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# Effect of Bromocriptine and Progesterone on the Length of the Ovarian Cycle in 4- and 5-Day Estrous Cyclic Rats

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To study the effect of prolactin and progesterone on the length of the reproductive cycle in the rat, rats of different estrous cycle length (four and five days, respectively) were injected daily (09.00 h) with either bromocriptine (1 mg/rat) or 70 % ethanol vehicle (0.25 ml) from the day of estrus onward, up to the appearance of the next ovulation. Each group of rats was then (16.00, metestrus) also injected with either progesterone (4 mg/rat) or 0.2 ml of olive oil. The effects of these treatments on the length of the estrous cycle was studied by both the recording of vaginal smears daily and by direct visualization of oocyte-cumulus complexes on the ensuing day of estrus (10.00 h-12.00 h). Bromocriptine treatment shortened the length of the cycle by one day in 5-day but not in 4-day cyclic rats, while progesterone treatment lengthened estrous cycles by one day in both groups of rats. Treatment with both bromocriptine and progesterone had no effect on the estrous cycle length of 5-day cyclic rats, but did prolong in one day the cycle of 4-day cyclic rats. These facts suggest that prolactin regulates the length of the ovarian reproductive cycle in the rat through its action on the secretion of progesterone by the corpus luteum.

Key words: Bromocriptine, Progesterone, Ovarian cycle.

The administration of progesterone to 4-day cyclic rats during the time of corpus luteum activity produces both a prolongation of the duration of the elevated levels of circulating progesterone during diestrous (18) and a slowing down of the growth of the follicle (10). These effects of progesterone result in a 1 day prolongation of the length of the cycle (4).

The withdrawal of the luteotrophic support of prolactin by means of bromocriptine injection (12) on estrus to 5-day cyclic rats produces both a shortening of the duration of the progesterone secretion

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by the corpus luteum of the cycle with a tendency to speed the follicular growth up (19), and accordingly a 1 day reduction of the length of the cycle (3, 19).

These findings suggest a dual role of prolactin and progesterone in regulating the length of the rat ovarian reproductive cycle. The purpose of this experiment was to study the effects of the combined treatment of progesterone and bromocriptine on the length of the estrous cycle in both 4- and 5-day cyclic rats.

## Materials and Methods

*Four-day cyclic rats.*— Forty virgin Wistar female rats from the colony of the Department of Physiology, Faculty of Medicine, University of Córdoba (Spain) were used. Animals were maintained in a light (light on 17.00-19.00 h) and temperature (20-22 °C) controlled room having free access to pelleted food and tap water. Only those rats showing at least two consecutive 4-day cycles were used (15).

*Five-day cyclic rats.*— Fifty virgin (RxU)$F_1$ female rats from the colony of the Department of Endocrinology, Growth and Reproduction, Faculty of Medicine, Erasmus University, Rotterdam (The Netherlands) were used. Animals were kept under standard conditions of light (light on 05.00-19.00 h) and temperature (20-22 °C) having free access to pelleted food and tap water. Under these conditions animals show almost exclusively 5-day estrous cycles and only 5-day cycles were used (19).

*Treatments.*— Bromocriptine (Sandoz, Basle) was dissolved in 70 % ethanol. To inhibit pituitary prolactin release, each rat was daily s.c injected with 1 mg/0.25 ml solvent at 09.00 h from the day of estrus until the ensuing day of proestrus. Injection of the vehicle served as control.

Progesterone (Sigma) was dissolved in olive oil at a concentration of 20 mg/ml. Each rat was once s.c injected at 16.00 h on metestrus with 4 mg/0.2 ml oil. Control injections consisted in 0.2 ml oil.

*Control and experimental groups.*— Rats from each estrous cycle type (4 or 5 days in length) were injected with either bromocriptine or progesterone, or with both bromocriptine and progesterone. Control injections were 0.25 ml 70 % ethanol and 0.2 ml olive oil.

*Ovulation.*— To asses whether ovulation had occurred and, accordingly, the length of the ovarian cycle, the fallopian tube was exposed through a small flank incision and the ampullary region searched for signs of ovulation on the morning (09.00-11.00 h) of the day of estrus. Both oviducts were surgically separated from the ovary and uterus and placed in glass dishes. The ampullary region was lanced with a miniblade, and the mass of adherent oocyte-cumulus complexes was expressed with a light presure. After that the number of ova were counted.

*Statistic.*— In order to assess the statistical significance of the effects of the different treatments, the exact probability Fisher's test was used. A value of P below 0.01 was considered significant.

