Pharmacology Biochemistry & Behavior, Vol. 41, pp. 69–74. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/92 $5.00 + .00

Effects of Neonatal Exposure to the Antiprogestin Mifepristone, RU 486, on the Sexual Development of the Rat

MARC A. WEINSTEIN,[1] ERIC T. PLEIM AND RONALD J. BARFIELD

Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855

Received 8 April 1991

WEINSTEIN, M. A., E. T. PLEIM AND R. J. BARFIELD. *Effects of neonatal exposure to the antiprogestin mifepristone, RU 486, on the sexual development of the rat.* PHARMACOL BIOCHEM BEHAV **41**(1) 69–74, 1992.—RU 38486 (RU 486, mifepristone) is a potent progesterone receptor antagonist that has been used in humans in the pharmacologic induction of abortion. The effects of exposure to RU 486 during the neonatal period of the rat has not been previously reported. We examined the consequences of such exposure in the context of sexual development. Long-Evans rat pups were subcutaneously injected with either 100 μg RU 486, 300 μg RU 486, 500 μg progesterone (P), or 50 μg testosterone propionate (TP) in 0.05 ml sesame oil, or oil vehicle alone within 8 hours of birth, and 24 and 48 hours later. Treatment with either dose of RU 486 significantly advanced the onset of vaginal opening in females and attenuated defeminization of the lordosis response measured in males castrated as adults. As expected, TP-treated subjects were masculinized and defeminized, with females displaying fused vaginas and neither males nor females demonstrating lordosis behavior. Treatment with P caused no significant alterations in either the timing of vaginal opening or sexual behavior. These results indicate that RU 486 has clear developmental effects in the rat. Since this may well be a result of progesterone receptor blockade, further research is needed to clarify the processes involved.

Mifepristone RU 486 Neonatal Development Sexual behavior Differentiation Vaginal opening Progesterone

THE development of the antiprogestin RU 38486 (RU 486, mifepristone) has provided the unique opportunity to study the effects of reversible progesterone blockade on a variety of physiological and pharmacological processes (39). The use of RU 486 followed by prostaglandin treatment in the pharmacologic induction of abortion has been highly successful and well documented (2). Recently, there has been some concern over the possible teratogenicity of RU 486 in failed abortion attempts (6,25). Evidence supporting this position is limited to a study which examined the effects of subabortive doses of RU 486 on the fetal development of rabbits (31). In this report a small number of fetuses were observed with morphologic anomalies ranging from small size to necrotic destruction of part of the brain and the absence of vertebral canal closure. However, there has been no evidence of a teratogenic or fetotoxic effect of RU 486 in humans (2). Recently, a report cited three women who gave birth to morphologically normal infants after treatment with RU 486 but not subsequent administration of prostaglandin (33). The use of RU 486 with oxytocin in the induction of parturition (54) also warrants concern about possible adverse effects of this substance on the neonate. To date, there has been no data on the effects of perinatal exposure to RU 486 on the development of behavior.

RU 486 also provides the opportunity to study the effects of progesterone receptor blockade on the development of sexual behaviors in rats. Progestin receptors have been isolated from the brain and hypophysis of the rat during the perinatal period sensitive for sexual organization (37). Thus it is possible that progesterone may exert an influence on feminine sexual development through its receptor.

The effects of androgens and estrogens in rodent sexual differentiation have been studied extensively (13, 18, 24, 30), but much less attention has been directed to a possible role for progesterone in this scheme. Early studies determined that progesterone can prevent androgenization in rat pups treated with exogenous testosterone (5), and that it can block sterility induced in female rats by exogenous testosterone and in male rats by estrogen (32). The latter authors suggested that endogenous progesterone may protect the fetus from the deleterious effects of androgens, and that low progesterone levels during pregnancy might be the cause of hypogonadal syndromes produced by androgenic and estrogenic alterations of the hypothalamic- hypophysial-gonadal axis. A similar study demonstrating the antiestrogenic properties of maternal progesterone concurs with these results (11). Other studies have shown that perinatal administration of progesterone or R5020, a synthetic progestin agonist, can impair ejaculatory frequency in male rats (8), attenuate the defeminizing effect of androgens (36), increase feminine sexual behavior in prenatally exposed females (16), impair masculine sexual behavior in male rats (28) and reduce typical male

[1]Current address: Yale University School of Medicine, 367 Cedar Street, New Haven, CT 06510.

aggressive behavior (29).

