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The preovulatory rise of ovarian ornithine decarboxylase is required for progesterone secretion by the corpus luteum

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Abstract

Ovarian progesterone secretion during the diestrus stage of the estrous cycle is produced by luteal cells derived from granulosa and thecal cells after the differentiation process that follows ovulation. Our results show that blockade of the preovulatory rise of ovarian ornithine decarboxylase (ODC), a key enzyme in polyamine biosynthesis, by treatment with the specific inhibitor α-difluoromethylornithine (DFMO) leads to a significant decrease in the ovarian progesterone content and a dramatic fall in the plasma levels of this hormone during the following diestrus. The same inhibition was produced in spite of the fact that both luteinizing and follicle stimulating hormones were given concomitantly with DFMO. On the other hand, the acute rise in the plasma progesterone levels observed after administration of human chorionic gonadotropin to mice at different periods of the estrous cycle was not affected by DFMO administration. Our results indicate that although elevated levels of ODC are not required for acute ovarian steroidogenesis, the preovulatory peak of ovarian ODC activity observed in the evening of proestrus may be critical for the establishment of a constitutive steroidogenic pathway and progesterone secretion by the corpus luteum during the diestrus stage of the murine estrous cycle.

Keywords: Ornithine decarboxylase; Polyamines; α-Difluoromethylornithine; Progesterone; Corpus luteum; Mouse ovary

Ornithine decarboxylase (ODC, EC 4.1.1.17) is a key enzyme in the biosynthetic pathway of polyamines, ubiquitous cations implicated in processes of cell growth and differentiation, and also in cell death [1–3]. In spite of recent exciting advances in the knowledge of the regulatory mechanisms of this enzyme [4–8], the molecular mechanisms by which these substances exert their actions are only partially understood. The generation of transgenic mice over-expressing ODC has provided an important tool for understanding the implication of polyamines in several physiological and pathological processes [9–17] but there are still many examples of normal tissues, such as male mouse kidney and rat placenta, that show very high levels of ODC activity in which the physiological role of the enzyme has not been convincingly defined.

Different studies using either transgenic or non-transgenic approaches have suggested that ODC and polyamines may exert important functions in the reproductive system. Thus transgenic mice with altered polyamine homeostasis have shown reproductive deficiencies [18,19]. Moreover, early studies clearly showed that ODC plays a critical role in rodent embryogenesis since treatment of gravid mice with α-difluoromethylornithine (DFMO), a specific and irreversible inhibitor of ODC, during a critical period of pregnancy produced marked contragestational effects [20]. In the rat ovary, ODC is induced in response to exogenous gonadotropins [21–24], or to endogenous luteinizing hormone (LH), as it occurs in the evening of proestrus following the preovulatory surge of this hormone [25]. While these findings suggested that the ovarian ODC elevation may play a role in the ovulatory process, the treatment of adult cycling rats with DFMO did not provide convincing evidence to support this possibility [26].

In previous studies, we found that potassium deficiency produced marked effects on sex steroid hormone secretion both in male and female mice [27–29]. In female mice the decrease in progesterone secretion by the

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corpus luteum was found to be associated with the absence of the preovulatory rise of ovarian ODC [29]. This fall in progesterone secretion at diestrus in the potassium-deficient mice could be related, in principle, with the diminution in the LH surge but it is also possible that the decrease in plasma progesterone levels at this stage of the estrous cycle could be the consequence of a reduced ODC induction in the mouse ovary. To test this possibility, we have studied the effect of ODC inhibition by DFMO on plasma and ovarian progesterone levels in female mice. Since DFMO treatment may affect LH secretion through the inhibition of pituitary ODC [30–32], we have also studied the effect of DFMO on progesterone secretion in animals treated concomitantly with exogenous LH. The effect of DFMO on the acute secretion of progesterone in response to human chorionic gonadotropin (hCG) was also addressed. Our results indicate that in mice the preovulatory rise of ovarian ODC may have a permissive role in some of the changes elicited by cycling gonadotropins on ovarian functions but this rise is critical for a secretory activity of the corpus luteum formed from the ovulatory follicle.

Materials and methods

Animals and treatments. Adult Swiss CD1 female mice were used in these experiments. Control animals were fed standard chow (UAR A03, Panlab, Barcelona, Spain) and water ad libitum. Animals were maintained at 22 °C ambient temperature and 50% relative humidity under a controlled 12-h light-dark cycle. Estrous cycles were monitored by daily vaginal smears and only mice exhibiting at least two consecutive 4-day estrous cycles were included in the study. Blood samples were collected under light ether anesthesia by cardiac puncture, at 9.00 and 18.00–24.00 h in proestrus or at 9.00 h in estrus and diestrus (one puncture per animal). Plasma was obtained by centrifugation at 4 °C and was kept frozen at −70 °C until analysis. Mice were killed by decapitation under ether anesthesia at different stages of the estrous cycle and the ovaries were quickly removed and weighed before homogenization.