## Results and Discussion

The effects of progesterone and/or bromocriptine treatment on the length of the ovarian reproductive cycle of 4- and 5-day cyclic rats are depicted in table I. Injection of progesterone on metestrus produces a 1 day prolongation of the length of the ovarian cycle in both 4- and 5-day cyclic rats ($p < 0.01$). Regardless whether the rats were injected or not with progesterone, bromocriptine treatment induces a 1 day shortening of the reproductive cycle in 5-day cyclic rats ($p < 0.01$),

Table I. *Effects of bromocriptine and progesterone on the length of the ovarian reproductive cycle in 4- and 5-day cyclic rats.*

* Ethanol 70 % (0.25 ml) or bromocriptine (1 mg) was injected daily at 09.00 h from the day of estrus until the ensuing day of proestrus. ** Olive oil (0.2 ml) or progesterone (4 mg) was injected at 16.00 h on the day of metestrus.

| Treatment | | n | Number of rats with cycles of | | |
|---|---|---|---|---|---|
| | | | 4 day | 5 day | 6 day |
| 4-DAY CYCLIC RATS | | | | | |
| oil | ethanol | 10 | 9 | 1 | 0 |
| oil | bromocriptine | 9 | 9 | 0 | 0 |
| progesterone | ethanol | 9 | 0 | 9[a] | 0 |
| progesterone | bromocriptine | 8 | 1 | 7[a] | 0 |
| 5-DAY CYCLIC RATS | | | | | |
| oil | ethanol | 10 | 0 | 9 | 1 |
| oil | bromocriptine | 10 | 10[a] | 0 | 0 |
| progesterone | ethanol | 13 | 0 | 3 | 10[a] |
| progesterone | bromocriptine | 12 | 0 | 10 | 2 |

[a] $p < 0.01$ vs vehicle injected rats (Fisher's test).

while it has no effect in 4-day cyclic rats. The number of ova released was 10-15 for ovulatory cycle and no differences were found between 4- and 5-day cycles.

In cyclic female rat the only difference in peripheral hormone levels between 4- and 5-day estrous cycles is the more prolonged duration of progesterone secretion by the corpus luteum in the latter (8, 9, 13). Because of that, it is generally agreed that the secretion of progesterone by the corpus luteum of the cycle is the main causative factor in determining the time of ovulation through a modulation of both the preovulatory desensitization to estrogen feed-back (5, 6) and the follicular growth and follicular steroidogenesis (4, 8, 10) and in consequence the length of the reproductive cycle (4, 8, 9). The experimental manipulation of the elevated levels of progesterone during diestrous corroborates the role of progesterone in the regulation of the length of the estrous cycle (3, 4, 14, 18, 19).

Recently, it has been pointed out that the cause of the different duration in the secretion of progesterone by the corpus luteum and accordingly the length of the cycle lies on the ability of the corpus luteum to respond to both prolactin and luteinizing hormone. Four day cycles are the result of an inability of the corpus luteum to respond to the antiluteolytic effects of prolactin during the afternoon of estrus against the luteolytic action of the luteinizing hormone during metestrus (15, 16). In contrast, a corpus luteum able to respond to such antiluteolytic effects of prolactin, results in 5-day cycles (7, 17).

The above explanation is based on both the effects of either bromocriptine (3, 15, 19) and/or anti-LH serum injection (15, 16) on the corpus luteum progesterone secretion in 4-day as well as in 5-day estrous cyclic rats and on the wide correlation between the duration of the corpus luteum progesterone secretion and the length of the estrous cycle (8, 9) but not on the effects of the anti-LH serum and/or bromocriptine on the length of the estrous cycle. This is because while during the estrous cycle of the rat, prolactin acts on the corpus luteum function only (19), the luteinizing hormone affects both the luteal and the follicular components of the ovary (11) and in accordance with the latter,

with the ensuing ovulation regardless the action of the luteinizing hormone on the corpus luteum function.

The results of this experiment evidence that the prolongation of the elevated peripheral levels of progesterone during the diestrous phase by exogenous progesterone (18) in a similar extend to that found by neutralization of the biological activity of early diestrous luteinizing hormone secretion (7, 15-17), produces a 24 hour lengthening of the rat ovarian cycle (table I).

Bromocriptine injection through the inhibitory action on prolactin release (12) produce different effects in 4- and 5-day cyclic rats. Thus, while bromocriptine affects neither the secretion of progesterone by the corpus luteum (1, 2, 15) nor the length of the cycle (3) in both 4-day and exogenous progesterone-induced 5-day cyclic rats, spontaneous 5-day cyclic and exogenous progesterone-induced 6-day cyclic rats suffer a shortening of the estrous cycle (table I) through a bromocriptine reduction of the secretion of progesterone by the corpus luteum (3, 17, 19).

## Resumen

Se estudia el efecto de la prolactina y la progesterona sobre la duración del ciclo estral en ratas con ciclos estrales de duración de 4 y 5 días, inyectadas diariamente (9 horas) con bromocriptina (1 mg/rata) o con etanol al 70 % (0,25 ml), desde el día de estro hasta la presentación de la siguiente ovulación. Los animales de cada grupo son tratados, además, el día de metestro a las 16 horas con progesterona (4 mg/rata) o con aceite de oliva (0,2 ml). El efecto de estos tratamientos sobre la longitud del ciclo estral, se determina por dos métodos: el diagnóstico citológico vaginal diario, y la visualización directa de los óvulos en los oviductos. La bromocriptina no tiene efecto alguno sobre la duración del ciclo en las ratas de 4 días, pero lo acorta en 1 día en las de 5. La progesterona prolonga los ciclos 1 día en ambos tipos de ratas. El tratamiento combinado de bromocriptina y progesterona no modifica la duración del ciclo en las ratas de 5 días, pero prolonga 1 día el de las de 4. Estos hechos sugieren que la prolactina regula la duración del ciclo estral de la rata a través de su acción sobre la secreción de progesterona por el cuerpo lúteo.

Palabras clave: Bromocriptina, Progesterona, Ciclo ovárico.

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