The role of ovarian secretions in the development of feminine behavioral potential has been elucidated by studies in which both male and female rats received ovarian grafts neonatally (12,15). Furthermore, the results of Shapiro et al. (47) suggested that the serum progesterone found in three-day-old female rats may not only serve to protect the female from the normally high levels of serum androgens and estrogens, but may exert a direct influence on feminine sexual development. However, this work has not been confirmed by others (10, 45, 52). Roh, Batten, Friedman and Kim (46) employed the use of RU 486 in the study of progesterone-dependent aspects of development. They found that RU 486 antagonism of progesterone had no effect on the maturation, cleavage, and fertilization of oocytes. In addition, RU 486 has been shown to cross the placenta in humans (14), and it may alter fetal aldosterone levels (26). However, no previous study has examined the consequences of neonatal exposure of rats to RU 486. An experiment of this nature might be useful in determining the role of progesterone in sexual differentiation and whether progesterone is required for normal development. The objective of this study was to investigate the developmental consequences of neonatal exposure to RU 486.

METHOD

Hormones

Testosterone propionate (TP) was obtained from Schering Corporation (Kenilworth, NJ), and 4-pregnen-3,20-dione (progesterone) was obtained from Steraloids, Inc. (Wilton, NH). RU 38486 was generously provided by Dr. D. Philibert of Roussel-Uclaf (Romainville, France).

General Methods

Long-Evans rats were obtained from Blue Spruce Farms, Inc. (Altamont, NY) in order to breed the subjects for this study. One week before the expected date of parturition, each mother was moved to a $25 \times 44 \times 25$ cm plastic tub with wood chip bedding where she was left undisturbed until birth.

Within eight hours after birth (day of birth = day 1) each litter was reduced to four females and four males, and the pups received experimental injections. Injection volumes were 0.05 ml, and all litter-mates received the same treatment. Injection holes were sealed with Flexible Collodion (Fisher Scientific Company, Fair Lawn, NJ) and the pups were coated in sesame oil before being returned to their mother. Each pup was given two additional injections twenty-four and forty-eight hours later.

Weaning took place on day 22, and ano-genital distance was measured at this time with calipers. The pups were housed in cages of two or three by treatment and sex, and Purina laboratory chow and water were provided ad lib. All pups were weighed once every other week until day 56 in Experiment 1, and only on day 22 and 30 in Experiment 2. Beginning on day 28 all females were examined daily for the presence of vaginal opening.

Bilateral ovariectomies were performed during the tenth week of life under Metofane anesthesia (Pitman-Moore Inc., Washington Crossing, NJ). Female sexual behavior tests commenced two weeks later. These involved injecting the ovariectomized females with 8 μg estradiol benzoate (EB) two days before testing and 200 μg progesterone (P) 4 h prior to each test. The animals were then placed in a testing chamber (a $50 \times 26 \times 30$ cm glass aquarium) with an experienced male, and, in a ten mount test, lordosis quotient (percent of lordoses per mount by the male) and lordosis score (lordosis rating from 0–3) (22,23) were recorded as well as the presence or absence of proceptive behaviors. Once a week for the next two weeks the animals received the same hormone treatments but were observed only during the second week.

One week following the second behavioral test each female was implanted with Silastic tubing (30 mm, 1.47 mm i.d., 1.96 mm o.d.; Dow Corning, Midland, MI) containing crystalline testosterone propionate. The Silastics were implanted subcutaneously in the back of the neck during Metofane anesthesia. Two weeks later, the females were tested for male sexual behavior. The experimental female was placed in a testing arena ($50 \times 26 \times 30$ cm glass aquarium) and allowed five minutes to adapt before the introduction of an estrous female. The following behaviors were observed and recorded on a four channel event recorder during a 30-minute test: mount latency (time to the first mount), intromission latency (time to the first intromission), ejaculation latency (time from the first intromission to ejaculation), vocalization latency (time from ejaculation to vocalization at 22 kHz), vocalization duration, and postejaculatory intromission latency (time from ejaculation to next intromission). Ultrasonic postejaculatory vocalizations were recorded with a bat detector tuned to 22 kHz (Model S200, QMC Instruments, Ltd., London, England). Each animal was tested once a week for two weeks.

At approximately 90 days of age the gonadally intact males were tested for male copulatory behavior. Each animal was placed in a $50 \times 26 \times 30$ cm glass aquarium and allowed five minutes to adapt. An estrous female was introduced into the arena and mount latency, intromission latency, ejaculation latency, vocalization latency, vocalization duration, and postejaculatory intromission latency were recorded as described above. Tests were terminated after the first intromission following ejaculation, after 30 minutes if no intromission occurred, or if 20 minutes expired since the most recent intromission. Each subject was tested once a week for four weeks.

At the conclusion of the masculine behavior tests each male was castrated under Metofane anesthesia and two weeks later tested for feminine behavior. For the first week of feminine testing each male was injected with 8 μg EB 48 h and 500 μg P 4 h prior to behavioral observation. The subject was placed in a testing arena with an experienced male, and lordosis quotient (LQ) and lordosis score (LS) were determined in a 10 mount test as detailed above. Since the dose of EB employed resulted in too few males displaying lordosis, the dose was increased to 30 μg for the following tests. The subjects were tested once a week for three weeks.