Potassium deficiency was produced by giving the mice a diet similar to the control but containing a low concentration of potassium (120 mg/kg potassium, UAR 212K, Panlab, Barcelona, Spain) for a period of 15 days. To study the effect of DFMO on acute steroidogenesis, animals were given hCG (6 IU/mouse, i.m.) at 9.00 h of all estrous stages and sacrificed 5 h after the injection. DFMO (2 mg/g, i.p., Illex Oncology, San Antonio, TX) was injected 30 min before hormone administration. To inhibit the preovulatory rise of ovarian ODC, DFMO was given under different schedules (see results) during the afternoon/evening of proestrus and blood was collected by cardiac puncture in the morning of the next diestrus. For hormone replacement experiments, three groups of potassium-deficient mice were treated with (a) saline, (b) 6 IU of LH, and follicle stimulating hormone (FSH), or (c) LH + FSH + DFMO at 16.00 h of proestrus and plasma was collected at 9.00 h of the next diestrus.

Analytical methods. Progesterone was determined by ELISA using the Enzymun-Test kit supplied by Boehringer–Mannheim Immunodiagnostics (Mannheim, Germany). Ovarian progesterone concentration was determined after homogenization of ovaries using a Polytron (Brinkmann Instruments, Westbury, NY) in ice-cold ethanol (1:20 w/v). The extract was centrifuged at 10,000*g* for 20 min, the supernatant was diluted in 50% ethanol containing 0.9% NaCl, and progesterone was measured. The intraassay variation and sensitivity were 10% and 5 pg/tube, respectively.

For ODC determination the ovaries were homogenized with the aid of a Polytron homogenizer in buffer containing 25 mM Tris pH 7.2, 2 mM dithiothreitol, 0.1 mM pyridoxal phosphate, 0.1 mM EDTA, and 0.25 M sucrose. The crude extract was centrifuged at 12,000*g* for 20 min and ODC activity was determined in the supernatant according to published methods [24,27] by measuring, $^{14}CO_2$ release from 0.07 mM L-[1-^{14}C]-ornithine (specific activity 56.2 mCi/mmol, Moravek Biochemicals, USA).

Statistics. Results are given as means ± SEM. The significance of differences observed was assessed by ANOVA, followed by the post hoc Newman–Keuls test. $p < 0.05$ was considered statistically significant.

Results

Fig. 1 shows plasma progesterone levels and ovarian ODC activity at different stages of the estrous cycle in

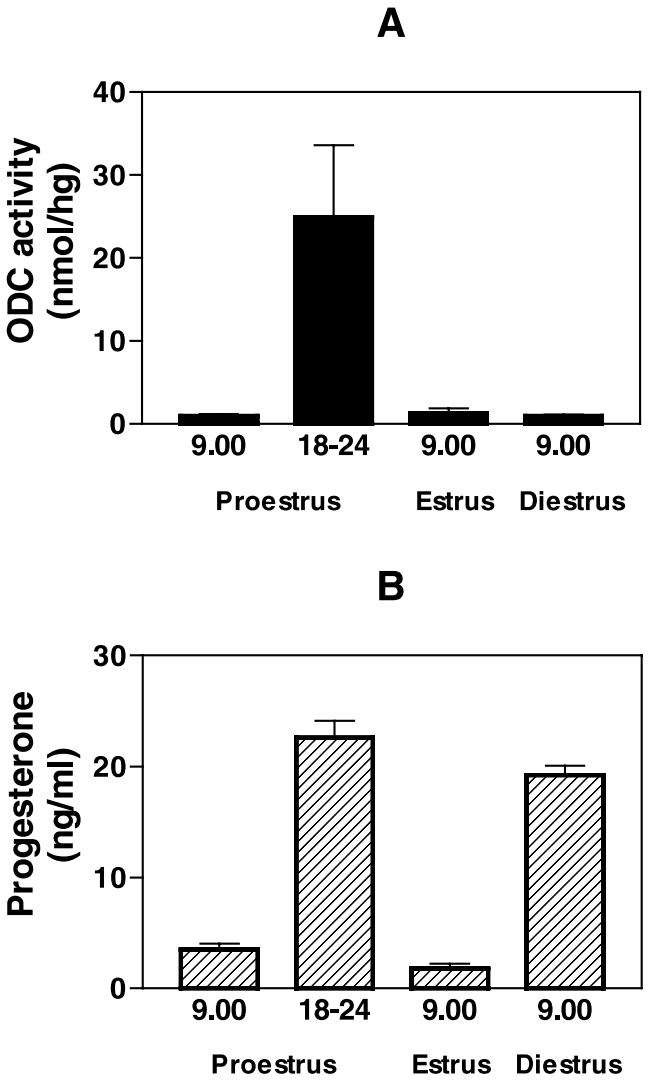

Fig. 1. Ovarian ODC activity (A) and plasma progesterone concentration (B) at different phases of the murine estrous cycle.

