α-Difluoromethylornithine Modifies FSH Secretion and Puberty Onset in the Female Rat (43954)

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Abstract. **FSH secretion is high in immature female rats from Postnatal Day 5 to 18 and decreases thereafter. This is a relatively steroid-independent event of cerebral origin and of importance for puberty onset. Polyamines, a group of ubiquitous amines, play an essential role in tissue growth and differentiation, body weight increment, brain organization, and molecular mechanisms of hormonal action. Polyamine levels as well as the activity of ornithine decarboxylase, the limiting enzyme in polyamines biosynthesis, are highest during development. Inhibition of their synthesis during this period by α-difluoromethylornithine (DFMO), a specific and irreversible inhibitor of ornithine decarboxylase, impairs normal brain development.**

The present study tested the hypothesis that polyamines play a role during brain organization of reproduction. DFMO was administered following different schedules in female newborn rats, and the effect on pituitary secretion, puberty onset, and fertility was evaluated. In three groups (daily injections from Day 1 to 9, or from Day 1 to 6, or injections on alternate days from Day 1 to 9), a delay in vaginal opening and first estrous was observed. When vaginal opening was plotted against body weight, it was evident that in groups daily injected with DFMO vaginal opening occurred at a lower body weight. In the group treated on alternate days, a delay occurred but at a higher body weight than in controls. In this group, serum FSH levels on Day 10 and 20, but not on Day 30, were higher in DFMO rats. In the group treated from Day 1 to 6 daily, DFMO increased serum FSH on Postnatal Day 20. After vaginal opening, estrous cyclicity in control and DFMO injected rats was similar. There was no significant effect of treatment on fertility and litter weight or number of offspring at birth.

It is concluded that DFMO, an inhibitor of ornithine decarboxylase, administered during the first week of life in female rats is followed by prolonged high FSH serum levels and delayed puberty, but once puberty occurs, fertility is normal.

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Participation of the brain in controlling early events of the development of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL) secretion, as well as on sexual development in mammals, is well documented (1, 2). FSH secretion is high in immature female rats from days 5 to 18, and decreases thereafter until adulthood. Those high FSH levels are considered important for ovarian maturation and puberty onset (3–5). Temporary elevation of FSH levels in the female is thought to be a relatively steroid-independent event of central origin (6), although several other components are thought to be involved. Furthermore, FSH secretion during development is sexually differentiated, as FSH hormone levels in the male are low in the first postnatal weeks and increase at the time when FSH levels decrease in the female (7, 8). Striking sexual differences have also been described for LH and PRL secretion in the prepubertal rat, and participation of sexual organization of brain structures has been related to the occurrence of such differences (3, 9–12).

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Polyamines (putrescine, spermidine, and spermine) are a group of ubiquitous aliphatic amines that play an essential role in tissue growth and differentiation, in body weight increment and brain organization (13–15). In addition, polyamines also play a role in the molecular mechanisms of hormonal action, intracellular signaling, and cell-to-cell communication (16, 17). Numerous studies have shown that polyamine levels as well as the activity of ornithine decarboxylase, the limiting enzyme in polyamines biosynthesis, are highest during development and decline after growth process stops (18, 19). Inhibition of their synthesis during the development period by α-difluoromethylornithine (DFMO), a specific and irreversible inhibitor of ornithine decarboxylase, impairs brain normal development (20, 21), and treatment of newborn rats with polyamines induces precocious somatic and neurobehavioral development (14). Polyamine participation in brain-endocrine interactions has also been described, including effects on pituitary hormones related to the estrous cycle: ornithine decarboxylase activity and polyamines in the pituitary fluctuate during the rat estrous cycle and polyamines have been related to gonadotropins release in the adult (22–25).

The present work studies the effect of DFMO, the most common drug used as inhibitor of ornithine decarboxylase, administered following different schemes to newborn rats, on pituitary secretion, puberty onset, and fertility in the female.

Materials and Methods

Animals. Pregnant Sprague Dawley rats were housed in an air-conditioned room with lights on at 07:00 hr and off at 19:00 hr. They were given free access to laboratory chow and tap water.

Mothers with 9–10 pups each were kept in individual cages. Day of birth was considered Day 1. Pups were allowed to remain with their mothers until 22 days of age. In the experiments that were performed before the age of weaning, pups were separated from their mothers 1 hr before sampling.