Experiment 1

The purpose of this study was to determine if neonatal treatment of rats with RU 486 would affect sexual differentiation. Pups were divided into three treatment groups and injected with either 100 μg RU 486, 50 μg testosterone propionate (TP), or oil control. TP was employed as a positive control in order to provide a group of masculinized animals for comparative study.

Experiment 2

After the completion of the behavioral testing in Experiment 1, it was determined that a higher dose of RU 486 might have been more effective. RU 486 does not dissolve easily in oil, and 100 μg per 0.05 ml was the best that could be obtained. Since an injection bolus of greater than 0.05 ml was undesirable, it was decided that a suspension of 300 μg RU 486 in 0.05 ml sesame oil would be used. This concentration provided a well

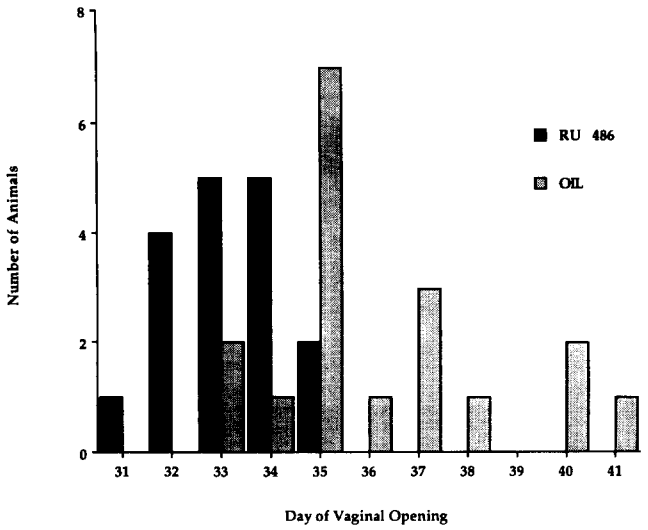

FIG. 1. Advancement of vaginal openings by RU 486. Female subjects were treated on postnatal days 1, 2, and 3 with either 100 μg RU 486 or control (oil).

distributed suspension and was mixed thoroughly before injection into the pups.

Recent work by our group (41) and others (7, 19, 20, 27) has demonstrated that RU 486 possesses not only antagonist activity, but progestin agonist activity. Thus it was difficult to interpret results of Experiment 1 as due to the antiprogesterone action of RU 486. It was certainly possible that any results obtained were due to progestimimetic activity. Therefore, in this experiment, progesterone was used to demonstrate the effects of a clear progestin agonist on parameters of sexual differentiation.

Neonatal pups were injected with either 300 μg RU 486, 500 μg P, or oil control.

Statistics

Statistical analyses were performed through the use of computer programs for the Macintosh SE. The statistical tests were carried out with Statview 512+ (BrainPower Inc., Calabasas, CA) and Statworks 1.2 (Cricket Software Inc., Philadelphia, PA). Where appropriate, nonparametric statistics were used (48).

RESULTS

Mortality

In Experiment 1, all treated pups survived at least until gonadectomy. In Experiment 2, the P-treated animals suffered 44 percent mortality with only 18 of 32 pups surviving the treatment by the time of weaning. In neither experiment did RU 486 result in mortality.

Effects of Treatment on Vaginal Introitus

Treatment with 100 μg RU 486 in Experiment 1 clearly advanced the time to vaginal opening in female rats (Mann-Whitney, $U=30.5$, $p<0.002$). The mean time to vaginal introitus for RU 486 subjects was 33 days as compared to 36 days in oil-treated controls (Fig. 1, Table 1). All females treated with TP displayed permanently fused vaginas and were therefore left out of the statistical analysis.

TABLE 1

FREQUENCY AND CUMULATIVE DISTRIBUTIONS OF THE DAY OF VAGINAL OPENING IN FEMALE SUBJECTS EXPOSED TO NEONATAL RU 486, PROGESTERONE, OR CONTROL

			Age (days) at Vaginal Opening										
Treatment	n	Distribution	31	32	33	34	35	36	37	38	39	40	41
RU 486		Frequency	1	4	5	5	2						
100 μg*	17	Cumulative	1	5	10	15	17						
Control		Frequency			2	1	7	1	3	1	0	2	1
Exp. 1	18	Cumulative			2	3	10	11	14	15	15	17	18
RU 486		Frequency			4	7	5						
300 μg†	16	Cumulative			4	11	16						
Control		Frequency			2	0	3	4	0	2			
Exp. 2	11	Cumulative			2	2	5	9	9	11			
Prog.		Frequency			1	3	2	2	1				
500 μg	9	Cumulative			1	4	6	8	9				

Females treated with 50 μg testosterone propionate developed permanently fused vaginas. All animals received the specified treatment on each of the first three days of life.

*Differed significantly from experiment 1 oil control ($p<0.002$).

†Differed significantly from experiment 2 oil control ($p<0.02$).

