase of the normal cycle. Absence of ovulation during

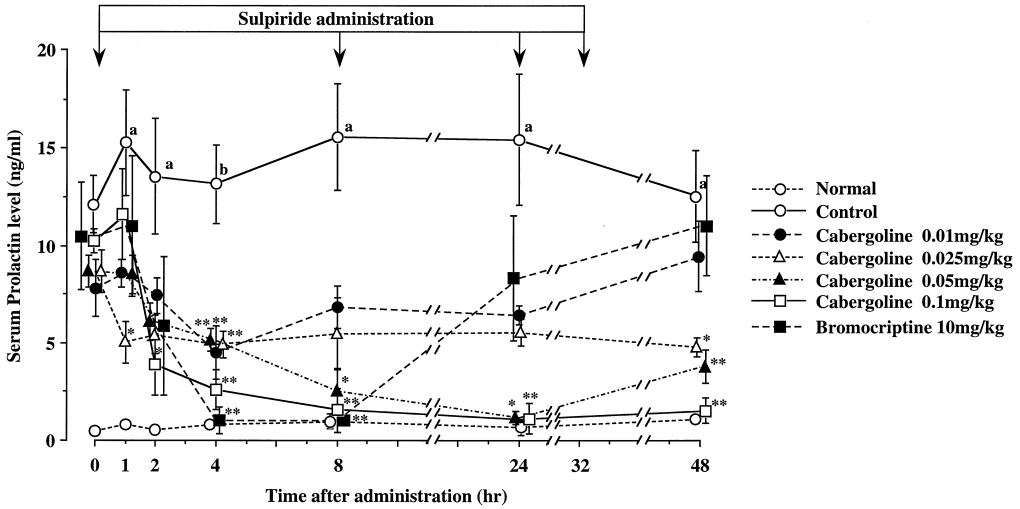


Fig. 2. Effects of cabergoline and bromocriptine on sulpiride (10 mg kg^{-1}, 2× day^{-1} for 6 days)-induced hyperprolactinemia in female common marmosets. Cabergoline and bromocriptine were administered orally at time 0, 5 days after the first sulpiride dose. Each point indicates the mean ± S.E.M. of data from four animals. ↓: Sulpiride was administered intramuscularly. * and **: significantly different from control at $P < 0.05$ and 0.01. [a] and [b]: significantly different from normal at $P < 0.05$ and 0.01.

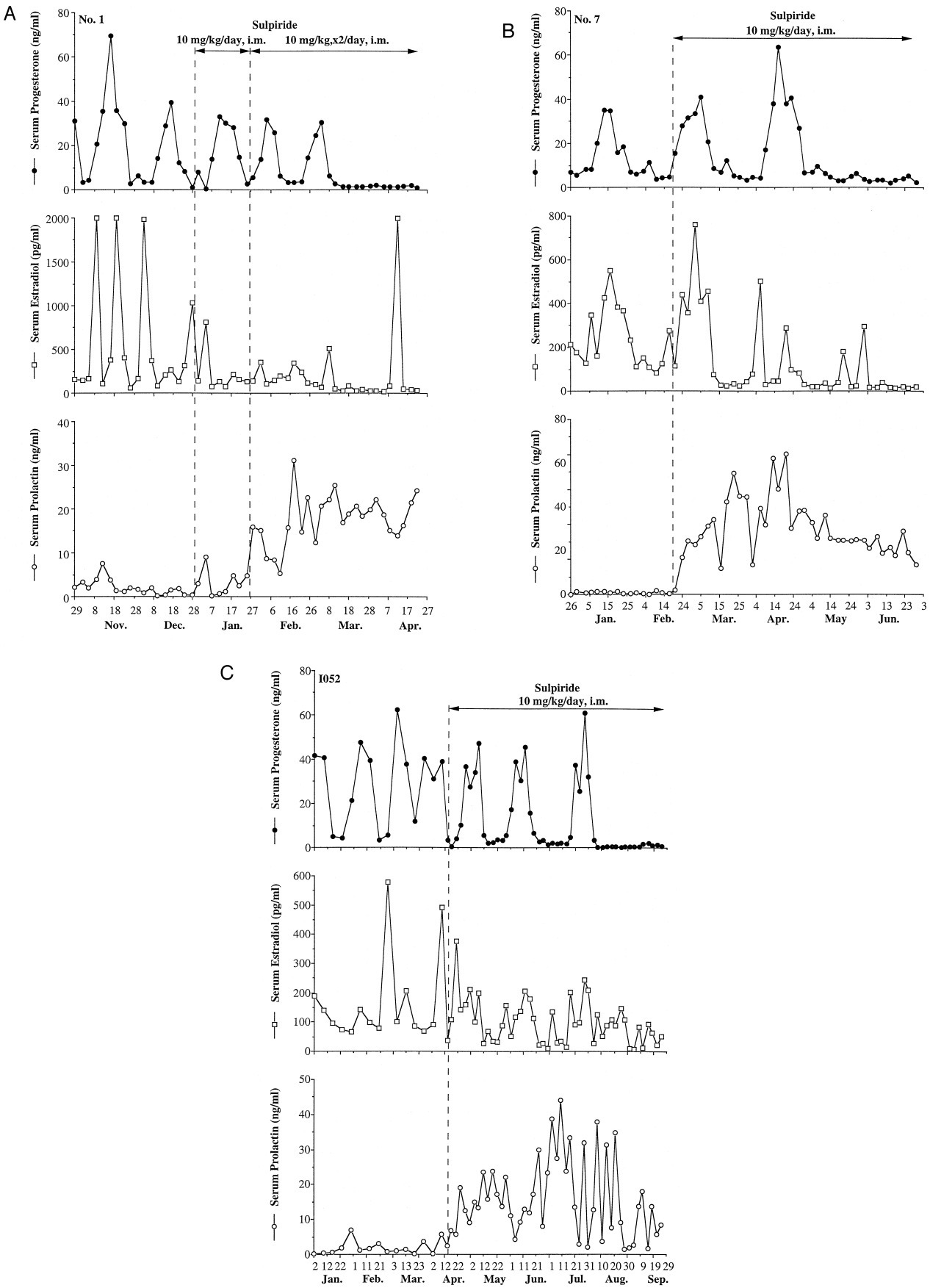
this period, was continued when the condition of the ovaries was observed by laparoscopy.

