Ovulation from Histamine Depleted Ovaries[1] (35206)

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Luteinizing hormone (LH) induced ovarian hyperemia has been long recognized (1). This response occurs within seconds of the administration of LH and persists for several hours (2). The effect on the microcirculation is very similar to that seen after induction of histamine secretion (3). Szego (4, 5) suggested that histamine release in the ovary is a necessary prerequisite for ovulation. This hypothesis has been tested by imposing on pregnant mare's serum (PMS)[2], human chorionic gonadotropin (HCG)[2] treated immature rats intensive treatment with compound 48/80[3] (condensation product of formaldehyde and *p*-methoxyphenethylmethylamine), a mast cell histamine releasing drug, and Benadryl (β-dimethylaminoethyl benzhydryl ether hydrochloride) an antihistamine.

Methods. All the animals used were either obtained from Sprague-Dawley, Madison, Wisconsin or were raised in our colony from Sprague-Dawley stock. The rats were kept on a 14:10-hr light:dark cycle and were fed Purina rat chow and water *ad libitum*.

All the animals were subjected to the same basal hormone regimen. They received 30 IU of PMS sc in 0.1 ml of saline at 1600 hr on day 24 of age and 30 IU of HCG in 0.5 ml of saline iv on day 26 at 0800 hr, or 40 hr later. Zero hour rats in all the groups were killed at 0800 hr without receiving any HCG. Group 1 served as a control group and received only the hormones. Group 2 received 4 mg of Benadryl/day divided in two doses administered in 12-hr intervals beginning 1 hr before hormone treatment was instituted. Group 3 received compound 48/80 beginning on day 22 with an initial dose of 50 μg; the second dose, 100 μg; and the third dose, 150 μg. On the succeeding days the rats received 600 μg in three uniformly spaced injections. Group 4 received compound 48/80 and Benadryl in the same doses as administered to groups 1 and 2 and begun on the same days as in these groups. Group 5 received no treatment at all. All pharmacological agents were administered through day 26. Glucose (80 mg/rat) was administered 10 min prior to the drugs to decrease their toxicity (6, 7).

The rats were anesthetiezd with ether, and blood was obtained from the abdominal aorta with a syringe whose air space was replaced with 5% sodium citrate. One ml of the blood was added to a test tube containing 0.9 ml of 10 *N* perchloric acid. The ovaries were then removed, chilled in a dish of ice, and dissected free of surrounding tissue. At this time the oviducts were routinely examined for evidence of ovulation by demonstration of distention of the ampulla and in several experiments, counts of ova were made (8).

The extraction of histamine from the ovarian homogenate and whole blood was performed as suggested by Shore *et al.* (9) except that potassium hydroxide and potassium chloride was substituted for sodium hydroxide and sodium chloride. This substitution gave a higher percentage recovery and made possible the removal of excess perchloric acid in the form of potassium perchlorate. Fluorescence was measured on the Farrand spectrofluorometer with activation wavelength of 360 μ and a fluorescence at 440 μ[4]. The data

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[2] Pregnant mare serum (PMS) and human chorionic gonadotropin (HCG) were obtained from Ayerst Laboratories, Inc.

[3] Compound 48/80 was generously provided by Dr. S. Winston Singleton of Burroughs Wellcome Company Inc.

[4] Although the peak fluorescence of histamine complexed with ophthalaldehyde (OPT) in acid solution is 450 μ, the peak was obtained at 440 μ with our Farrand spectrofluorometer.

are analyzed statistically with Student's *t* test.

Concentration of histamine in blood. Untreated immature rats (24–26 days) have about 0.100 μg histamine/ml of blood (Fig. 1). Forty hr after administration of 30 IU of PMS the histamine concentrations are elvated to 0.150 μg/ml of blood and 3 hr after administration of HCG the histamine concentration is elevated to 0.225 μg/ml; this concentration is maintained for 8 hr. Benadryl retards the disappearance of histamine from the circulation since it causes the concentration to go up to 0.250 μg/ml and 8 hr after administration of HCG it is approximately 0.225 μg/ml. No increase in the blood histamine concentation at 3, 5, or 8 hr after HCG was obtained in the compound 48/80 treatment group. No difference was noted in group 4 between the 0-hr histamine blood concentration and the 3-, 5-, or 8-hr intervals after HCG administration or between the combined treatment and compound 48/80 treatment.

Concentration of histamine in the ovary. The concentation of histamine in immature rat ovaries was below detectable levels. Treatment with PMS and HCG caused an increase in the concentration of histamine to 7.51 ± 0.25 ng/mg of ovary and by 8 hr after the HCG was administered, the histamine concentration had declined by 16% to 6.01 ± 0.48 ng/mg. After administration of Benadryl the ovarian histamine concentration was elevated to 9.10 ± 0.6 ng/mg but at 8 hr after HCG had fallen to a level of 7.51 ± 0.49 ng/mg seen with PMS and HCG alone. Administration of HCG after chronic treatment with compound 48/80 resulted in further depletion of greatly reduced tissue concentrations; the decline was from 1.10 ± 0.17 to 0.35 ±0.47 ng/mg over a period from 0 to 8 hr. Combined treatment with compound 48/80 and Benadryl prevented the extreme depletion of histamine from the ovary and even resulted in some reaccumulation of histamine. The concentration was 1.80 ± 0.7 ng/mg at 0 hr and 2.30 ± 0.5 ng/mg 8 hr after the HCG treatment.

The effect of drug treatment on ovarian weight. Eight hr after HCG was administered to PMS-primed rats, the ovaries weighed 65.6 ± 4.6 mg compared to ovaries of untreated rats which weighed 22.2 ± 2.4 mg. Benadryl added to the hormone regimen caused a lesser increase to 53.5 ± 3.8. After discharge of the histamine with compound 48/80 the ovarian weight was 36.2 ± 4.8 mg while combined treatment with Benadryl and compound 48/80 resulted in a weight of 28.0 ± 1.3 mg, a value not significantly greater than the ovarian weight of the untreated group 5.