adult female mice. The time-course of plasma progesterone values in mice was similar to that reported in rats [33]. There was a transient rise in plasma progesterone concentration at the evening of proestrus and a period with sustained elevated levels during diestrus. Mean values of ovarian ODC activity from 18.00 to 24.00 h at proestrus were remarkably higher than those measured at 9.00 h at any stage of the estrous cycle. The increases in plasma progesterone and ovarian ODC activity in the late proestrus may be the consequence of the preovulatory surge of LH. Fig. 2 shows that ovarian ODC activity and progesterone secretion increased in response to the administration of hCG. It must be noted that while ODC activity was remarkably elevated in the ovary after 5 h of hCG injection at any stage of the estrous cycle (Fig. 2A), progesterone secretion increased dramatically at estrus, only moderately at proestrus, and did not change at all at diestrus (Fig. 2B). The concomitant administration of DFMO with hCG totally abolished the increase in ovarian ODC but only produced a moderate effect on progesterone secretion at proestrus. These results indicate that the inhibition of the rise of ovarian ODC activity in response to exogenous hCG produced by DFMO does not affect the acute increase in progesterone secretion elicited by the peptide hormone.

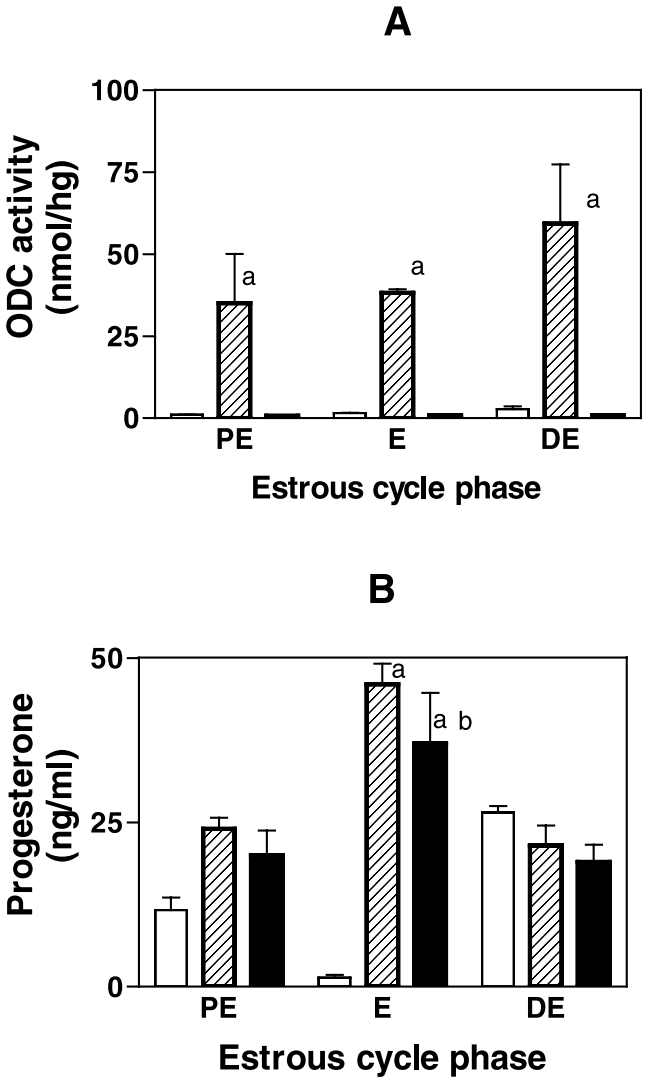

Fig. 2. Effect of DFMO on the changes induced by hCG in the ovaries and plasma of female mice. (A) Ovarian ODC activity in control (open bars), hCG-treated mice (hatched bars) and hCG + DFMO-treated mice (solid bars); (a) $p < 0.001$. (B) Plasma progesterone concentration in control (open bars), hCG treated mice (hatched bars) and hCG+DFMO treated mice (solid bars); (a) $p < 0.001$ vs control; (b) $p < 0.05$ vs hCG group.

