

The Influence of Estrogen and Prolactin on Hageman Factor (Factor XII) Titer in Ovariectomized and Hypophysectomized Rats

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The synthesis of prothrombin in hepatic microsomes is augmented in intact estrogen-treated rats and in hypophysectomized rats treated with purified prolactin. We investigated the influence of these gonadal and pituitary hormones on the titer of Hageman factor (factor XII), reportedly elevated in women using oral contraceptives. Rats were ovariectomized to minimize the influence of endogenous estrogen and progesterone on the Hageman factor titer. The administration of progesterone did not alter the plasma concentration of Hageman factor. In contrast, the infusion of 17β -estradiol induced a marked elevation of the plasma Hageman factor titer, as measured functionally and immunologically. The titer of Hageman factor was directly related to both plasma estradiol and prolactin concentrations, indicating that prolactin may play

a role in the regulation of plasma Hageman factor titers. In agreement with this, hypophysectomy induced a marked decrease in the Hageman factor level. In hypophysectomized ovariectomized animals, the administration of estradiol restored the Hageman factor titer to normal levels, whereas the infusion of prolactin induced a dramatic rise in the Hageman factor titer to the degree observed in nonhypophysectomized estrogen-treated rats. No further increase in the Hageman factor titer was observed in rats treated with both estradiol and prolactin. These data indicate that estrogens increase the plasma Hageman factor titer both directly and through its release of prolactin and that prolactin may also increase the titer of Hageman factor through estrogen-independent mechanisms.
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MULTIPLE HEMOSTATIC DEFECTS observed in oral contraceptive users have been attributed by many investigators to the estrogenic component of oral contraceptives and, by some, to the progestogens. Recently, we demonstrated elevated titers of Hageman factor (factor XII) among premenopausal asymptomatic women using cyclic estrogen-progestogen compounds for birth control.¹ In such women, the high Hageman factor titer was strongly related to the exaggerated cryoactivation of factor VII² and plasma prorenin³ and to spontaneous shortening of the prothrombin time.²

In 1977, Nishino⁴ observed that estrogen and prolactin increased prothrombin synthesis in liver microsomes. Using isolated liver perfusion techniques, Owens and Cimino⁵ demonstrated augmented synthesis of factor VII and plasminogen in estrogen-treated rats. In the present study, the individual effects of estrogen, progesterone, and prolactin on the plasma titer of Hageman factor in rats were studied. Since estrogens are potent stimuli for prolactin secretion, it would be difficult to distinguish the influence of each hormone in rats with intact pituitary glands. These effects can, however, be easily evaluated in hypophysectomized rats.

Using this approach, we found that estradiol and prolactin can independently raise the titer of Hageman factor in rats.

MATERIALS AND METHODS

Sixty-eight intact and 65 hypophysectomized adult female Sprague-Dawley rats, weighing 250 to 300 g, were supplied by Zivic-Miller Co, Allison Park, Pa. Hypophysectomy was performed through the parapharyngeal approach. Experiments on these rats were performed seven days after hypophysectomy. Ovariectomy was performed under light ether anesthesia through bilateral flank incisions. One or two mini-osmotic pumps (Alza Pharmaceuticals, Palo Alto, Calif) containing a hormone or the solvent in which the hormone was dissolved were placed into the peritoneal cavity. The mini-osmotic pumps used were regulated to deliver 1 μ L of solution per hour and had a volume capacity of 200 μ L per pump, making possible a continuous infusion of hormone for seven days. The rats were fed a normal mouse-rat diet (Teklad Test Diets, Madison, Wis) and water ad libitum. Seven days after oophorectomy or the insertion of the mini-osmotic pumps, or after both procedures, 5 to 8 mL of blood was drawn from the abdominal aorta through a wide abdominal incision under ether anesthesia. The rats were then killed using ether overdose.

Lyophilized purified 17β -estradiol or progesterone (purities >99% by thin-layer chromatography, Sigma Chemical Co, St Louis) were dissolved in warm polyethylene glycol (Sigma) and 200- μ L aliquots were injected into mini-osmotic pumps. The calculated delivery doses were 20, 200, and 400 ng/h of estradiol and 400 ng/h of progesterone.

Purified ovine prolactin (35 IU/mg, supplied by the National Institute of Arthritis, Metabolism, Diabetes and Digestive Diseases, Bethesda, Md) was dissolved in polyethylene glycol. The calculated pump delivery dosage was 2 IU of prolactin per hour.