3.3. Effects of cabergoline and bromocriptine on hyperprolactinemia and hyperprolactinemic anovulation

As shown in Fig. 2, cabergoline lowered the sulpiride-induced serum prolactin levels in a dose-dependent manner at doses from 0.01 to 0.1 mg kg^{-1}, with significant inhibitory effects being observed at 4 h after its administration at all doses. At doses of 0.05 mg kg^{-1} or more, this effect of cabergoline was long-lasting, prolactin levels being still significantly lower than the control at 48 h. Bromocriptine also lowered the high serum prolactin levels at 4 and 8 h after its administration, but that effect had disappeared by 24 h. The effective dose of cabergoline was about 100 time lower than the dose of bromocriptine used here. Moreover, at one-two hundredth the dose of bromocriptine, cabergoline had a longer-lasting effect on prolactin secretion. The changes with time in the serum progesterone, estradiol, and prolactin levels under various conditions are shown for two typical cases in Fig. 4A and B. In our common marmosets, long-term treatment with sulpiride inhibited the cyclic secretion of progesterone and estradiol and made the animals anovulatory. The serum levels of progesterone and estradiol were for the most part within the range seen in the follicular phase of the normal cycle. In animal cord no. H036 (Fig. 4A), cabergoline at a dose of 0.05 mg kg^{-1} daily caused a dramatic decline in the serum prolactin level, which returned to the normal value within 8 days. Then, the serum estradiol level rose progressively to a peak and the serum estradiol and progesterone levels were elevated following ovulation. It was observed that this particular marmoset had two ovulations during or just after the 0.05 mg kg^{-1} daily administration of cabergoline. The cessation of cabergoline administration caused an increase in the serum prolactin level followed again by anovulation. Neither cabergoline at a dose of 0.01 mg kg^{-1} daily nor bromocriptine at a dose of 1 mg kg^{-1} daily changed the elevated prolactin level or the suppressed progesterone and estradiol levels. However, bromocriptine at a dose of 10 mg kg^{-1} daily reduced the serum prolactin level and restored ovulation. In the animal cord no. I901 (Fig. 4B), during sulpiride-induced hyperprolactinemic anovulation, cabergoline (0.01 mg kg^{-1} daily for 41 days) and bromocriptine (1 mg kg^{-1} daily for 34 days followed by 10 mg kg^{-1} daily for 38 days) failed to change the serum progesterone, estradiol, and prolactin levels. However, cabergoline at a dose of 0.05 mg kg^{-1} daily reduced the elevated serum prolactin level, and ovulation occurred as a result of the restoration of normal ovarian function.

The mean of the serum prolactin levels seen in five common marmosets was 1.53 ± 0.16 ng ml^{-1} during the normal cycle (Table 1). During sulpiride-induced hyperprolactinemia, the mean of the serum prolactin levels was 21.19 ± 1.11 ng ml^{-1}, significantly ($P < 0.01$) greater than that seen in normal animals during the normal cycle. The serum prolactin levels in hyperprolactinemic anovulatory marmosets did not fall into the normal range during

Fig. 3. Changes in serum progesterone, estradiol, and prolactin during the normal ovarian cycle and following daily injections of sulpiride (10 mg kg^{-1}, once or twice a day) in three common marmosets (A: animal cord no. 1; B: animal cord no. 7; C: animal cord no. I052).


A
No. 1
Sulpiride
10 mg/kg/day, i.m.
10 mg/kg,x2/day, i.m.
Serum Progesterone (ng/ml)
Serum Estradiol (pg/ml)
Serum Prolactin (ng/ml)
29 8 18 28 8 18 28 7 17 27 6 16 26 8 18 28 7 17 27
Nov. Dec. Jan. Feb. Mar. Apr.
B
No. 7
Sulpiride
10 mg/kg/day, i.m.
Serum Progesterone (ng/ml)
Serum Estradiol (pg/ml)
Serum Prolactin (ng/ml)
26 5 15 25 4 14 24 5 15 25 4 14 24 4 14 24 3 13 23 3
Jan. Feb. Mar. Apr. May Jun.
C
I052
Sulpiride
10 mg/kg/day, i.m.
Serum Progesterone (ng/ml)
Serum Estradiol (pg/ml)
Serum Prolactin (ng/ml)
2 12 22 1 11 21 3 13 23 2 12 22 2 12 22 1 11 21 1 11 21 31 10 20 30 9 19 29
Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep.

