Influence of Augmented Hageman Factor (Factor XII) Titers on the Cryoactivation of Plasma Prorenin in Women Using Oral Contraceptive Agents

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ABSTRACT Prolonged cold storage of plasma may induce the conversion of plasma prorenin (inactive renin) to renin. This phenomenon is exaggerated in oral contraceptive (OC) users; the titer of Hageman factor (HF, Factor XII) in OC users is higher than in nonusers. The present study relates these observations. The increment in plasma renin activity (PRA) during cold storage, as measured by generation of angiotensin I, correlated strongly with the initial plasma titer of HF. Increasing the HF titer of nonusers to that observed in OC users by addition of purified HF increased cold-induced PRA at least twofold, while reducing the plasma HF titer of OC users correspondingly decreased cold-induced PRA. Thus, in OC users, the enhanced conversion of plasma prorenin to renin during cold storage reflects the elevated plasma titer of HF.

INTRODUCTION

Activation of prorenin (inactive renin, big renin) can be induced in vitro by acidification (1–3), trypsinization (4, 5), or prolonged cold storage of plasma (5–8). Cold storage or acidification appears to activate prorenin via enzyme-mediated reactions in which Hageman factor (HF, Factor XII)[1] participates (4, 9, 10). Acid-induced activation requires the presence in plasma of prekallikrein (Fletcher factor); this indicates that kallikrein may activate prorenin (9–12). Cryoactivation, however, has been detected in Fletcher trait plasma (13), as if plasma prekallikrein were not an absolute requirement (14). Nonetheless, the cold-induced increase in plasma renin activity (PRA) in vitro can be blocked by protease inhibitors (13, 15, 16). Perhaps, as Osmond et al. (17) suggested, cryoactivation is mediated by plasminogen or vitamin K-dependent clotting factors.

Osmond and co-workers (5, 6) observed enhanced PRA in cold-stored oral contraceptive (OC) users' plasmas. Van Royen et al. (18) correlated this phenomenon with the shortening of the Thrombotest time that is sometimes seen in cold-stored plasmas. The cold activation of Factor VII and the shortening of the prothrombin time observed in women using OC is related to their high titer of HF (19). The present study was therefore undertaken to determine the relationship between HF coagulant titer and the cold activation of plasma prorenin in the plasma of OC users.

METHODS

16 white women using OC, 15 white women not using OC, 10 normal oriental subjects, 12 normal white males, and two patients that were deficient in HF were studied. The OC agents contained 0.5–1.0 mg of a progestin (norethindrone, ethynodiol acetate, or norgestrel) and 0.035–0.08 mg of an estrogen (ethinyl estradiol or mestranol). Citrated plasma was prepared as described previously (20) from venous blood that was drawn in the afternoon after informed consent for the procedure was obtained. The plasma was either immediately frozen at −70°C in polyethylene tubes that were rinsed in silicone oil (control) or incubated at −4°C for 60 h (cold-stored) and then frozen at −70°C until used. A pool of 24 normal male plasmas was used as the standard for

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[1] *Abbreviations used in this paper:* C1-INH, C1 esterase inhibitor; HF, Hageman factor; OC, oral contraceptive; PRA, plasma renin activity.

measuring HF procoagulant activity (21). 1 U of HF clot-promoting activity was defined as the amount present in 1 ml of this pooled plasma.

Radioiodinated angiotensin I was supplied by New England Nuclear, Boston, MA. PRA was quantified in duplicate in control and cold-stored plasmas by radioimmunoassay of angiotensin I that was generated after incubation at 37°C for 3 h at pH 6.0 (22). The coefficient of variation for duplicate determinations of PRA was 11.2% in 40 plasma samples. The cold-promoted change in PRA was expressed as:

Percentage change in PRA = [(PRA in cold-stored plasma − PRA in control plasma)/PRA in control plasma] × 100.

Trypsinization of plasma was performed by using a modification of Osmond's methods (5): 2 mg of trypsin (Worthington Biochemical Corp., Freehold, NJ, from bovine pancreas) mixed in 0.2 ml of 0.002 N HCl was added to 2 ml of plasma; 0.01 ml of 2.6 M Tris acetate buffer (pH 7.4) was added; and the pH was then adjusted to 7.4 as necessary. The mixture was then incubated at 37°C for 3 h after which inhibitors of trypsin were added (5) before assay of plasma renin activity.

Purified human HF (sp act 152.7 and 142 U/mg protein) was prepared as described previously (23). The preparations were devoid of detectable amounts of other clotting factors and formed a single band of 80,000 mol. wt. on sodium dodecylsulfate polyacrylamide gel electrophoresis in both reduced and nonreduced samples.

