Effect of Phenoxybenzamine on Luteinizing Hormone Release in the Female Rat[1] (36035)

ALBERT RATNER
(Introduced by S. Solomon)
Department of Physiology, The University of New Mexico, School of Medicine, Albuquerque, New Mexico 87106

In the spontaneously ovulating rat treatment with the alpha adrenergic blocking agent, Dibenamine, prior to the critical period on the day of proestrus inhibits ovulation that would normally occur that evening (1), Dibenamine was also shown to be capable of inhibiting estrogen-induced (2) and progesterone-induced ovulation in the rat (3). In laying hens, both spontaneous and progesterone-induced ovulation could also be blocked by injection of Dibenamine (4). The ability of the drug to block ovulation was ascribed by these authors to the blockade of a neurogenic stimulus involved in the activation of the adenohypophysis to release ovulating hormone.

Recent data by Ferrando and Nalbandov (5) show that treatment with Dibenzyline, which is 6–10 times more potent as Dibenamine, blocks ovulation in laying hens not only when it is injected systemically but also when it is injected directly into the wall of preovulatory follicle. The blocking effect of Dibenzyline could be overcome by injection of luteinizing hormone (LH) systemically, These observations suggest that the drug may be working at the level of the ovary, as well as at the hypothalamus.

In an attempt to further elucidate the possible mode of action of alpha adrenergic blocking agents, studies were carried out to investigate the effects of phenoxybenzamine (Dibenzyline) upon plasma LH levels in the ovariectomized and proestrus rat.

Materials and Methods. Experimental animals. Female Sprague-Dawley rats (body wt, 200–250 g) were used in these studies. All rats were caged in an air-conditioned, temperature-controlled room with a lighting schedule of 14 hr of light and 10 hr of darkness, and supplied with Purina laboratory chow and water *ad libitum*. Under these conditions, the ovulatory surge of LH is expected to occur on the day of proestrus between 1400 and 1600 (6), the "critical" period.

Normal rats. Vaginal smear records were taken each day and animals were used in the experiment on the day of proestrus, provided they displayed: (a) a clear 4-day cycle in 3 consecutive cycles; (b) a typical proestrus smear; and (c) a clearly distended uterus. The uterus was inspected on the morning of proestrus by laparotomy approached by a single lateral incision while the rat was under ether anesthesia. Blood was collected between 1500 and 1600; and plasma LH was measured. Effects upon ovulation were determined by removing the oviducts on the following day (estrus) and counting the ova under a microscope. Ovulation was considered complete if 6 or more ova could be counted in the 2 tubes.

Ovariectomized rats. The rats were used 3–6 weeks postovariectomy. Phenoxybenzamine was administered for 2 days at a dose of 20 mg/kg/day for 8 days at a dose of 10 mg/kg/day. Control rats were injected with an equal volume of saline. There was no appreciable difference in body weight between the control and phenoxybenzamine-treated animals. One day after the last injection, the rats were bled; and plasma LH was measured.

LH assay. At the designated time, the rats were anesthetized and bled from the jugular vein into a heparinized syringe. Blood from 3 or more donors was pooled and the plasma was separated by centrifugation. Two milliliters of plasma were injected into suitably

[1] This work was supported by a research grant from the National Science Foundation, GB 8084.

TABLE I. Effect of Phenoxybenzamine Alone and in Combination with HCG on Ovulation in Cycling Rats.

Group no.	Treatment	Dose	Injection time	No. of rats Treated	No. of rats Ovulating	Ovulating (%)
1	Saline	—	1300	5	5	100
2	Propranolol	20 mg/kg, ip	1300	4	4	100
3	MJ 1999	20 mg/kg, ip	1300	4	4	100
4	Phenoxybenzamine	20 mg/kg, ip	1300	7	1	14
5	Phenoxybenzamine	20 mg/kg, ip	1700	7	5	71
6	Phenoxybenzamine + HCG	20 mg/kg, ip 20 IU, iv	1300 1500	6	5	83

primed immature female rats to determine the percentage ovarian ascorbic acid depletion (OAAD) by a modification of the method of Parlow [cited in Ref. (7)]. This served as an index of plasma LH concentration. Control and treated plasma samples were always run during the same day.