In Experiment 2, treatment with 300 μg RU 486 also accelerated time to vaginal introitus compared to controls (Mann-Whitney, $U=35.5$, $p<0.02$) and there was an overall effect of treatment on day of vaginal opening (Kruskal-Wallis, $H's=7.708$, $p=0.021$). The mean age of RU 486-treated subjects at the time of vaginal opening was 34 days compared to 36 days for oil-treated controls (Table 1). The average age for the P-treated group was 35 days which was not significantly different than the control. By day 35, 100 percent of both RU 486 treatment groups had displayed vaginal openings as compared to 67 percent of the P-treated subjects and 50 percent of oil-treated controls (Table 1).

Feminine Sexual Behavior

The ability of castrated males to display feminine behavior after sequential injections of estrogen and progesterone was significantly affected by the treatments of Experiment 1. Neonatal treatment with 100 μg RU 486 increased the lordosis response of adult males. One-way ANOVA revealed significant effects of treatment each week on lordosis quotient [week 1: $F(2,43)=4.86$, $p=0.013$; week 2: $F(2,43)=4.39$, $p=0.018$; week 3: $F(2,43)=3.84$, $p=0.029$] and lordosis score [week 1: $F(2,43)=4.76$, $p=0.014$; week 2: $F(2,43)=4.44$, $p=0.018$; week 3: $F(2,43)=4.63$, $p=0.015$]. Since the mean LQ scores for the

TABLE 2

MEAN (SEM) LORDOSIS SCORE RATINGS OF EXPERIMENT 1 RESPONDING MALES ONLY

Treatment	n	Week 1	n	Week 2	n	Week 3
RU 486	5	6.4 (1.9)	5	8.2 (1.2)	5	12.8 (1.1)
Oil	0	0	3	3.3 (0.9)*	2	5 (2)*
TP	0	0	1	2*	3	2.7 (0.9)*

*Significantly less than 100 μg RU 486 scores (Fischer PLSD $p<0.05$).

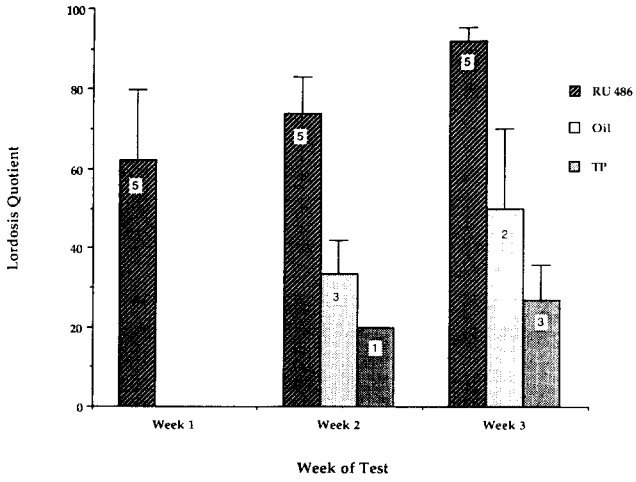

FIG. 2. Feminine behavior induced in males (Experiment 1). The data includes only those animals that responded to sequential estrogen and progesterone administration following castration. The number of responding animals appears in each bar. The animals were tested three times as outlined in the text.

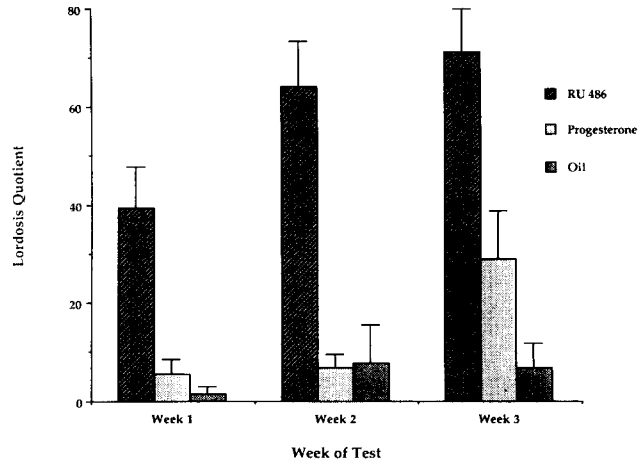

FIG. 3. Feminine behavior induced in males (Experiment 2). The data includes all animals (responders and nonresponders). The animals were tested three times as outlined in the text.

subjects were quite low, due to relatively few responders in each group, it was decided that all nonresponders would be discarded from the post hoc statistical analysis. Fischer PLSD post hoc tests were performed between groups and the results are summarized in Fig. 2 and Table 2. Additionally, only the RU 486 treatment group demonstrated perfect LQ scores of 100.