Citrated plasma was prepared as described previously⁶ from blood drawn from the abdominal aorta. These plasmas were immediately frozen at -70°C in polyethylene tubes rinsed in silicone oil. A pool of plasmas from 18 intact rats was prepared using earlier methods⁷ and was used as the standard for measuring Hageman factor procoagulant and antigenic titers. One rat unit of coagulant or antigenic Hageman factor was defined as the amount present in 1 mL of this pooled plasma. The unit used was not the same as that used to describe the titer of human Hageman factor, one unit of which is defined as the amount in 1 mL of human pooled plasma.

Procoagulant titers of Hageman factor were measured by a modification of the kaolin partial thromboplastin time technique.⁸

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Purified rat Hageman factor (specific activity, 22.3 U/mg protein) was prepared by Dr Hidehiko Saito (University Hospitals of Cleveland), as described previously.⁹ This preparation was devoid of detectable amounts of other clotting factors and formed a single band at 80,000 mol wt on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in both reduced and nonreduced samples.

Radioimmunoassay for rat Hageman factor antigen was performed as described previously¹⁰ using monospecific anti-rat Hageman factor antibody. Plasma prolactin level was measured by a specific radioimmunoassay technique using the kits and procedures provided by the National Institute of Arthritis, Metabolism, Diabetes and Digestive Diseases.¹¹ Plasma estradiol titers were measured by Nicols Institute, San Juan Capistrano, Calif, using a direct radioimmunoassay technique (Pantex Immuno ¹²⁵I kits, Pantex, Santa Monica, Calif).

The significance of differences among groups was tested by the Student's *t* test.¹² Pearson's coefficient of correlation was used to compare plasma estradiol, prolactin, and coagulant Hageman factor titers in estrogen-treated and untreated ovariectomized rats.¹³ Our results are expressed as the arithmetic mean \pm SD.

RESULTS

The effect of 17 β -estradiol and progesterone on Hageman factor coagulant and antigenic titers in ovariectomized rats. Ovariectomized rats were selected for studies to minimize the possible influence of endogenous estrogen and progesterone on Hageman factor titer. The Hageman factor coagulant (mean \pm SD, 0.93 \pm 0.09 U/mL) and antigenic (mean \pm SD, 0.97 \pm 0.07 U/mL) titers of ovariectomized rats were not significantly different from those of intact female rats (mean coagulant titer, 0.96 \pm 0.24 U/mL; mean antigen titer, 0.93 \pm 0.12 U/mL) (Fig 1). The ovariectomized rats had estradiol levels of 6 pg/mL or less at the time of blood drawing. There was no change in the coagulant titer of Hageman factor in rats that were ovariectomized for three months (mean, 1.03 \pm 0.15 U/mL).

The continuous infusion of 17 β -estradiol at 20, 200, and 400 ng/h for seven days into ovariectomized rats induced a significant increase in the Hageman factor titer compared to the nontreated ovariectomized group (Table 1). Although the mean level of Hageman factor was slightly higher in rats receiving 200 and 400 ng/h, this trend was not significant.

In a subset of plasmas from six untreated ovariectomized and seven estrogen-treated ovariectomized animals in which the Hageman factor and estradiol titers were studied simultaneously, the Hageman factor coagulant titer tended to be directly related to the plasma estradiol level ($r = .79$; $P < .001$).

Infusion of progesterone at 400 ng/h for seven days did not alter the plasma Hageman factor titer (mean coagulant titer, 0.85 \pm 0.16 U/mL; mean antigen titer, 0.90 \pm 0.15 U/mL).

The relationship between Hageman factor coagulant titer and plasma prolactin level in estrogen-treated ovariectomized rats. Estrogens increase prolactin secretion by the pituitary gland.¹⁴ Because prolactin may be a mediator of the estrogen-induced elevation in Hageman factor titer, prolactin levels were measured in untreated and estrogen-treated ovariectomized rats. A direct relationship was observed between the Hageman factor coagulant titer and the plasma prolactin level ($r = .96$, $P < .001$; Fig 2).