Injected Materials. Phenoxybenzamine hydrochloride (Dibenzyline, Smith Kline and French), propranolol hydrochloride (Inderal, Ayerst), MJ 1999 (Sotalol, Mead Johnson), and HCG (Ayerst) were dissolved or diluted in saline and injected. Control animals received an equal volume of saline. The actual doses, times and routes of administration are mentioned in the text and Tables I–III.

Results. Effect of phenoxybenzamine alone and in combination with HCG on ovulation in cycling rats (Table I). A single injection of phenoxybenzamine (20 mg/kg) given at 1300 on the afternoon of proestrus clearly blocked ovulation (14% ovulating). Ovulation failed to occur in only 2 animals (out of 7 treated) which received phenoxybenzamine after the critical period. An injection of HCG during the critical period effectively prevented the blockade of ovulation by phenoxybenzamine alone (83% ovulating). Treatment with beta blocking agent propranolol or MJ 1999 failed to prevent ovulation.

TABLE II. Effect of Phenoxybenzamine on Plasma LH Activity (% ovarian ascorbic acid depletion) in Ovariectomized Rats.

Controls	Phenoxybenzamine	t^a	p
Treated for 2 days (20 mg/kg/day, ip)			
13.6 ± 2.4 (5)[b]	17.6 ± 2.1 (4)	1.2	NS[c]
16.2 ± 11.3 (4)	18.1 ± 3.4 (4)	0.5	NS
Treated for 8 days (10 mg/kg/day, ip)			
13.6 ± 2.4 (5)	15.9 ± 3.1 (5)	0.58	NS

[a] t = Student's t test (control vs phenoxybenzamine).
[b] Mean ± SEM (no. of assay rats).
[c] NS = not significant.

TABLE III. Effect of Phenoxybenzamine on Plasma LH Activity (% ovarian ascorbic acid depletion) in Cycling Rats.

Controls	Phenoxybenzamine[a] (20 mg/kg, ip)	t^b	p
12.6 ± 2.7 (5)[c]	4.8 ± 3.7 (6)	1.6	NS[d]
12.1 ± 3.2 (5)	13.8 ± 2.3 (5)	0.4	NS
10.8 ± 1.7 (5)	11.1 ± 1.6 (5)	0.2	NS

[a] Given at 1300 on the day of proestrus.
[b] t = Student's t test (control vs phenoxybenzamine).
[c] Mean ± SEM (no. of assay rats).
[d] NS = not significant.

Effect of phenoxybenzamine on plasma LH activity in ovariectomized rats (Table II). Significant levels of LH were present in plasma from ovariectomized rats as previously reported (8). Phenoxybenzamine injections for 2 days (20 mg/kg/day) or for 8 days (10 mg/kg/day) were ineffective in preventing the rise in plasma LH activity.

Effect of phenoxybenzamine on plasma LH activity in cycling rats (Table III). Significant levels of LH were present in the plasma from cyclic rats on the afternoon of proestrus as previously reported (9). In three experiments, plasma LH levels were not significantly affected by a single injection of phenoxyben-

zamine (20 mg/kg) given at 1300 on the day of proestrus.

Discussion. The finding that a drug, if injected before the critical period but not after, is capable of preventing ovulation in the rat has been used to infer that the drug is acting to block the hypothalamic neurogenic stimulus essential for LH release. In these studies we were unable to demonstrate a significant effect upon plasma LH levels in either ovariectomized or proestrus rats after treatment with phenoxybenzamine. If phenoxybenzamine does not block the release of LH, how might one explain the fact that it does block ovulation if administrated before the critical period and that exogenous administration of HCG is capable of overcoming the effect? It is conceivable that the drug may be acting at the ovarian level to somehow interfere with normal ovulatory events. Evidence in support of a local ovarian effect was presented by Ferrando and Nalbandov (5), who were able to inhibit ovulation in laying hens by injecting Dibenzyline directly into preovulatory follicles.

LH has been shown to increase ovarian blood flow in the rat (10). This action was suggested as possibly being of great importance in regulating how much of certain circulating substances, which might be necessary for ovulation, become available to the ovary. It has even been suggested that one general mechanism of hormone action involves expansion of the microcirculation of the target organ (11). The possibility exists that phenoxybenzamine may be acting on alpha receptors leading to a reduction in blood flow through the ovary. At this point we do not have information as to the effect of phenoxybenzamine upon ovarian blood flow, though the reports show that the dose used in these experiments would cause substantial hemodynamic effects (12).