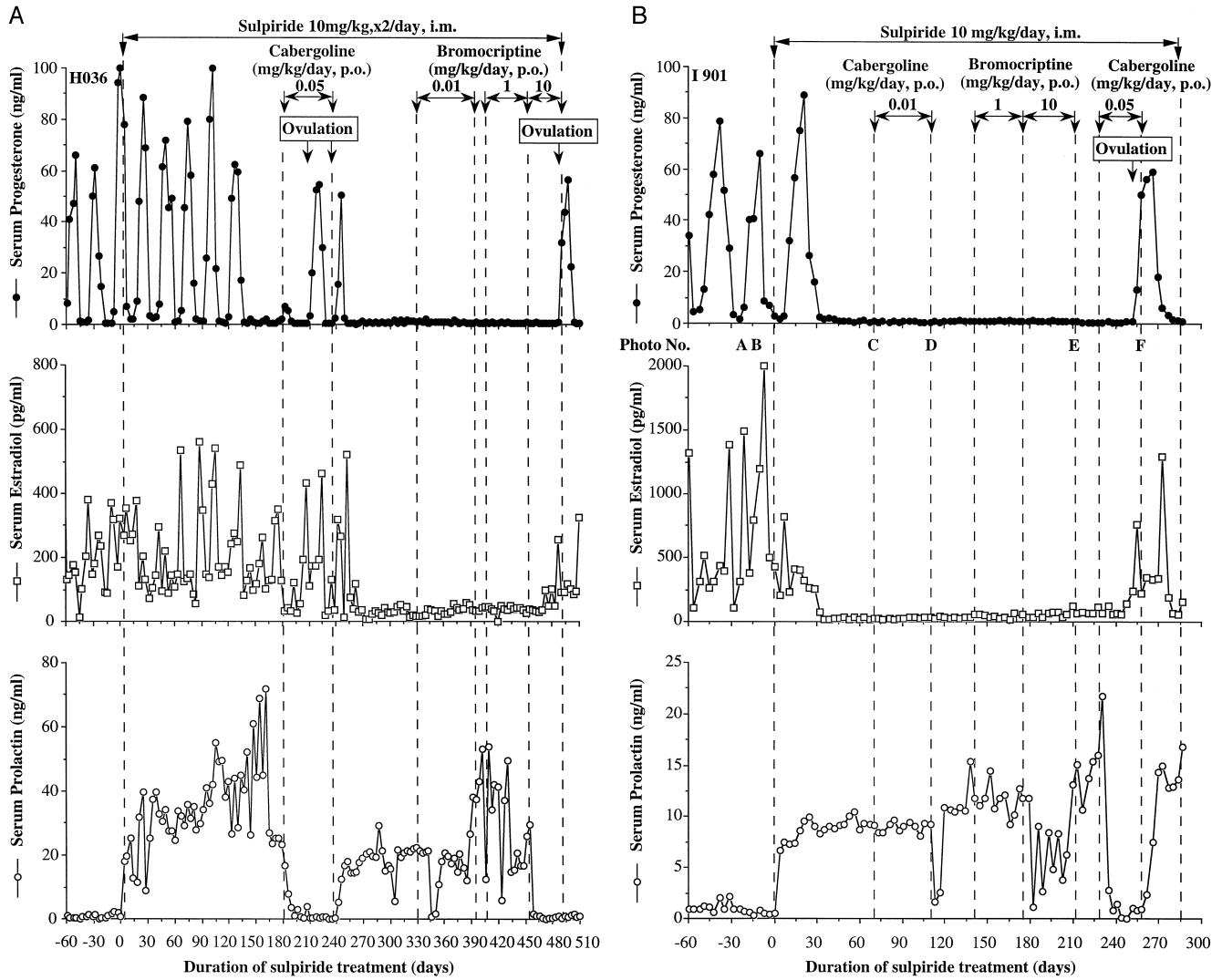


Fig. 4. Effects of cabergoline and bromocriptine on sulpiride-induced hyperprolactinemic anovulation in marmosets (A: animal cord no. H036; B: animal cord no. I901). Serum levels are shown for progesterone, estradiol, and prolactin. 'Photo Nos.' in B show the time-points at which laparoscopic photographs were taken.

the administration of either cabergoline at a dose of 0.01 mg kg^{-1} day^{-1} or bromocriptine at 1 mg kg^{-1} day^{-1} (each for periods of 1 month or more). However, cabergoline at 0.05 mg kg^{-1} day^{-1} and bromocriptine at 10 mg kg^{-1} day^{-1} each significantly ($P < 0.01$) lowered the elevated serum prolactin level (21.19 ± 1.11 to 1.92 ± 0.36 and 2.74 ± 0.59 ng ml^{-1}, respectively). Furthermore, repeated administration of cabergoline at 0.05 mg kg^{-1} day^{-1} restored ovarian function and suppressed the sulpiride-induced anovulation in all four animals tested. Bromocriptine at 10 mg kg^{-1} day^{-1} suppressed anovulation in 67% (2/3) of the animals tested.