Barbital-saline buffer (pH 7.5) contained 7.8 g sodium chloride, 2.76 g barbital, and 2.06 g sodium barbital per liter of distilled water.

To determine the effect of the elevated HF titer of OC users upon generation of PRA in cold-stored plasma, normal plasma was mixed with sufficient purified HF in barbital-saline buffer to raise its coagulant titer to 2.00 U/ml, or with an equal volume of barbital-saline buffer. Conversely, the HF coagulant titer in OC users' plasma was reduced to a variable degree by absorption with insoluble rabbit anti-human HF immunoglobulin. Briefly, crude monospecific rabbit immunoglobulin against human HF (24) was coupled to cyanogen bromide agarose (Pharmacia Fine Chemicals, Piscataway, NJ). Then, 1.4 ml of OC users' plasma was mixed for 1 h with varying amounts of the insoluble rabbit anti-HF immunoglobulin. The plasma was then separated by centrifugation (2,000 *g*, 10 min, room temperature), assayed for residual HF coagulant activity, and PRA was measured in control, cold-stored, and trypsin-activated plasmas.

To determine whether Hageman factor possesses intrinsic angiotensin I-generating activity at pH 6.0, 50 μg (80 μl) of popcorn inhibitor, a potent inhibitor of activated HF (25) was added to 0.5 ml of a cold-activated OC users' plasma before incubation at 37°C for 3 h. An equal volume of barbital-saline buffer was added to the control cold-activated plasma.

Procoagulant titers of HF were measured in control plasmas by a modification of the kaolin partial thromboplastin time technique (26).

C1 esterase inhibitor (C1-INH) activity in control and cold-stored plasmas was measured, after incubation of C1 at 37°C for 15 min, in an esterolytic assay described previously (27). 1 U of inhibitory activity was that amount which inhibited 10 U of C1. Acidometric titration of the free acid in mixtures in formaldehyde was carried out with an automatic titrator (model ABU 1a) and pH stat (Radiometer Co., Copenhagen, Denmark).

The significance of difference in titers between OC users and nonusers was tested by *t* test (28). Pearson's coefficient of correlation was tested for significance in comparison of the logarithmic percent change in PRA in cold-stored plasmas and the HF titer in control plasmas (29). Results are reported as arithmetic mean±SD.

RESULTS

The effect of cold storage upon plasma renin activity in the plasmas of women using OC agents. The PRA in the control plasmas of OC users ranged from 0.33 to 3.87 ng/ml per hour (mean 1.49±1.02) of angiotensin I generated, and from 0.25 to 3.80 ng/ml per hour (mean 1.59±0.96) of angiotensin I in nonusers. There was no difference in the base-line PRA values of OC users and nonusers. In contrast, the PRA in cold-stored plasmas of OC users ranged from 2.24 to 8.42 ng/ml per hour (mean 4.60±1.93) of angiotensin I, while PRA in nonusers ranged from 0.48 to 11.00 ng/ml per hour (mean 2.78±2.73) of angiotensin I generated. The increase in PRA during cold storage, which represents the amount of active renin formed from prorenin or inactive renin, was significantly greater in OC users compared with nonusers ($P < 0.005$).

Hageman factor clot-promoting activity in plasmas of women using OC agents. The HF coagulant titer in women using OC was significantly increased compared with nonusers in agreement with our earlier experience (30). The HF coagulant titer in 16 control plasmas of women using OC ranged from 1.10 to 3.08 U/ml (mean 1.84±0.54) and the HF titer in 15 nonusers ranged from 0.43 to 1.56 U/ml (mean 0.97±0.30) ($P < 0.001$).

The relationship of Hageman factor coagulant activity and the cold-promoted increase in plasma renin activity. The HF coagulant titer in 55 control plasmas, among them the plasmas of 10 oriental subjects who were known to have reduced HF titers (31), tended to be directly related to the percentage change in PRA upon cold storage of plasma ($r = 0.72$; $P < 0.001$) (Fig. 1).