In Experiment 2, males treated during the first three days of life with 300 μg RU 486 had significantly larger LQ scores than the P-treated group or controls during all three tests [Fig. 3; one-way ANOVA; week 1: $F(2,35)=11.45$, $p=0.0001$; week 2: $F(2,35)=17.73$, $p=0.0001$; week 3: $F(2,33)=18.41$, $p=0.0001$]. The same trend was observed with LS scores. The proportion of animals responding (LQ>0) to estradiol and progesterone by the final test were 13 of 15 RU 486 subjects, 6 of 9 in the P group, and 2 of 12 control animals responding by the final test, $\chi^2(2)=13.78$, $p<0.01$. (At the time of the last test one animal from the oil group had died and one from the RU 486 group was sickly; these were not tested during week 3.)

The treatment of neonatal female rats with TP in the dose used on Experiment 1 virtually abolished the ability of female adults to display feminine sexual behavior after ovariectomy and hormone replacement. Neither treatment with RU 486 in Experiment 1 or RU 486 or P in Experiment 2 resulted in any significant alterations in feminine behavior in the females when compared to controls.

Masculine Sexual Behavior

The neonatal treatment of male rat pups with RU 486 in Experiment 1 and 2 resulted in no significant alterations in normal male sexual behavior.

As in the masculine behavioral tests of males, similar tests with the females revealed no significant differences between RU 486 and controls, and no differences in Experiment 2. Not surprisingly, the only animals to have displayed ejaculatory motor patterns and 22 kHz vocalizations were the females treated at birth with TP. An equal number of animals in each group showed the ability to mount and intromit.

Effects of Treatment on Anogenital Distance and Body Weight

In Experiment 1 and Experiment 2 no significant effect of neonatal treatment with RU 486 was found on the ano-genital distance of the 22-day-old male or female rats. There was also no significant effect of RU 486 on the body weight of the developing animals.

DISCUSSION

We report here that neonatal exposure to RU 486 in rats accelerates time to vaginal opening and induces alterations in differentiation of sexual behavior. However, in both males and females, neonatal treatment with 100 μg RU 486 had no significant effect on the anogenital distance of 22-day-old rat pups. Similarly, the results of Experiment 2 show that early postnatal treatment with progesterone plays little role in the regulation of anogenital distance.

Perhaps most surprising and significant was the effect of the neonatal treatments on the day of vaginal introitus. It is difficult to explain the advancement of vaginal opening in the RU 486-treated females since it has been shown that TP exposure on day 4 or 5 caused advancement of vaginal opening in rats (24, 32, 35, 51), but when administered on day 1, it resulted in delayed vaginal opening (36). Exogenous estrogen during early development (day 5) has also been shown to induce advancement of vaginal introitus (42). Therefore, pubertal advancement, displayed through vaginal opening, seems to be a concomitant of defeminization in this group.

It is possible that in this study RU 486 has blocked an active role of progesterone in the regulation of pubertal timing by interrupting a progestin-mediated mechanism that postpones vaginal opening in normal females. It is also of interest that neonatal P administration did not alter the timing of puberty when compared with oil controls. Although treatment with 300 μg RU 486 differed insignificantly from the P subjects, treatment with 100 μg RU 486 significantly advanced vaginal opening compared to P subjects. The progesterone already present in the pup may be at a sufficient level for delaying vaginal opening, therefore any increase in progesterone would have little effect on the timing of vaginal opening. This supports the interpretation that RU 486 interrupts a progesterone mediated process.

RU 486 may exert this effect by altering the timing of the maturation of ovarian hormone feedback on gonadotropin release. The prevailing view of timing of puberty is that shortly before the onset of puberty an estrogen-positive hypothalamic feedback mechanism matures (1). In addition, an increase in the responsiveness of the ovaries to gonadotropins occurs which results in elevated estrogen levels (38). This is followed by a LH surge which results in the onset of puberty (17,34). By blocking progesterone at birth, RU 486 may significantly alter the sensitivity of the hypothalamic and hypophyseal tissues to steroids by interrupting a progesterone-dependent developmental process. It has been shown that neonatal androgens can affect this sensitivity in a way that produces a premature LH surge, and thus, advancement of puberty (51). It has also been shown that neonatal intracerebral implantation of testosterone was more effective in advancing vaginal opening than subcutaneous injections suggesting a neural site for regulation of vaginal introitus (51).

RU 486 has been reported to bind to androgen receptors and also to behave as a weak androgen antagonist (40). Therefore, the acceleration of vaginal opening by RU 486 is probably not due to an androgenic defeminizing action. Recent evidence has revealed that RU 486, in the presence of P, also acts as a progestin-like antiestrogen (53). Since the female neonate does not provide a progestin-free environment, the antiestrogenic property of RU 486 may be significant. However, in light of earlier studies which showed that estrogen advances vaginal introitus, this property of RU 486 is probably not relevant here. In addition, antiprogesterone serum has been shown to delay the onset of vaginal opening when given to female rats on the day of birth (50). It is difficult to compare this experiment to the present study, but it demonstrates that absence of progesterone and blockade of progesterone by RU 486 have different consequences which suggests progesterone agonist properties of RU 486 in this setting. It is certainly possible that this may interrupt the development of a progesterone dependent mechanism of pubertal timing.