Table 1
Effects of cabergoline and bromocriptine on sulpiride-induced hyperprolactinemic anovulation in common marmosets

Drugs	Dose (mg kg^{-1} day^{-1})	Serum PRL (ng ml^{-1})	Ovulation[a]	(%)
Normal		1.53 ± 0.16[b]	5/5[b]	100
Control		21.19 ± 1.11	0/5	0
Cabergoline	0.01	15.77 ± 1.26	0/3	0
	0.05	1.92 ± 0.36[b]	4/4[b]	100
Bromocriptine	1	20.38 ± 2.42	0/3	0
	10	2.74 ± 0.59[b]	2/3	67

Cabergoline and bromocriptine were administered orally; sulpiride was administered intramuscularly ('control').
[a]Each value indicates number of animals showing ovulation/number of animals.
[b]Significantly different from control at $P < 0.01$.

In marmoset cord no. I901, the morphological changes occurring in the ovary under various conditions are shown in laparoscopic photographs.

A developing follicle was distinguishable from the other parts of the ovary as a slightly translucent blue-gray tissue a few days prior to ovulation in the normal cycle. As the follicle filled with follicular fluid, the distended membrane of the ovary stretched (Fig. 5A). Bright red protrusions

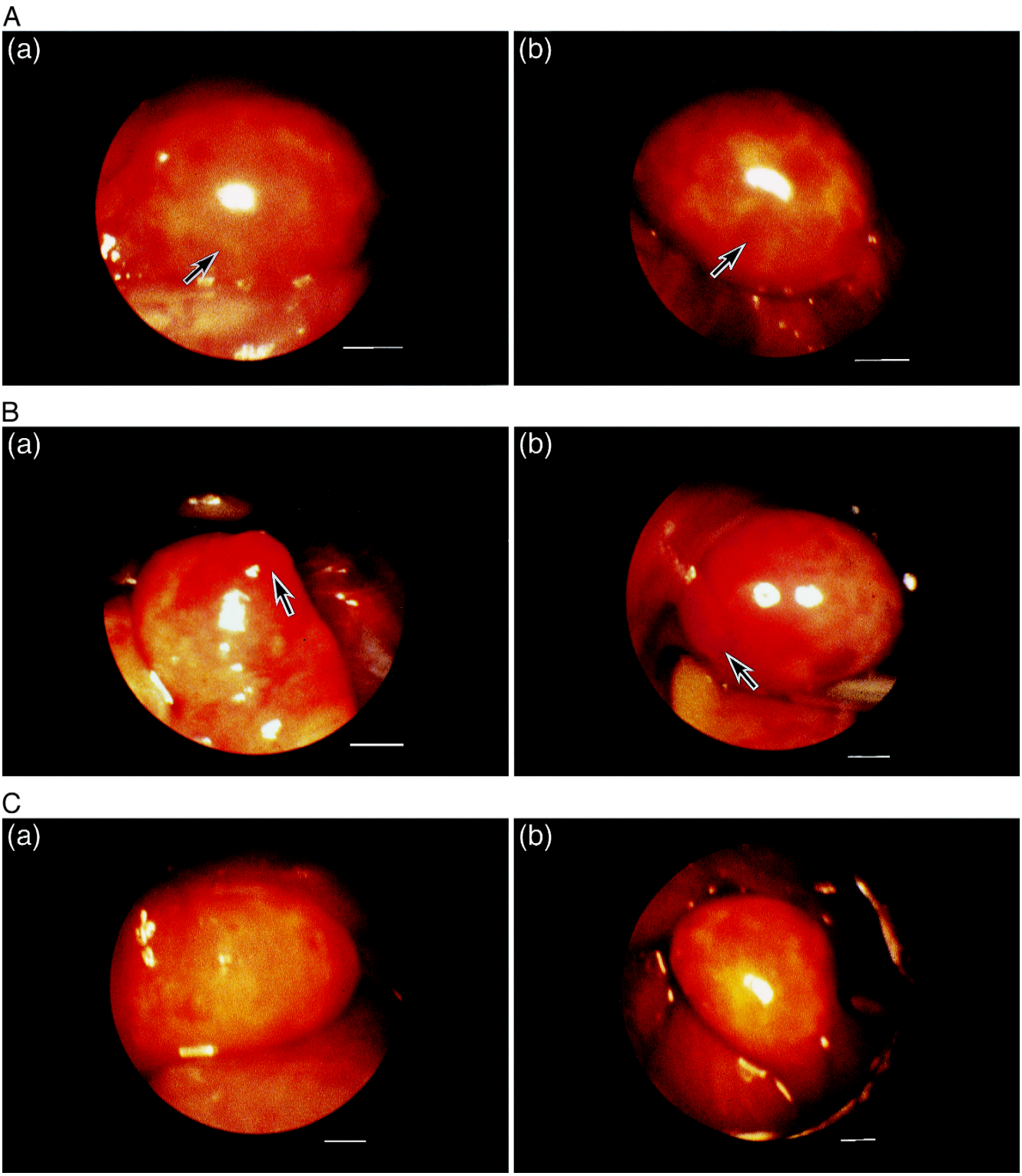