When the HF coagulant titer of two OC users' plasmas was reduced to varying HF levels by treatment of plasma with monospecific rabbit anti-human HF immunoglobulin, the cold-promoted increase in PRA was correspondingly reduced. The dose-response curve demonstrated a direct relationship between HF coagulant titer and the percentage change in cold-induced PRA ($r = 0.93$; $P < 0.001$) (Fig. 2). The reduction in titer of HF was not accompanied by any change in the renin activity that was achieved by treating plasma with trypsin. Thus, trypsin-activated PRA in two OC users' plasmas was 3.27 and 3.02 ng/ml per hour whereas that of the same plasmas, which were treated to reduce their HF coagulant titer from 2.00

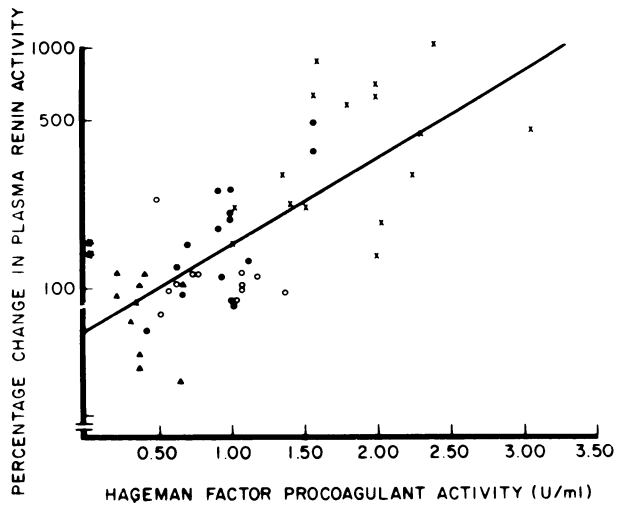

FIGURE 1 The relationship of Hageman factor coagulant activity and percentage change in plasma renin activity in cold-stored plasmas. Hageman factor coagulant activities, which were measured in clotting assays in 55 asymptomatic individuals, are plotted on the horizontal axis and the percentage change in plasma renin activity is plotted on the vertical axis of a semilogarithmic graph. ×, 16 white OC users; ●, 15 white women nonusers; *, two individuals with Hageman trait; O, 12 white males; ▲, 10 oriental subjects. To accommodate the percentage decrease in plasma renin activity on this graph, we have arbitrarily chosen 100% to represent no change in plasma renin activity. $r = 0.72$; $P < 0.001$.

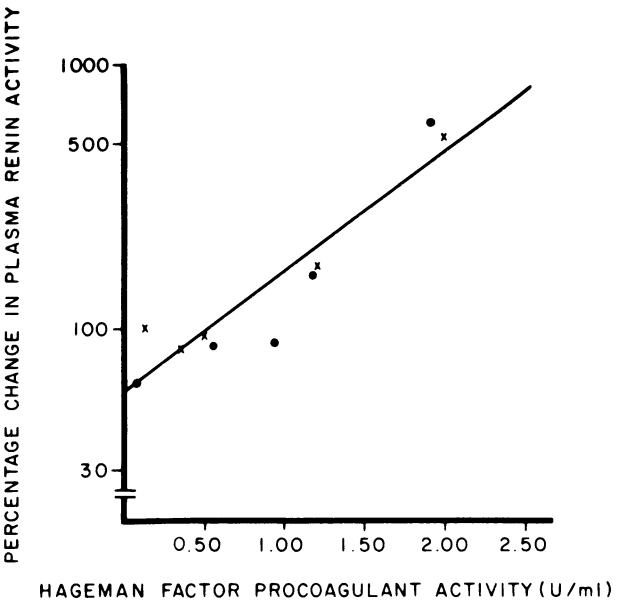

FIGURE 2 The relationship of Hageman Factor coagulant activity and percentage change in plasma renin activity in Hageman factor-depleted OC user's plasma. Hageman factor coagulant activities in OC user's plasma, that were depleted of Hageman factor to different degrees by treatment with insoluble antiserum against HF, are plotted on the horizontal axis, and the percentage change in plasma renin activity is plotted on the vertical axis of a semilogarithmic graph. The results are the means of duplicate determinations. Two separate experiments, represented respectively by × and ●, were performed. $r = 0.93$; $P < 0.001$.

to 1.00 U/ml, was 3.40 and 3.08 ng/ml per hour, respectively.

The effect of addition of purified Hageman factor upon plasma renin activity in cold-stored normal plasma. The addition of sufficient purified HF to normal plasma to raise the coagulant titer to ~2.00 U/ml resulted in at least a twofold increase in cold-activated PRA compared with untreated plasma (Table I). In contrast, the addition of an equal volume of buffer did not promote cryoactivation of prorenin.

Studies to determine whether Hageman factor possesses intrinsic angiotensin-generating activity. Conceivably, the role of HF in the generation of angiotensin I was a direct action upon renin substrate rather than upon plasma prorenin. An inhibitor derived from popcorn blocks the enzymatic properties of activated forms of HF (25). Addition of this inhibitor to the plasmas of OC users after cold activation did not prevent the subsequent elaboration of angiotensin I (Table II).