An interesting finding of this study was the complete lack of toxicity of both doses of RU 486 in the rat pups. Despite the ability of RU 486 to act as a potent antiglucocorticoid (4), no adverse side effects were observed in any treated animal. The extreme toxicity of progesterone observed in this study is consistent with other reports. Diamond and Wong (9) observed 40 to 90 percent mortality within 50 days when they exposed 3-day-old rat pups to 1.25 mg or 5.0 mg progesterone. They later suggested that this was due to anesthetic properties of the particular doses of progesterone during neonatal life (8). The pups in this study most likely suffered the same fate. There also were no differences in the sex of those pups treated with P that died as fifty percent of the mortality was seen in each sex.

The increase of feminine behavior in males by neonatal treatment with RU 486 may be due to antidefeminization. There exist two possible explanations for this effect. First, RU 486 may act at the androgen receptor which would result in the attenuation of androgenic defeminization and lead to the elevated lordosis quotients observed in this study. This would be consistent with the view that progesterone acts as an antiandrogen in the developing rat and demonstrates progestimimietic activity of RU 486. This is certainly plausible in light of the work in our laboratory (41) and others (7, 19, 27, 49) that has demonstrated progestin agonist properties of RU 486. Therefore, RU 486 may act as a weak progestin by attenuating the defemininizing actions of testoterone and influence feminine development in this manner.

Second, Gerall, Dunlap, and Hendricks (15) have demonstrated that ovarian secretions, which include progesterone, influence the expression of feminine behavioral potential in male and female rats. Male and female rats that were gonadectomized at birth were implanted with ovarian grafts or sham operated or use as controls. Both sexes displayed an increase in feminine behavior as adults when challenged with sequential injections of EB and P after the grafts were removed. Although the control males responded to female hormone therapy, those with ovarian grafts during development demonstrated enhanced female receptivity (12). Thus ovarian secretions including progesterone may actively promote the development of maximum female potential, a process that might be termed feminization. Again, in our males, RU 486 may have behaved as a progestin agonist.

To our knowledge this is the first report to describe the consequences to rats of neonatal exposure to RU 486. Morphologic abnormalities were observed in a small proportion of rabbit fetuses from mothers treated with RU 486 during mid to late gestation when abortion did not occur (31). Although infrequent, failure of RU 486 to induce abortion in humans does occur and one study has identified risk factors for failure (21). There has been a recent report of three women treated with RU 486 for early pregnancy termination who decided not to receive prostaglandin, given as an adjunct to RU 486 to promote abortion, and did not subsequently abort the fetus (33). All three offspring have shown no abnormalities in development at least to 15 months. However, since RU 486 crosses the placenta (14,26), since it has been implicated as an adjunct to oxytocin in late gestation to induce parturition (3,54), and since it has been shown that in utero exposure of human fetuses to synthetic progestins can result in subtle behavioral changes (43,44), similar effects in humans as reported here in rodents should be considered. Futher investigation on the mechanisms of RU 486 in producing the effects cited in this study is needed.

ACKNOWLEDGEMENTS

This work was supported by USPHS Grant HD-04484 to R.J.B. The authors are grateful to Dr. D. Philibert of Roussel-UCLAF for the generous gift of RU 486. We also thank Ellen Leiman for her excellent technical assistance.

REFERENCES

1. Andrews, W. W.; Mizejewski, G. J.; Ojeda, S. R. Development of estradiol-positive feedback on luteinizing hormone release in the female rat: A quantitative study. Endocrinology 109:1404–1413; 1981.
2. Baulieu, E. E. Editorial: RU486 and the early nineties. Endocrinology 127:2043–2046; 1990.
3. Baulieu, E. E. RU 486 as an antiprogesterone steroid: From receptor to contragestion and beyond. JAMA 262:1808–1814; 1989.
4. Bertagna, X.; Bertagna, C.; Luton, J. P.; Husson, J. M.; Girard, F. The new steroid analogue RU 486 inhibits glucocorticoid action in man. J. Clin. Endocrinol. Metab. 59:25–28; 1984.
5. Cagnoni, M.; Fantini, F.; Morace, G.; Ghetti, A. Failure of testosterone propionate to induce the "early-androgen" syndrome in rats previously injected with progesterone. J. Endocrinol. 33:527–528; 1965.
6. Cole, P. French government approves abortion pill for commercial use. Nature 335:486; 1988.
7. Collins, R. L.; Hodgen, G. D. Blockade of the spontaneous midcycle gonadotropin surge in monkeys by RU 486: A progesterone antagonist or agonist? J. Clin. Endocrinol. Metab. 63:1270–1276; 1986.
8. Diamond, M.; Llacuna, A.; Wong, C. L. Sex behavior after neonatal progesterone, testosterone, estrogen, or antiandrogens. Horm. Behav. 4:73–88; 1973.
9. Diamond, M.; Wong, C. Neonatal progesterone: Effect on repro-