Fig. 5. Photographs taken by laparoscopy showing both ovaries of a common marmoset (cord no. I901). (A) Left (a) and right (b) ovaries during a normal ovarian cycle. A developing follicle (arrow) can be seen (Fig. 4B photo no. A). (B) Left (a) and right (b) ovaries after ovulation in a normal ovarian cycle. A new corpus luteum (arrow) can be seen as the bright-red corpus hemorrhagicum (photo no. B). (C) Left (a) and right (b) ovaries during sulpiride-induced hyperprolactinemic anovulation. Neither follicle nor corpus luteum is present (photo no. C). (D) Left (a) and right (b) ovaries during period of anovulation after repeated administration of cabergoline (0.01 mg kg^{-1}, p.o. daily for 41 days). Neither follicle nor corpus luteum is present (photo no. D). (E) Left (a) and right (b) ovaries during period of anovulation after repeated administration of bromocriptine (1 mg kg^{-1}, p.o. daily for 32 days, then 10 mg kg^{-1}, p.o. for 38 days). Neither follicle nor corpus luteum is present (photo no. E). (F) Left (a) and right (b) ovaries after ovulation following repeated administration of cabergoline (0.05 mg kg^{-1}, p.o. daily for 32 days). A new corpus luteum (arrow) can be seen as the bright-red corpus hemorrhagicum (photo no. F). Bar = 1 mm.

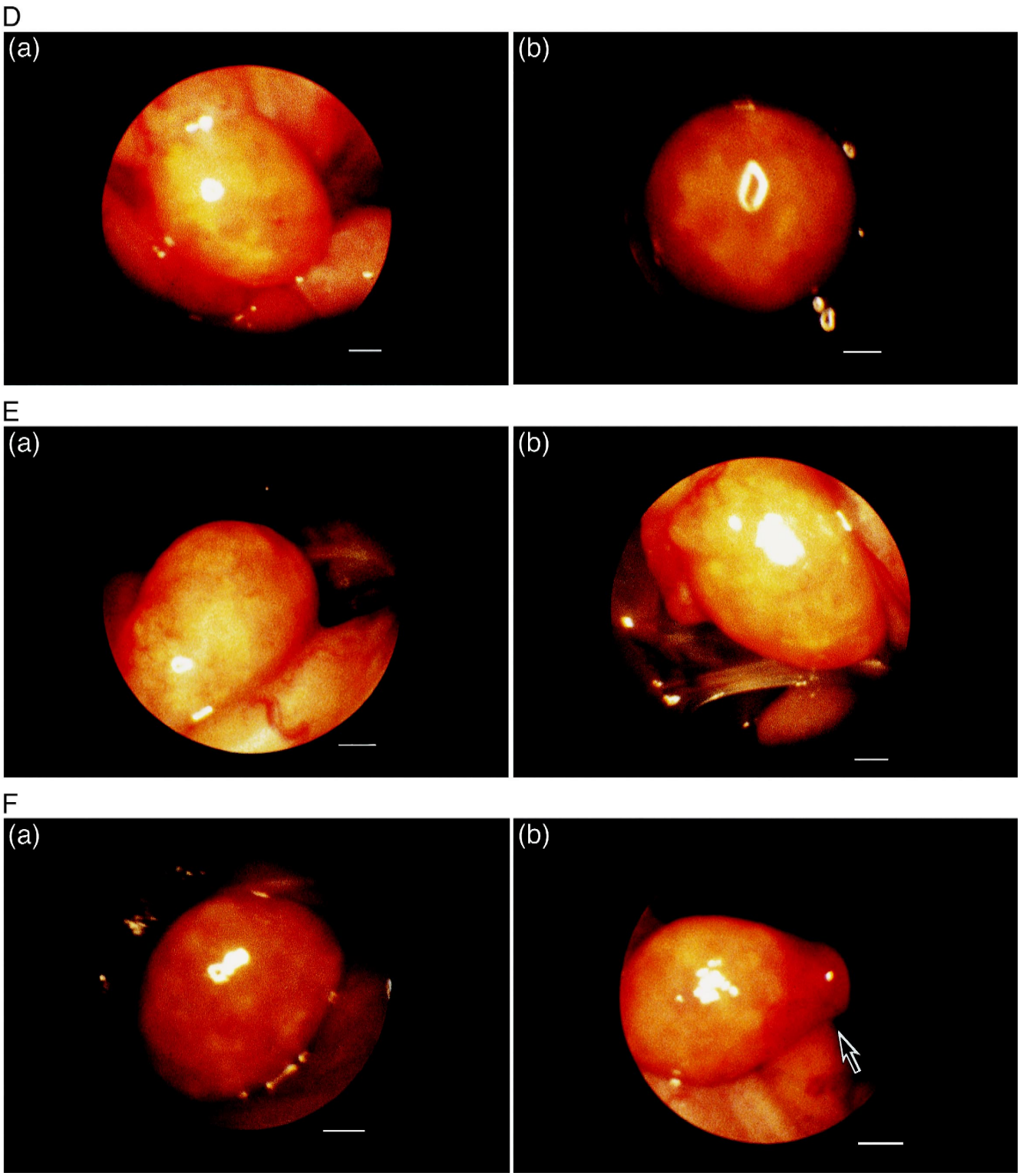


Fig. 5 (continued).