Effect of drug treatment on ovulation. The appearance of fluids in the uterine horn and oviducts was reduced by the administration of benadryl and compound 48/80, however, the distention of the ampulla of the oviducts generally associated with ovulation was unaffected. Distended ampullae were seen in the oviducts of almost all the animals subjected to the drug regimen.

This portion of the experiment was repeated contrasting the response of PMS–HCG-treated rats with a combined treatment group. All the rats in the control group ovulated with a mean of 28 ova/rat. In the combined treatment group, six of seven rats ovulated but only 7 ova/rat were released.

Discussion. Thonnard-Neumann (10) reported a decline in the number of circulating basophils in human females at midcycle coinciding with the time of ovulation; while Baseila (11) noted that in female rabbits stimulated to ovulate, there was a fall in the number of circulating basophils 12 hr after the stimulus. Zachariae *et al.* (12) found an accumulation of basophils around postovulatory rabbit follicles. It is probable that the hyperemia seen in the preovulatory follicle is due to histamine release by basophils. These workers did not note the histamine levels of ovaries or blood in their subjects, however, Szego and Gitin (5) observed a decrease in histamine concentation of rat ovaries. In the rat there occurs simultaneously with a decrease in ovarian histamine concentration an increase in the histamine concentration of the blood as a result of the administration of HCG (Fig. 1).

The concentration of histamine in the ovary is dependent on hormonal treatment.

PMS, manifesting both FSH- and LH-like activities, causes the histamine concentration to rise in the ovary and blood; and HCG with its predominantly LH-like action causes the ovarian histamine concentration to fall and blood concentration to rise. Although no correlations have been made in primates it is interesting to speculate that the sharp fall in body temperature at ovulation may be related to a similar histamine release mechanism.

The ovaries of rats treated with PMS and then HCG are hyperemic at the time of ovulation; compound 48/80 does not prevent the hyperemia while Benadryl does. Increase in ovarian weight as a result of treatment with the hormones is apparently dependent on an increase in blood flow (2) and increased capillary surface area due to release of histamine. In animals depleted of histamine, or depleted and with histamine receptors blocked, there was minimal growth of the ovary. The completeness of blockade with benadryl is subject to some question even though the ovaries were pale since they were almost as large as the hormone-treated ovaries.

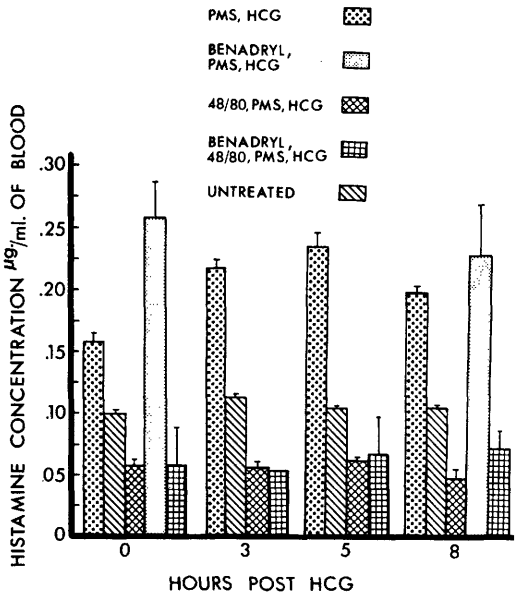

FIG. 1. The effect of hormones and various pharmacologic compounds on the histamine concentration of blood at different times after the administration of HCG. The 0-hr interval represents the histamine concentrations in the absence of HCG in both above and in Fig. 2.

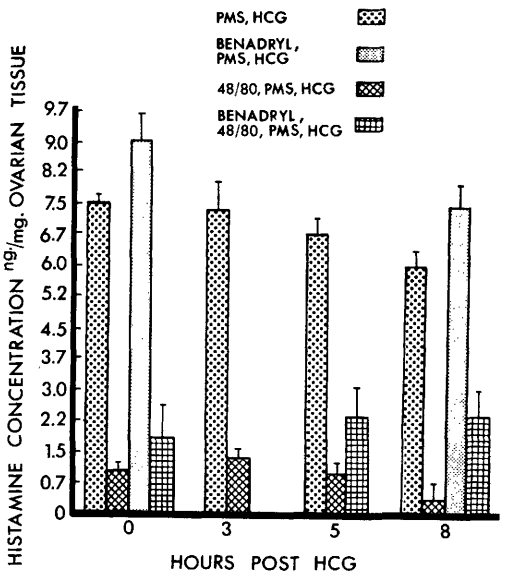

FIG. 2. The effect of hormones and various pharmacologic compounds on the histamine concentration of immature rat ovaries at different times after the administration of HCG.

Ovulation occurs from ovaries that are under the influence of compound 48/80 and Benadryl. They show marked reduction in histamine concentation (Fig. 2) and a failure to increase in weight. It is therefore probable that histamine plays an essential role in the increase in ovarian weight but probably has no direct role in the induction of ovulation.

Summary. Administration of compound 48-80 to PMS–HCG-treated immature rats causes an inhibition of the ovarian weight increase due to the stimulation exerted by HCG. Benadryl causes a slight reduction in this ovarian response but combination with 48/80 results in a profound inhibition. The combined treatment reduced the number of ova shed, but all the rats ovulated. The ovarian histamine content decreased but the blood concentrations rose after treatment with HCG. Histamine apparently plays a major role in the acute increase in ovarian weight but not in ovulation.

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