To test whether the blockade of the preovulatory rise in ODC activity observed in the evening of proestrus may affect the constitutive progesterone secretion observed at diestrus, we treated female mice with DFMO during proestrus following different schedules. Table 1 shows that oral administration of a single dose of DFMO (2 mg/g) given at 14.00 h of proestrus produced a marked fall in plasma progesterone during the next diestrus (about 39% of control values). This effect was further increased by repeated administration of DFMO (2 mg/g, three times 3 h apart); in this case, plasma progesterone concentrations decreased to 19% of the values found in saline-treated animals. Repeated administration of a lower dose (0.5 mg/g, three times) also produced a significant reduction in progesterone (to 36% of control group). Strikingly, the decrease in plasma progesterone was greater than that found in ovarian progesterone, which fell from 11.07 ± 0.81 μg/g in the control group to 8.21 ± 0.31 μg/g ($p < 0.01$) in the group treated repeatedly with 2 mg/g DFMO. The time of DFMO administration was critical to induce the fall in plasma progesterone concentration, since the administration of the enzyme inhibitor at estrus or diestrus did not affect progesterone values at the next diestrus (Fig. 3) or proestrus (data not shown). No apparent macroscopic differences between the ovaries of control and DFMO-treated mice were observed.

To explore the possibility that the changes elicited by DFMO treatment on progesterone secretion might be

Table 1
Effect of DFMO treatment at proestrus on diestrus plasma progesterone levels

Treatment	[Progesterone] (ng/ml)
Vehicle	18.5 ± 0.9
0.5 mg/g, 3 doses	6.7 ± 0.7[a]
2 mg/g, 1 dose	7.2 ± 3.5[a]
2 mg/g, 3 doses	3.5 ± 1.0[a]

DFMO dissolved in 0.9% NaCl was given by gavage to regularly cycling female mice at different concentrations starting at 15.00 h of proestrus (the first dose) and the others 3 h apart. Blood was collected at 9.00 h of the next diestrus. Results are the means ± SEM from 6 to 8 animals per group.

[a] $p < 0.001$ vs saline.

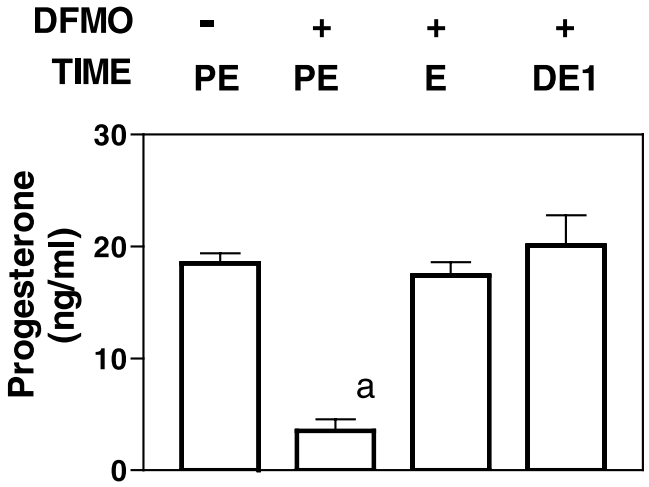

Fig. 3. Time dependence of DFMO administration on diestrus plasma progesterone levels. Three doses of DFMO (2 mg/g) were given by gavage between 14.00 and 20.00 h in the evening of proestrus (PE), estrus (E), or diestrus (DE) and plasma progesterone levels were determined in the morning of the following diestrus; (a) $p < 0.001$ vs control.

Table 2
Effect of proestrus treatment with DFMO and gonadotropins on diestrus plasma progesterone levels in control and potassium-deficient mice

Treatment	Control	Potassium-deficient
Vehicle	26.5 ± 0.96	1.80 ± 0.38
LH + FSH	24.1 ± 1.72	26.0 ± 0.75[a]
LH + FSH + DFMO	7.8 ± 1.65[a]	5.7 ± 0.94[b]

Control and potassium-deficient mice were treated with 6 IU of LH and FSH at 16.00 h of proestrus. DFMO (2 mg/g) was given by gavage at 14.00, 17.00, and 20.00 h. Progesterone was analyzed in the blood collected at 9.00 h in the next diestrus. Results are the means ± SEM from 5 to 8 animals per group.

[a] $p < 0.001$ vs vehicle.