indicating the presence of the corpus luteum were found after ovulation (Fig. 5B). The ovaries of this marmoset during sulpiride-induced hyperprolactinemic anovulation are shown in Fig. 5C. At this stage, the ovaries were small and oval, and the surface exhibited a marbled mosaic-like pattern of yellow and dark-red. Neither large follicles nor corpora lutea were detectable. Long-term treatment with both cabergoline (0.01 mg kg^{-1} daily for 41 days) and bromocriptine (1 mg kg^{-1} daily for 34 days followed by 10 mg kg^{-1} daily for 38 days) failed to change the

condition of the ovaries (Fig. 5D and E). However, following long-term treatment with cabergoline at 0.05 mg kg^{-1} daily for 35 days, bright red protrusions indicating the presence of the corpus hemorrhagicum were found after ovulation in the right ovary (Fig. 5F).

4. Discussion

The common marmoset is a useful model for endocrinological research because it exhibits the same pattern of hormonal changes during the ovarian cycle as do humans (Moro et al., 1995). In this study, we found that sulpiride-induced hyperprolactinemia caused both inadequate ovarian function and anovulation in common marmosets. In contrast, Kholkute et al. (1988) reported that metoclopramide-induced hyperprolactinemia failed to affect ovarian function in the same species, although sulpiride-induced hyperprolactinemia has also been found to disrupt the menstrual cycle and ovulation in the baboon (Aso et al., 1982). Interestingly, Aono et al. (1978) reported that menstrual disturbances were more common in patients on sulpiride than in patients on metoclopramide. Thus, the difference between our results and those of Kholkute et al. (1988) may be a consequence of the different hyperprolactinemic drug used and/or the treatment period (Kholkute et al. gave metoclopramide for 60 days).

Inhibitors of prolactin secretion by the prolactin-producing cells of the anterior pituitary are thought to produce their effects by a direct agonistic action on dopamine D_2 receptors on lactotroph cells (Enjalbert and Backaert, 1983). Bromocriptine has been the most widely used prolactin-lowering agent since its introduction in 1972 (Besser et al., 1972). Worldwide experience indicates that bromocriptine is highly effective for normalizing or reducing prolactin levels in hyperprolactinemic patients, and restores normal gonadal function in approximately 70–90% of patients (Vance et al., 1984; Ferrari and Crosignani, 1986). However, the occurrence of side-effects and the need for two or three daily doses of bromocriptine remain important problems in the long-term management of hyperprolactinemic patients.

The new dopaminergic ergoline derivative, cabergoline, has been found to suppress serum prolactin levels in hyperprolactinemic patients and several reports concerning women (Ferrari et al., 1986; Mattei et al., 1988; Ciccarelli et al., 1989; Ferrari et al., 1989, 1992; Webster et al., 1994; Biller et al., 1996) have been published; however, only a few preclinical experiments have been reported so far. Furthermore, no investigation into the effect of cabergoline on hyperprolactinemia and hyperprolactinemic anovulation in a non-human primate such as the common marmoset has yet been reported.

In the present study, a single oral dose of 0.1 mg kg^{-1} cabergoline reduced sulpiride-induced prolactin secretion to less than 20% of the pretreatment level at 48 h after its administration. Cabergoline had a longer-lasting effect than bromocriptine, since the former was still effective 48 h after its administration, whereas the effect of bromocriptine lasted 8 h. These results are consistent with the experience with these drugs in hyperprolactinemic patients (Ferrari et al., 1986; Mattei et al., 1988; Ferrari et al., 1989; Ciccarelli et al., 1989) and with the results of a comparative study in rats (Di Salle et al., 1984). This long-lasting action might be related to the thorough distribution of cabergoline within highly perfused organs, including the pituitary, and to its particularly slow elimination from the pituitary (Ferrari et al., 1986). Indeed, in vitro data indicate that cabergoline binds firmly and for a very prolonged period to monolayer cultures of prolactin-secreting cells (Di Salle et al., 1984).

In the present study, repeated administration of cabergoline at 0.05 mg kg^{-1} day^{-1} reduced serum prolactin levels, normalizing them within 1 week, in hyperprolactinemic anovulatory common marmosets. Thereafter, we observed ovulation following an increase in the serum estradiol level. This reflected the resumption of the follicular development that had been depressed during the preceding hyperprolactinemic state. In association with this ovulation, we observed a normal pattern of secretion of ovarian hormones, and we also directly confirmed the presence of corpora lutea by laparoscopy. On the basis of these results, it can be inferred that the increased serum progesterone levels represent secretion from the corpus luteum.