ductive functions in the female rat. Anat. Rec. 163:178; 1969.
10. Dohler, K. D.; Wuttke, W. Changes with age in levels of serum gonadotropins, prolactin, and gonadal steroids in prepubertal male and female rats. Endocrinology 97:898–907; 1975.
11. Dorfman, R. I. The antiestrogenic and antiandrogenic activities of progesterone in the defence of the normal fetus. Anat. Rec. 157: 547–557; 1967.
12. Dunlap, J. L.; Gerall, A. A.; McLean, L. D. Enhancement of female receptivity in neonatally castrated males by prepuberal ovarian transplants. Physiol. Behav. 10:701–705; 1973.
13. Feder, H. H. Hormones and sexual behavior. Annu. Rev. Psychol. 35:165–200; 1984.
14. Frydman, R.; Taylor, S.; Ulmann, A. Transplacental passage of mifepristone. Lancet II(8466):1252; 1985.
15. Gerall, A. A.; Dunlap, J. L.; Hendricks, S. E. Effect of ovarian secretions on female behavioral potentiality in the rat. J. Comp. Physiol. Psychol. 82:449–465; 1973.
16. Gladue, B. A.; Clemens, L. G. Androgenic influences on feminine sexual behavior in male and female rats: defeminization blocked by prenatal antiandrogen treatment. Endocrinology 103:1702–1709; 1978.
17. Goldman, B. D. Puberty. In: Adler, N. T., ed. Neuroendocrinology of reproduction: Physiology and behavior. New York: Plenum Press; 1981:229–239.
18. Goy, R. W.; McEwen, B. S. Sexual differentiation of the brain. Cambridge, MA: MIT Press; 1980.
19. Gravanis, A.; Schaison, G.; George, M.; DeBrux, J.; Satyaswaroop, P. G.; Baulieu, E. E.; Robel, P. Endometrial and pituitary responses to the steroidal antiprogestin RU 486 in postmenopausal woman. J. Clin. Endocrinol. Metab. 60:156–163; 1985.
20. Gray, G. O.; Leavitt, W. W. RU 486 is not an antiprogestin in the hamster. J. Steroid Biochem. 28:493–497; 1987.
21. Grimes, D. A.; Bernstein, L.; Lacarra, M.; Shoupe, D.; Mishell, D. R. Predictors of failed attempted abortion with the antiprogestin mifepristone (RU 486). Am. J. Obstet. Gynecol. 162:910–917; 1990.
22. Hardy D. F.; DeBold, J. F. Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat. Physiol. Behav. 7:643–645; 1971.
23. Hardy D. F.; DeBold, J. F. The relationship between levels of exogenous hormones and the display of lordosis by the female rat. Horm. Behav. 2:289–297; 1971.
24. Harris, G. W.; Levine, S. Sexual differentiation of the brain and its experimental control. J. Physiol. 181:379–400; 1965.
25. Henrion, R. RU 486 abortions. Nature 338:110; 1989.
26. Hill, N. C. W.; Selinger, M.; Ferguson, J.; MacKenzie, I. Z. The placental transfer of mifepristone (RU 486) during the second trimester and its influence upon maternal and fetal steroid concentrations. Br. J. Obstet. Gynaecol. 97:406–411; 1990.
27. Horwitz, K. B. The antiprogestin RU 38486: Receptor-mediated progestin versus antiprogestin actions screened in estrogen insensitive T47D$_{co}$ human breast cancer cells. Endocrinology 116:2236–2245; 1985.
28. Hull, E. M. Effects of neonatal exposure to progesterone on sexual behavior of male and female rats. Physiol. Behav. 26:401–405; 1981.
29. Hull, E. M.; Franz, J. R.; Synder, A. M.; Nishita, J. K. Perinatal progesterone and learning, social and reproductive behavior in rats. Physiol. Behav. 24:251–256; 1980.
30. Hutchison, J. B., ed. Biological determinants of sexual behaviour. New York: John Wiley and Sons; 1978.
31. Jost, A. Animal reproduction—New data on the hormonal requirement of the pregnant rabbit: partial pregnancies and fetal anomalies resulting from treatment with a hormonal antagonist, given at a subabortive dosage. CR Acad. Sci. Paris (III) 7:281–284; 1986.
32. Kincl, F. A.; Maqueo, M. Prevention by progesterone of steroid-induced sterility in neonatal male and female rats. Endocrinology 77:859–862; 1965.
33. Lim, B. H.; Lees, D. A. R.; Bjornsson, S.; Lunan, C.B.; Cohn, M. R.; Stewart, P.; Davey, A. Normal development after exposure to mifepristone in early pregnancy. Lancet 336:257–258; 1990.