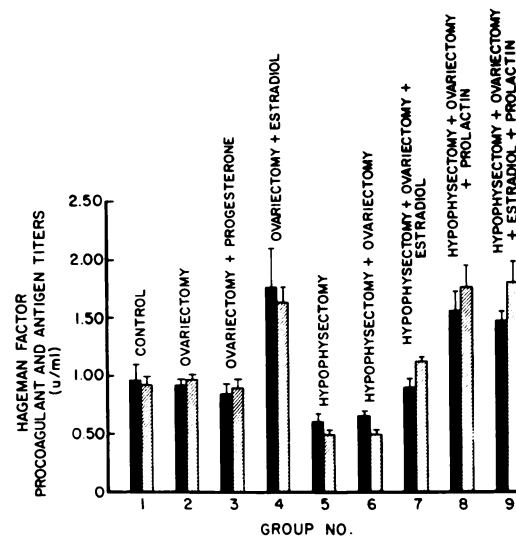


Fig 1. The procoagulant and antigen titers of Hageman factor in estrogen-, progesterone-, and prolactin-treated rats after ovariectomy and hypophysectomy. ■, Mean coagulant titer; □, mean antigen titer; and T, standard deviation. The rats were divided into the following groups: (1) control intact rats ($n = 18$); (2) ovariectomized rats ($n = 8$); (3) ovariectomized progesterone-treated rats ($n = 10$); (4) ovariectomized estradiol-treated rats ($n = 15$); (5) hypophysectomized rats ($n = 5$); (6) hypophysectomized ovariectomized rats ($n = 6$); (7) hypophysectomized ovariectomized estradiol-treated rats ($n = 10$); (8) hypophysectomized ovariectomized prolactin-treated rats ($n = 9$); (9) hypophysectomized ovariectomized estradiol- and prolactin-treated rats ($n = 8$).

The effect of hypophysectomy and prolactin infusion on Hageman factor coagulant and antigenic titers. Hypophysectomy alone induced a marked decrease in plasma Hageman factor titer (mean coagulant titer, 0.62 \pm 0.17 U/mL; mean antigen titer, 0.49 \pm 0.07 U/mL) compared to control intact rats ($P < .001$; Fig 1). There was no difference between the Hageman factor titer of hypophysectomized rats and that of the hypophysectomized ovariectomized group (mean coagulant titer, 0.66 \pm 0.13 U/mL; mean antigen titer, 0.51 \pm 0.19 U/mL).

The infusion of estradiol to hypophysectomized ovariectomized rats at 400 ng/h for seven days raised the titer of

Table 1. The Effect of Varying Doses of Estrogen on the Coagulant and Antigenic Titers of Hageman Factor in Ovariectomized Sprague-Dawley Rats

Plasma Sample	No. of Rats	Hageman Factor	
		Coagulant Activity (U/mL)	Antigen (U/mL)
Control	8	0.93 \pm 0.09	0.97 \pm 0.07
17 β -Estradiol (20 ng/h)	8	1.52 \pm 0.55 ($P < .005$)	—
17 β -Estradiol (200 ng/h)	15	1.75 \pm 0.69 ($P < .005$)	1.63 \pm 0.23 ($P < .001$)
17 β -Estradiol (400 ng/h)	9	1.90 \pm 0.59 ($P < .001$)	—

Results are expressed as arithmetic means \pm SD. Each assay was done in duplicate.

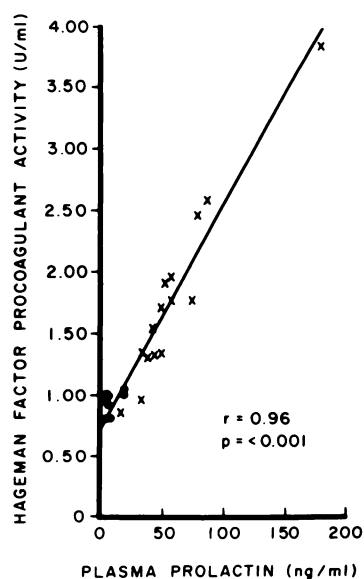


Fig 2. The relationship of Hageman factor coagulant titer and plasma prolactin titer in ovariectomized rats. The Hageman factor coagulant titers, measured by clotting assays, are plotted on the vertical axis, and plasma prolactin titers are plotted on the horizontal axis of a linear graph. ●, Ovariectomized untreated rats; x, ovariectomized estrogen-treated rats.

Hageman factor (mean coagulant titer, 0.90 ± 0.16 U/mL; mean antigen titer, 1.13 ± 0.07 U/mL; $P < .005$ and $.001$, respectively) to that observed in intact rats. In these rats, the mean prolactin titer (7.3 ± 4.7 ng/mL) was not significantly different from that of untreated hypophysectomized rats. The infusion of prolactin to hypophysectomized ovariectomized rats induced a dramatic rise of Hageman factor titer above the normal level (mean coagulant titer, 1.55 ± 0.21 U/mL; mean antigen titer, 1.75 ± 0.39 U/mL; $P < .001$). No further elevation of the plasma Hageman factor titer was observed when both estradiol (400 ng/h) and prolactin (2 IU/h) were administered to hypophysectomized ovariectomized rats (mean coagulant titer, 1.47 ± 0.18 U/mL; mean antigen titer, 1.79 ± 0.35 U/mL).