The existence of adrenergic receptors in the ovary was first suggested as a result of observations that sympathetic nerve fibers are found in close proximity to smooth muscle surrounding blood vessels and also ramifying throughout the ovarian stroma (13–16). The type of fluorescence in the nerves is characteristic of norepinephrine, leading the investigators to conclude that the ovarian stroma is supplied by a noradrenergic plexus. Regarding the function of these fibers, there is little that can be deduced from the meager supply of evidence now available. Evidence for the existence of alpha and beta adrenergic receptors in the ovary was first suggested by the work of Rocerto *et al.* (17). They showed that stimulation of alpha receptors caused an increase in the contractions of the isolated cat ovary, while the addition of phenoxybenzamine was shown to block this effect. The physiological significance of these contractions is uncertain but it was suggested that they may play a role in the ovulatory process. Our results further suggest that alpha receptors in the ovary may play an important role in ovulation. Whether phenoxybenzamine may be acting on adrenergic nerves terminating in the ovary or is exerting a direct effect on ovarian vasculature or musculature remains to be determined.

Summary. The present experiments were carried out to determine the effects of phenoxybenzamine (an alpha adrenergic blocking agent) upon ovulation and the concentration of LH in the blood of ovariectomized and proestrus rats. Ovulation was blocked when phenoxybenzamine (20 mg/kg) was administered prior to the assumed critical period of LH release. This effect could be overcome by treatment with HCG (20 IU) during the critical period. Treatment with propranolol or MJ 1999 (beta adrenergic blocking agents) had no effect on ovulation. Phenoxybenzamine injections for 2 days (20 mg/kg/day) or for 8 days (10 mg/kg/day) was shown to be ineffective in preventing the rise in plasma LH levels that occurs in ovariectomized controls. A single injection of phenoxybenzamine before the critical period in cycling rats also was shown to have no significant effect on the level of LH present in the blood during the afternoon of proestrus. These results suggest that phenoxybenzamine may be acting directly on the ovary to prevent ovulation and that alpha adrenergic receptors located in the ovary may play an important role in the ovulatory process.

PMS and HCG were kindly supplied by Dr. John Jewell of Ayerst Laboratories. The phenoxybenzamine

was donated by G. June Oswald of Smith Kline and French Laboratories. The author is grateful to Miss Marjory Lucci for technical assistance during this study.

1. Everett, J. W., Sawyer, C. H., and Markee, J. E., Endocrinology 44, 234 (1949).

2. Sawyer, C. H., Everett, J. W., and Markee, J. E., Endocrinology 44, 218 (1949).

3. Everett, J. W., and Sawyer, C. H., Endocrinology 45, 581 (1949).

4. Van Tienhoven, A., Nalbandov, A. V., and Norton, H. W., Endocrinology 54, 605 (1954).

5. Ferrando, G., and Nalbandov, A. V., Endocrinology 85, 38 (1969).

6. Everett, J. W., Endocrinology 59, 580 (1956).

7. Crighton, D. B., Watanabe, S., Dhariwal, A. P. S., and McCann, S. M., Proc. Soc. Exp. Biol. Med. 128, 537 (1968).

8. Taleisnik, S., and McCann, S. M., Endocrinology 68, 263 (1961).

9. Ramirez, V. D., and McCann, S. M., Endocrinology 74, 814 (1964).

10. Wurtman, R. J., Endocrinology 75, 927 (1967).

11. Szego, C. M., and Lawson, D. A., Endocrinology 74, 372 (1964).

12. Nickerson, M., "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, eds.), 3rd ed., p. 546. Macmillan Co., New York (1966).

13. Jacobowitz, D., and Walach, E. E., Endocrinology 81, 1132 (1967).

14. Owman, C., Rosengren, E., and Sjöberg, N. O., Obstet. Gynecol. 30, 763 (1967).

15. Rosengren, E., and Sjöberg, N. O., Amer. J. Anat. 121, 271 (1967).

16. Neilson, D., Jones, G. S., Woodruff, J. D., and Goldberg, B., Obstet. Gynecol. Surv. 25, 889 (1970).

17. Rocerto, T., Jacobowitz, D., and Walach, E. E., Endocrinology 84, 1336 (1969).

Received July 12, 1971. P.S.E.B.M., 1971, Vol. 138.