α-Difluoromethylornithine (Eflornithine HCl, a gift of Marion Merrel Dow Pharmaceuticals, Inc.), a specific and irreversible inhibitor of ornithine decarboxylase, was administered to test the effect on puberty onset and fertility, according to the following schedules in order to select the appropriate treatment:

1) Group 1, injected on the first day of life (only one dose);
2) Group 1–3 D, between Day 1 and 3 (three doses, daily);
3) Group 1–9 D, between Day 1 and 9 (nine doses);
4) Group 11–19 D, between Day 11 and 19 (nine doses);
5) Group 1–6 D, between Day 1 and 6 (six doses);
6) Group 1–9 A, between Day 1 and 9 on Alternate Days 1,3,5,7,9 (five doses, alternate);
7) Group 11–16 D, between Day 11 and 16 (six doses);
8) Group 11–19 A, between Day 11 and 19 on alternate days 11,13,15,17,19 (five doses).

DFMO was administered sc, between 10:00 and 12:30 hr, in a dose of 500 mg/day/kg body wt. Control rats were injected with an equal volume of solvent (saline solution [5 μl/g]). Rats were weighed periodically from the first week of life. Starting on Day 30 rats were examined daily for vaginal opening. The day when the vagina was fully open was recorded and vaginal smears were performed thereafter for several consecutive cycles. Finally at 50–60 days of life, saline- or DFMO-injected females were tested for fertility. Each treated rat was mated with a normal male. Females were checked for pregnancy and the day, number, and sex of offspring were recorded.

Hormone Assays. All the blood samples were taken between 10:00 and 12:00 hr. Blood samples are obtained under ether fumes from the jugular vein. Serum FSH, LH, and PRL were measured by RIA using kits provided by the NIDDK. Results are expressed in terms of FSH RP2, LH RP2, and PRL RP3.

Statistical Analysis. Differences between two groups were analyzed using Student's t test. Longitudinal differences were analyzed with one way analysis of variance followed by Student-Neuman-Keuls multiple comparison test for unequal replications. $P < 0.05$ was considered significant.

Results

Effects of Different Treatments with DFMO on Body Weight and on Vaginal Opening. Treatment with DFMO reduced body weight gain if it was administered daily from Day 1 to 9, or from Day 1 to 6 (Group 1–9 D and 1–6 D) (Fig. 1). In the rest of the six different treatments, including Group 1–9 A, no significant differences were observed in this parameter.

In three, out of the eight experimental groups used, a delay in vaginal opening and first estrous was observed. Such groups were 1–9 D, 1–6 D, and 1–9 A (Fig. 2).

When vaginal opening was plotted against body wt (Fig. 3), it was found that in Group 1–9 D and 1–6 D, vaginal opening occurred at a lower body weight than their respective control. In contrast, in Group 1–9 A a delay of vaginal opening was observed and body weight at vaginal opening was greater (mean ± SEM [g]: control, 137 ± 3, $n = 27$; DFMO, 146 ± 3, $n = 29$, $P < 0.05$). Since time of vaginal opening is related to body mass (6), hormonal levels were studied in Group 1–9 A in which body wt gain was similar to controls, and in Group 1–6 D, in which DFMO reduced body weight gain.

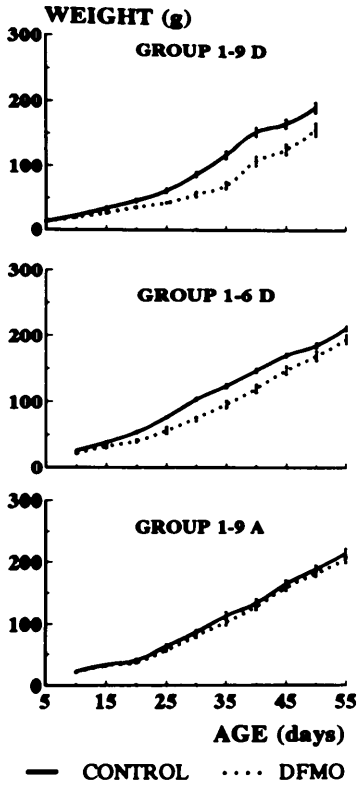

Figure 1. Body weight gain in developing female rat treated with DFMO. There is an inhibition by treatment in Group 1–9 D and 1–6 D, but not in Group 1–9 A. $P < 0.05$ from Day 10 and thereafter. For this and following figures, the point (or the height of the bar) indicates the mean and the vertical line 1 SEM. Number of rats for each group: 10 or more.