[b] $p < 0.001$ vs LH + FSH.

related to a possible reduction in the preovulatory surge of gonadotropins rather than to the decrease of ovarian ODC, we studied the effect of DFMO on progesterone secretion after exogenous administration of LH + FSH. For this experiment, we used potassium-deficient mice because they exhibited very low values of progesterone secretion at diestrus as a consequence of reduced LH secretion in the evening of proestrus [29]. Table 2 shows that administration of DFMO during the evening of proestrus almost abolished the dramatic increase in plasma progesterone at diestrus produced by the administration of gonadotropins.

Discussion

Progesterone secretion by the corpus luteum is of crucial importance in the early steps of pregnancy. Although it is well known that corpus luteum cells are formed by the differentiation of granulosa and thecal cells derived from graafian follicles [34,35], the molecular mechanisms implicated in this process are poorly known. The pulsatile release of gonadotropins from the pituitary and the action of different types of growth factors are critical for the onset and maintenance of cyclic ovulatory activity [36]. In the rodent ovary, the steroidogenic pathway is stimulated by the binding of LH to specific receptors which are coupled to the activation of the cAMP-signalling cascade [34–36] and different experiments have shown clearly that LH and hCG induce ODC activity in the ovary of immature and adult rats [21–24]. Moreover, ovarian ODC also presents a cyclic pattern with a preovulatory peak of activity that follows the LH surge in the evening of proestrus [25]. These data suggested that ovarian ODC may play an important role in the ovulatory process; however, blockade of ovarian ODC by DFMO, an irreversible and specific inhibitor of this enzyme [37], did not appear to affect either ovulation or fertility in the adult rat [26].

Our present results reveal that the preovulatory rise of ovarian ODC in mice is necessary to achieve elevated plasma progesterone levels during diestrus, which are thought to be essential for the implantation of the blastocyst. This effect of DFMO on progesterone secretion appears to be due mainly to the inhibition of ovarian ODC rather than due to a hypothetical alteration of LH or FSH secretion which could be produced by the inhibition of pituitary ODC [30–32], since reduced progesterone secretion was also observed when DFMO and gonadotropins were given concomitantly. Our results also indicate that while after hCG administration a potent induction of ovarian ODC was obtained at all stages of the estrous cycle, the increases in progesterone secretion were evident only in the periods with low basal progesterone levels in plasma, mainly at diestrus. Moreover, the fact that DFMO did not alter the changes in progesterone elicited by hCG, in spite of the total inhibition of ovarian ODC, suggests that ODC does not play a critical role in the rapid stimulation of progesterone secretion by gonadotropins. On the other hand, the finding that abolishment of the preovulatory rise of ovarian ODC produced a delayed response in the secretory capacity of the ovary at diestrus suggests that a marked increase in ovarian ODC activity may be necessary for the formation of a functional secreting corpus luteum. Since the origin of the elevated plasma progesterone levels at diestrus is the luteinized cells derived from the ovulatory follicles, it is likely that the increase in ovarian ODC that takes place in the evening of proestrus is necessary for activating the differentiation process that leads to the transformation of granulosa and thecal cells into luteic cells. In this regard, it has been suggested that ODC and polyamines may participate in the different stages of cellular differentiation in a

variety of systems, although the molecular mechanisms by which these organic cations exert their actions have not been precisely defined [38]. One possibility is that polyamines may be required for the changes in the expression of key regulatory enzymes or proteins in the steroidogenic pathway, such as $P450_{scc}$, StAR, $P450_{17\alpha}$, or $P450_{arom}$, which take place at the onset of the luteinizing process [36,39]. It is also possible that the alteration of the cycling pattern of ovarian ODC may affect the angiogenic process that is associated with the formation of a functional corpus luteum. The latter assumption is in agreement with recent evidence which suggests that ODC and polyamines exert a relevant role in the angiogenic process, at least in tumors [40,41]. The fact that the reduction in progesterone concentration at diestrus in the ovary is less than the decrease observed in plasma could be explained by assuming that both processes, steroidogenesis and angiogenesis, may be dependent on the rise of ovarian ODC prior to ovulation.

A link between ovarian ODC and progesterone secretion has also been observed while studying the effect of potassium deficiency on reproductive functions in mice [29]. In the present study, the reduction in plasma progesterone levels was slightly smaller than that observed in the potassium-deficient mice, but preliminary experiments have shown that blockade of the preovulatory rise of ovarian ODC results in transient infertility [42], probably by the diminution of plasma progesterone levels. Alterations in female reproductive ability in different species caused by DFMO treatment have been recently reported [43,44]. These results and those obtained from transgenic mice over-expressing ODC or spermidine/spermine acetyl transferase [18], a key enzyme in the polyamine retroconversion pathway, support the view that polyamines play a relevant role in the mammalian reproductive system.

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