The dose of cabergoline needed to restore ovulation in 100% of the group was 0.05 mg kg^{-1} day^{-1}, whereas even 10 mg kg^{-1} day^{-1} of bromocriptine restored ovulation in only 67% of the animals tested. Thus, cabergoline was about 200 times more effective than bromocriptine to suppress sulpiride-induced anovulation in marmosets. In contrast, Webster et al. (1994) was reported a potency ratio between cabergoline and bromocriptine which was approximately 35-fold in hyperprolactinemic amenorrheic women. The differences between our results and those of Webster et al. (1994) may be a consequence of species and metabolism differences between marmosets and humans or of differences in the drug treatment schedule of. We obtained similar results with a sulpiride-induced hyperprolactinemic anovulation model in rats and in a pituitary-transplanted hyperprolactinemic anovulation model in rats; cabergoline was 100–1000 times more effective than bromocriptine (unpublished data). Taken together, our data suggest that cabergoline restored ovulation as a result of an indirect action on the ovaries that is secondary to its suppression of elevated serum prolactin levels. Our data also showed that the ovaries returned to the anovulatory condition following a further elevation in serum prolactin levels when cabergoline administration was discontinued. This strongly suggests that the occurrence of ovulation during cabergoline treatment in the sulpiride-induced anovulation model was due to the cabergoline itself, and was not simply a spontaneous resumption of ovulation.

In conclusion, the present report describes a useful experimental model for the study of hyperprolactinemic anovulation. The inhibitory effect of prolactin hypersecretion and the resumption of ovulation during cabergoline treatment validate this marmoset model involving sulpiride-induced hyperprolactinemic anovulation. Our results suggest that the new dopamine D_2 receptor agonist, cabergoline, has a potent and long-lasting action, and thus could be a useful drug for the treatment of the problems experienced by hyperprolactinemic subjects, including galactorrhea, amenorrhea, and anovulation.

References

Aono, T., Shioji, T., Kinugawa, T., Onishi, T., Kurachi, K., 1978. Clinical and endocrinological analyses of patients with galactorrhea and menstrual disorders due to sulpiride or metoclopramide. J. Clin. Endocrinol. Metab. 47, 675–680.

Aso, T., Matsuoka, M., Su, J., Horie, K., Taii, S., Motohashi, T., Nishimura, T., 1982. Influence of sulpiride-induced hyperprolactinemia on baboon menstrual cycle: a longitudinal study. J. Med. Primatol. 11, 20–34.

Barrow, D.L., Tindall, G.T., Kovacs, K., Thorner, M.O., Horvath, E., Hoffman, J.C. Jr., 1984. Clinical and pathological effects of bromocriptine on prolactin-secreting and other pituitary tumors. J. Neurosurg. 60, 1–7.

Besser, G.M., Parke, L., Edwards, C.R.W., Forsyth, I.A., MacNeilly, A.S., 1972. Galactorrhea: successful treatment with reduction of plasma prolactin levels. Br. Med. J. 3, 669–672.

Bevan, J.S., Webster, J., Burke, C.W., Scanlon, M.F., 1992. Dopamine agonists and pituitary tumor shrinkage. Endocr. Rev. 13, 220–240.

Biller, B.M.K., Molitch, M.E., Vance, M.L., Cannistraro, K.B., Davis, K.R., Simons, J.A., Klibanski, A., 1996. Treatment of prolactin-secreting macroadenomas with the once-weekly dopamine agonist cabergoline. J. Clin. Endocrinol. Metab. 81, 2338–2343.

Ciccarelli, E., Giusti, M., Miola, C., Potenzoni, F., Sghedoni, D., Camanni, F., Giordano, G., 1989. Effectiveness and tolerability of long-term treatment with cabergoline, a new long-lasting ergoline derivative, in hyperprolactinemic patients. J. Clin. Endocrinol. Metab. 69, 725–728.

Cooper, P.R., 1991. Nonoperative management of prolactinomas. In: Cooper, P.R. (Ed.), Contemporary Diagnosis and Management of Pituitary Adenomas. AANS Publications Committee, IL, pp. 83–100.

Crosignani, P.G., Ferrari, C., 1990. Dopaminergic treatments for hyperprolactinemia. Clin. Obstet. Gynecol. 4, 441–455.

Dall'Ara, A., Lima, L., Cocchi, D., Di Salle, E., Cancio, E., Devesa, J., Müller, E.E., 1988. Inhibitory effect of cabergoline on development of estrogen-induced prolactin-secreting adenomas of the pituitary. Eur. J. Pharmacol. 151, 97–102.

Di Salle, E., Ornati, G., Briatico, G., 1982. FCE 21336, a new ergoline derivative with a potent and long-acting lowering effect on prolactin secretion in rats. J. Endocrinol. Invest. 5 (Suppl. 1), 45, Abstract.

Di Salle, E., Ornati, G., Giudici, D., Ragazzi, P., Briatico, G., 1983. Effect of the new ergoline derivative FCE 21336 on prolactin and LH secretion in the rats. J. Endocrinol. Invest. 6 (Suppl. 1), 6, Abstract.