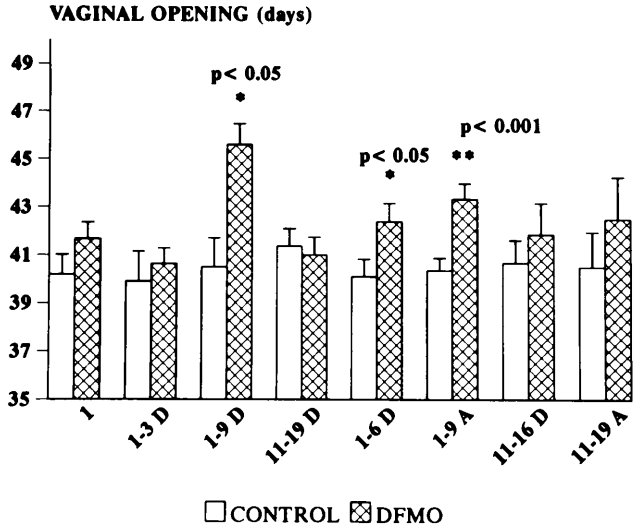

Figure 2. Day of vaginal opening in the eight studied group. In three of them, 1–9 D, 1–6 D, and 1–9 A, a delay was observed.

Serum Gonadotropins and Prolactin During and After DFMO Treatment. In Group 1–9 A (Fig. 4), in saline-injected controls basal levels of FSH were high at 6 days and higher at 10 days of age in female rats, and decreased markedly at 20 and 30 days. In DFMO-treated females, serum FSH at 10 and 20 post-

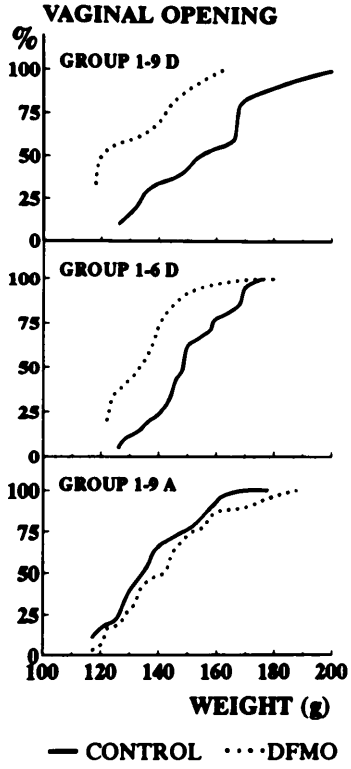

Figure 3. Vaginal opening plotted against body weight. In Group 1–9 D and 1–6 D, vaginal opening occurred at a lower body weight than their respective controls. In Group 1–9 A, a delay of vaginal opening was observed but body weight did not significantly differ between groups.

natal days was higher than in controls (10-day-old rats, ng/ml serum: control saline, 30.56 ± 2.86, $n = 27$; DFMO-treated, 45.04 ± 3.01, $n = 28$, $P < 0.002$). By 30 days no differences were observed. In Group 1–6 D (Fig. 5), serum FSH was elevated on Postnatal Day 20 in DFMO-treated rats. In contrast, daily treatment between 11 and 16 or 11 and 19 postnatal days, or on Alternate Days 11–19, did not modify FSH levels (data not shown).

The only change in serum LH observed was a small increment on Day 20 in Group 1–9 A (ng/ml serum: control, 0.82 ± 0.06, $n = 18$; DFMO, 1.04 ± 0.08, $n = 22$, $P < 0.05$). In group 1–6 D, PRL levels in females treated with DFMO showed a decrease at 20 days.

Cyclicity and Fertility After Puberty. After vaginal opening, 5-day cycles were similar in control and DFMO-injected rats. There was no significant effect of treatment on the fertility of females in any group, or on litter weight or number.

Discussion

The ability of DFMO, a specific and irreversible inhibitor of ornithine decarboxylase, to delay vaginal opening and first estrous was observed in groups in which treatment was performed during the first postnatal days when daily injections were given on Day 1

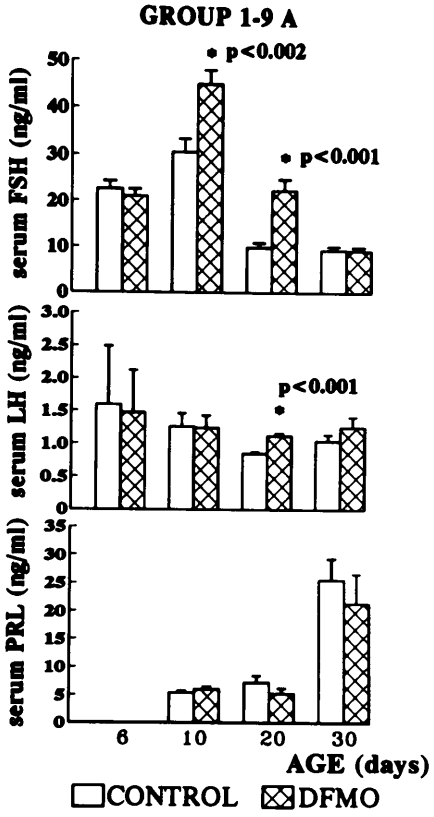

Figure 4. Serum FSH, LH, and prolactin in controls and Group 1–9 A at different ages during and after treatment.