In a subset of plasmas from hypophysectomized and intact rats in which the Hageman factor and prolactin titers were studied simultaneously, the prolactin-treated rats that had higher prolactin titers also had higher Hageman factor titers. Thus, the mean Hageman factor titer of intact rats was 0.98 ± 0.23 U/mL, while the corresponding mean prolactin titer was 52.0 ± 25.9 ng/mL. In contrast, the mean Hageman factor titer (0.65 ± 0.09 U/mL) and mean prolactin titer (5.1 ± 1.2 ng/mL) of hypophysectomized animals were significantly lower than those of intact rats ($P < .0001$).

DISCUSSION

Earlier, we reported a marked elevation of Hageman factor titer in the plasma of women using oral contraceptive agents.¹ These hormone preparations contained both estrogenic and progestational compounds. The high titer of Hageman factor appeared to be responsible for certain *in vitro* phenomena observed in the plasmas of oral contraceptive

users, such as enhanced fibrinolysis,¹ exaggerated cold activation of factor VII and spontaneous shortening of the prothrombin time,² a decrease in C1 esterase inhibitor (C1-INH) titer,¹ and enhanced cryoactivation of plasma prorenin.³ Whether these defects contribute toward the thrombotic tendency of oral contraceptive users is uncertain.

Elevated titers of fibrinogen,^{15,16} prothrombin,¹⁵⁻¹⁸ factor VII,¹⁵⁻¹⁸ Christmas factor (factor IX),¹⁹ and Stuart factor (factor X)¹⁵⁻¹⁹ in humans have also been associated with oral contraceptive use. Whether the observed plasma elevations of these clotting factors are due to the estrogenic or progestational component of oral contraceptives has not been clarified.²⁰ In rats, Nishino⁴ and Jolly et al²¹ demonstrated that estrogens protected male rats that were fed a vitamin K-deficient diet from developing hypoprothrombinemia. Further, Nishino⁴ observed that, although estrogen administration did not protect hypophysectomized rats from hypoprothrombinemia, purified prolactin provided this protection. Owens and Cimino⁵ later reported increased hepatic synthesis of prothrombin, factor VII, and plasminogen in diethylstilbestrol-treated rats. Neither Nishino nor Owens and Cimino examined the influence of progesterone on the hepatic synthesis of these clotting factors.

Since oral contraceptive agents contain both an estrogen and a progestogen, we studied the effect of individual infusions of estrogen or progesterone on the plasma titer of Hageman factor in ovariectomized rats. Since estrogens stimulate prolactin secretion by the hypophysis,¹⁴ we also studied the possible role of prolactin in the regulation of Hageman factor titer in plasma. With the exception of the control intact and untreated hypophysectomized rats, all rats were ovariectomized to minimize the possible influence of endogenous estrogen and progesterone on the titer of Hageman factor and to increase the sensitivity of their clotting system to estradiol.²² Ovariectomy alone for as long as three months or in combination with the infusion of progesterone at 400 ng/h for seven days did not alter the titer of Hageman factor. In contrast, the infusion of 17β -estradiol to ovariectomized rats induced a dramatic rise in the titer of Hageman factor, measured both functionally and immunologically. The titers of Hageman factor achieved were directly related to both plasma estradiol and prolactin titers, suggesting that prolactin may play a role in the modulation of the titer of this clotting factor.

Hypophysectomy alone as well as in combination with ovariectomy, which reduced the titer of plasma prolactin, induced a significant reduction in the plasma Hageman factor titer. The infusion of estrogen to hypophysectomized ovariectomized rats restored the titer of Hageman factor to normal levels. The administration of prolactin alone to hypophysectomized ovariectomized rats induced a more dramatic rise in the Hageman factor titer, to the degree observed in nonhypophysectomized estrogen-treated rats. No further rise was observed upon further addition of estrogen.

These data suggest that estradiol and prolactin raise the Hageman factor titer directly and independently but that estradiol may act maximally through the release of prolactin. Conceivably, the trace amounts of prolactin in the plasma of hypophysectomized rats may play some role in raising the

titer of Hageman factor, but the mean prolactin titer of hypophysectomized rats was not significantly increased by estrogen infusion. The liver is a major site of Hageman factor synthesis,²³ and estrogen and prolactin receptors are normally found on hepatic cells.^{24,25} Estrogens have been shown to induce an increase in the number of prolactin receptors in intact rats.²⁵ Further, prolactin itself can induce its own receptor in the liver of hypophysectomized rats.²⁶ Perhaps the mechanism by which Hageman factor titer is augmented

is through a specific effect of estrogens and prolactin on protein synthesis. Isolated liver perfusion studies are in progress to test this hypothesis.

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