Di Salle, E., Ornati, G., Giudici, D., 1984. A comparison of the in vivo and in vitro duration of prolactin lowering effect in rats of FCE 21336, pergolide and bromocriptine. J. Endocrinol. Invest. 7 (Suppl. 1), 32, Abstract.

Eguchi, K., Kawamoto, K., Uozumi, T., Ito, A., Arita, K., Kurisu, K., 1995. In vivo effect of cabergoline, a dopamine agonist, on estrogen-induced rat pituitary tumors. Endocr. J. 42, 153–161.

Enjalbert, A., Backaert, J., 1983. Pharmacological characterization of the D2 dopamine receptor negatively coupled with adenylate cyclase in rat anterior pituitary. Mol. Pharmacol. 23, 576–584.

Ferrari, C., Crosignani, P.G., 1986. Review: Medical treatment of hyperprolactinemic disorders. Hum. Reprod. 1, 507–514.

Ferrari, C., Barbieri, C., Caldara, R., Mucci, M., Codecasa, F., Paracchi, A., Romano, C., Boghen, M., Dubini, A., 1986. Long-lasting prolactin-lowering effect of cabergoline, a new dopamine agonist, in hyperprolactinemic patients. J. Clin. Endocrinol. Metab. 63, 941–945.

Ferrari, C., Mattei, A., Melis, G.B., Paracchi, A., Muratori, M., Faglia, G., Sghedoni, D., Crosignani, P.G., 1989. Cabergoline: long-acting oral treatment of hyperprolactinemic disorders. J. Clin. Endocrinol. Metab. 68, 1201–1206.

Ferrari, C., Paracchi, A., Mattei, A.M., de Vincentiis, S., D'Alberton, A., Crosignani, P.G., 1992. Cabergoline in the long-term therapy of hyperprolactinemic disorders. Acta Endocrinol. 126, 489–494.

Ho, K.I., Thorner, M.O., 1988. Therapeutic applications of bromocriptine in endocrine and neurological diseases. Drugs 36, 67–82.

Kholkute, S.D., Patil, R.P., Desai, M.P., Puri, C.P., 1988. Metachlopromide-induced hyperprolactinemia fails to affect ovarian cyclicity in common marmosets (*Callithrix jacchus*). Primates 29, 549–555.

Kinukawa, M., Moro, M., Kojima, M., 1996. Effect of cabergoline on hyperprolactinemic model in rats. Clin. Rep. 30, 2525–2530.

Lloyd, H.M., Meares, J.D., Jacobi, J., 1975. Effects of oestrogen and bromocriptine on in vivo secretion and mitosis in prolactin cells. Nature 255, 497–498.

MacLeod, R.M., Lobyn, C., 1977. Mechanism of increased prolactin secretion by sulpiride. J. Endocrinol. 72, 273–277.

Mancini, A.M., Guitelman, A., Vargas, C.A., Debeljuk, L., Aparicio, N.J., 1976. Effect of sulpiride on serum prolactin levels in humans. J. Clin. Endocrinol. Metab. 42, 181–184.

Mattei, A.M., Ferrari, C., Baroldi, P., Cabioni, V., Paracchi, A., Galparoli, C., Romano, C., Spellechia, D., Gerevini, G., Crosignani, P.G., 1988. Prolactin-lowering effect of acute and once weekly repetitive oral administration of cabergoline at two dose levels in hyperprolactinemic patients. J. Clin. Endocrinol. Metab. 66, 193–198.

Molitch, M.E., 1989. Management of prolactinomas. Annu. Rev. Med. 41, 255.

Moro, M., Torii, R., Koizumi, H., Inada, Y., Etoh, Y., Miyata, H., Tanioka, Y., 1995. Serum levels of prolactin during the ovarian cycle, pregnancy, and lactation in the common marmoset (*Callithrix jacchus*). Primates 36, 249–257.

Oseko, F., Morikawa, K., Motohashi, T., Aso, T., 1988. Effects of chronic sulpiride-induced hyperprolactinemia on menstrual cycles of normal women. Obstet. Gynecol. 72, 267–271.

Philosophe, R., Seibel, M.M., 1991. Novel approaches to the management of hyperprolactinemia. Curr. Opin. Obstet. Gynecol. 3, 336–342.

Robert, E., Musatti, L., Piscitelli, G., Ferrari, C., 1996. Pregnancy outcome after treatment with the ergot derivative, cabergoline. Reprod. Toxicol. 10, 333–337.

Tallo, D., Malarkey, W.B., 1981. Adrenergic and dopaminergic modulation of growth hormone and prolactin secretion in normal and tumor-bearing human pituitaries in monolayer culture. J. Clin. Endocrinol. Metab. 53, 1278–1284.

Torii, R., Abbott, D.H., Nigi, H., 1996. Morphological changes of the ovary and hormonal changes through the ovarian cycle of the common marmoset (*Callithrix jacchus*). Primates 37, 49–56.

Vance, M.L., Evans, W.S., Thorner, M.O., 1984. Drugs five years later: bromocriptine. Ann. Int. Med. 100, 78–91.

Webster, J., Piscitelli, G., Polli, A., Ferrari, C., Ismail, I., Scanlon, M.F., 1994. A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhea. New Engl. J. Med. 331, 904–909.