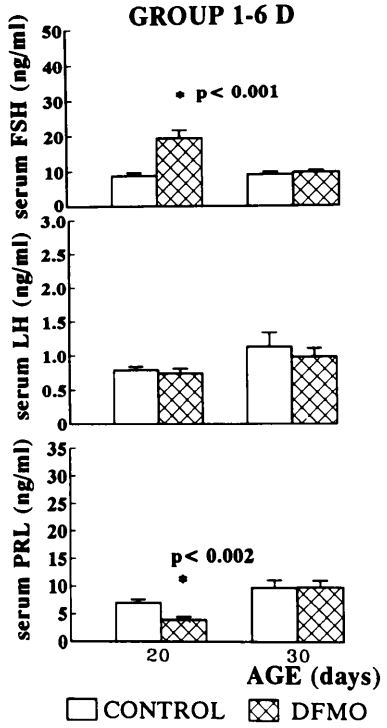

Figure 5. Serum FSH, LH, and prolactin in control and 1–6 D groups, at different ages and after treatment.

to 9, or on Day 1 to 6, or on Alternate Days 1, 3, 5, 7, and 9. Since no effects were observed when treatments were shorter (treatment on the first postnatal day, or for the first three postnatal days), it is inferred that the action of DFMO on the neuroendocrine mechanism(s) that regulate pituitary secretion and puberty should last at least until the sixth postnatal day in the female rat and it seems to be important to include days 3 to 6. The fact that in control groups in which treatment was initiated after the 10th postnatal day (daily injections of DFMO on Days 11–19; treatment for 6 days starting on Day 11 or treatment on alternate days after Day 11) there were no changes in body weight or puberty onset, indicates that DFMO's action extends only during the critical period of sexual organization until the 10th postnatal day.

As delayed puberty onset and increased FSH titers were observed both in Group 1–9 A, in which body weight gain was similar to that of controls, and in Group 1–6 D, in which treatment had reduced body weight gain, it could be suggested that those changes are independent of body weight gain.

Although the possibility exists that DFMO may act at different levels, evidence exists which indicates a direct effect of the drug on hypothalamus and pituitary. Polyamines were able to release FSH both *in vivo* and *in vitro* in two independent pioneering works (22, 26). More recently it was shown that *in vitro* DFMO inhibits the response to LHRH of the pituitary of cycling females and *in vivo* it blunted the preovulatory peaks of LH and prolactin, and that DFMO inhibited ornithine decarboxylase activity and putrescine content of the pituitary gland (24, 25). In a series of *in vitro* experiments, we found that both pituitary glands and hypothalamic explants of animals pretreated with DFMO (1–9 A regimen) changed they response to secretagoges. Pituitaries of those DFMO treated rats released more FSH in response to LHRH, and the release of LHRH induced by unspecific depolarization with ion K^+ was altered in the hypothalami (27).

FSH seems to be the main hormone involved in DFMO retarded puberty, since in DFMO treated females serum FSH was higher than in controls for several days even after the last DFMO injection in all studied groups. FSH secretion is sexually differentiated during these stages of development. FSH secretion is high in control immature female rats from Day 5 to 18, and decreases thereafter until adulthood. High FSH levels are considered a relatively steroid-independent event of cerebral origin (6) and of importance for ovarian maturation and puberty onset (3–5). It has been described that when high serum FSH levels decreased at a later stage due to chronic treatment with ethanol, puberty was also retarded (28). Alternatively, inhibition of gonadotropins levels in the female rat by sc administration of an antagonist of GnRH on Day 6, 9, 12, and 15 (10) or due to dopaminergic treatment between Day 11 and 20 of life (3) advanced vag-

inal opening. Thus, it seems that when high FSH levels persist after Day 18, as is in DFMO treated rats, there is a delay in puberty onset.

In our developing rats, an increment of LH was observed in only one group (1–9 A) and a decrease of PRL was found in Group 1–6 D. How those changes could be related to a delay in vaginal opening and first estrous is matter of further research. How does DFMO modify FSH secretion and puberty onset? The first answer is by inhibiting polyamine synthesis which is required for the maturation and differentiation of the brain. It has been reported that the LHRH neuronal network and gonadotropes are immature during the first 2 weeks of life, and polyamines may be involved in the maturation of this system (29). Interestingly enough, changes described after treatment were transient since after vaginal opening female cycles were normal and fertility maintained. Thus, it seems that DFMO treatments retard in a temporary way the maturation of brain control of puberty in the female rat.

It is concluded that DFMO, an inhibitor of ornithine decarboxylase, administered during the first week of life in female rats is followed by prolonged high FSH serum level and a delayed puberty, but once puberty does occur, fertility is normal.

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