34. McCann, S. M. Development and maturation of the hypothalamo-hypophyseal control of the reproductive system. Ann. Biol. Anim. Biochem. Biophys. 16:279–289; 1976.
35. McDonald, P. G.; Doughty, C. Comparison of the effect of neonatal administration of testosterone and dihydrotestosterone in the female rat. J. Reprod. Fertil. 30:55–62; 1972.
36. McEwen, B. S.; Lieberburg, I.; Chaptal, C.; Davis, P. G.; Krey, L. C.; MacLusky, N. J.; Roy, E. J. Attenuating the defeminization of the neonatal rat brain: Mechanisms of action of cyproterone acetate, 1,4,6-androstatriene-3,17,-dione and a synthetic progestin, R5020. Horm. Behav. 13:269–281; 1979.
37. MacLusky, N. J.; McEwen, B. S. Progestin receptors in the developing rat brain. Brain Res. 189:262–268; 1980.
38. Ojeda, S. R.; Advis, J. P.; Andrews, W. W. Neuroendocrine control of the onset of puberty in the rat. Fed. Proc. 39:2365–2371; 1980.
39. Philibert, D. RU-38486: An original multi-faceted antihormone in vivo. In: Agarwal, M. K.; Gruyter, W. D., eds. Adrenal steroid antagonism. New York: Walter de Gruyter; 1984:77–101.
40. Philibert, D.; Mouguilewsky, M.; Mary, I.; Lecaque, D.; Tournemine, C.; Secchi, J.; Deraedt, R. Pharmacological profile of RU 486 in animals. In: Baulieu, E. E.; Segal, S. J., eds. The antiprogestin steroid RU 486 and human fertility control. New York: Plenum Press; 1984:49–68.
41. Pleim, E. T.; Cailliau, P. J.; Weinstein, M. A.; Etgen, A. M.; Barfield, R. J. Facilitation of receptive behavior in estrogen-primed female rats by the antiprogestin, RU 486. Horm. Behav. 24:301–310; 1990.
42. Ramirez, V. D.; Sawyer, C. H. Advancement of puberty in the female rat by estrogen. Endocrinology 76:1158–1168; 1975.
43. Reinisch, J. M. Prenatal exposure of human foetuses to synthetic progestin and oestrogen affects personality. Nature 266:561–562; 1977.
44. Reinisch, J. M. Prenatal exposure to synthetic progestins increases potential for aggression in humans. Science 211:1171–1173; 1981.
45. Rhoda, J.; Corbier, P.; Roffi, J. Gonadal steroid concentrations in serum and hypothalamus of the rat at birth: Aromatization of testosterone to 17B-estradiol. Endocrinology 114:1754–1760; 1984.
46. Roh, S. I.; Batten, B. E.; Friedman, C. I.; Kim, M. H. The effects of progesterone antagonist RU 486 on mouse oocyte maturation, ovulation, fertilization, and cleavage. Am. J. Obstet. Gynecol. 159: 584–1589; 1988.
47. Shapiro, B. H.; Goldman, A. S.; Bongiovanni, A. M.; Marino, J. M. Neonatal progesterone and feminine sexual development. Nature 264:795–796; 1976.
48. Siegel, S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill; 1956.
49. Thomas, M.; Monet, J. D.; Brami, M.; Dautigny, N.; Assailly, J.; Ulmann, A.; Bader, C. A. Comparative effects of 17β-estradiol, progestin R5020, tamoxifen, and RU38486 on lactate dehydrogenase activity on MCF-7 human breast cancer cells. J. Steroid Biochem. 32:271–277; 1989.
50. Turkelson, C. M.; Dunlap, J. L.; MacPhee, A. A.; Gerall, A. A. Assay of perinatal testosterone and influence of antiprogesterone and theophylline on induction of sterility. Life Sci. 21:1149–1158; 1977.
51. Wagner, J. W.; Erwin, W.; Critchlow, V. Androgen sterilization produced by intracerebral implants of testosterone in neonatal female rats. Endocrinology 79:1135–1142; 1966.
52. Weisz, J.; Ward, I. L. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 106:306–316; 1980.
53. Wolf, J. P.; Hsiu, J. G.; Anderson, T. L.; Ulmann, A.; Baulieu, E. E.; Hodgen, G. D. Noncompetitive antiestrogenic effect of RU 486 in blocking the estrogen-stimulated luteinizing hormone surge and the proliferative action of estradiol on endometrium in castrate monkeys. Fertil. Steril. 52:1055–1060; 1989.
54. Wolf, J. P.; Sinosich, M.; Anderson, T. L.; Ulmann, A.; Baulieu, E. E.; Hodgen, G. D. Progesterone antagonist (RU 486) for cervical dilation, labor induction, and delivery in monkeys: Effectiveness in combination with oxytocin. Am. J. Obstet. Gynecol. 160:45–47; 1989.