The effect of cold storage upon C$\bar{1}$-INH activity in women using OC agents. The C1-INH activity in the control plasmas of OC users ranged from 1.55 to 7.25 U/ml (mean 4.89±1.86) and that of nonusers ranged from 4.60 to 10.30 U/ml (mean 7.23±1.85). The C$\bar{1}$-INH activity of OC users' control plasmas was significantly decreased compared with nonusers ($P < 0.02$), as reported earlier (30). Furthermore, in cold-stored plasmas, the C$\bar{1}$-INH activity of OC users ranged from 0.00 to 1.90 U/ml (mean 0.76±0.71) and from 5.70 to 9.40 U/ml (mean 7.31±1.32) in nonusers. Thus, the C$\bar{1}$-INH activity in cold-stored OC users' plasma was further reduced from its initial low titer ($P < 0.001$), whereas reduction in C$\bar{1}$--INH activity was not observed during cold storage of nonusers' plasma.

The effect of cold storage upon Hageman factor coagulant titer in women using OC agents. The Hageman factor titer in cold-stored plasmas was significantly decreased compared with control plasmas in the same subset of OC users ($P < 0.001$), while the Hageman factor titer in control and cold-stored plasmas was not significantly different in nonusers. Thus, the Hageman factor coagulant titer of OC users ranged from 1.36 to 2.40 U/ml (mean 1.97±0.31) in control plasmas and 0.83 to 1.40 U/ml (mean 1.04±0.16) in cold-stored plasmas. In contrast, the Hageman factor coagulant titer of nonusers ranged from 0.64 to 1.12

TABLE I
Plasma Renin Activity in Hageman Factor-fortified Normal Plasma

Plasma sample	Plasma renin activity	
	Control	Cold-stored
	ng/ml per hour angiotensin I	
Normal plus purified HF (153.7 U/mg protein)	0.53	1.47
Normal plus buffer	0.54	0.78
Normal plus purified HF (142 U/mg protein)	0.57	1.55
Normal plus buffer	0.59	0.36

1 ml of normal plasma contained ~1 U of Hageman factor-clotting activity. 50 μl of purified Hageman factor (specific clotting activities of 30.7 and 21.3 U/ml) in barbital-saline buffer was added to 1 ml normal plasma to raise the Hageman factor coagulant titer to 2.16 and 1.84 U/ml in the resultant mixtures. An equal volume of barbital-saline buffer (pH 7.4) was added to the untreated plasma with resultant Hageman factor coagulant titers of 0.84 and 0.76 U/ml, respectively.

U/ml (mean 0.90±0.17) in control plasmas and 0.48 to 1.14 U/ml (mean 0.84±0.18) in cold-stored plasmas.

DISCUSSION

Increase in plasma renin activity in vitro can be induced by acid (1–3), trypsin (4, 5), and by prolonged cold storage of plasma (5–8). These phenomena occur as independent processes (5) and are attributed to the activation of endogenous prorenin, an inactive form of renin that is found in normal plasma. The cryoactivation of plasma prorenin is mediated by neutral serine proteases, possibly prekallikrein (11, 12), plasmin (17, 32) or the vitamin K-dependent clotting factors (17), and is comparable with trypsin-activation in some studies (11, 16). This enzyme-mediated reaction can be blocked in the presence of protease inhibitors, such as diisopropylfluorophosphonate (16). Recent studies demonstrated that cold-activation of plasma prorenin is minimal in plasmas that are deficient in Hageman factor (4). In two deficient plasmas, HF deficiency did not lower the basic angiotensin-generation rate in plasma, but rather lowered the level of attainable cold-induced prorenin activation. This suggests that optimum cryoactivation of plasma prorenin requires adequate amounts of Hageman factor.

TABLE II
Plasma Renin Activity in Popcorn Inhibitor-treated, Cold-activated OC Users' Plasma

Plasma sample	Plasma renin activity
	ng/ml per hour angiotensin I
OC user plus popcorn inhibitor (0.5 ml) (50 μg in 80 μl buffer)	6.83
OC user plus buffer (0.5 ml) (80 μl)	6.42
OC user plus popcorn inhibitor (0.5 ml) (50 μg in 80 μl buffer)	1.70
OC user plus buffer (0.5 ml) (80 μl)	1.71

The tabulated results are the means of duplicate experiments; the buffer was barbital-saline (pH 7.4).

In 1978, Osmond and co-workers observed enhanced cryoactivation of plasma prorenin in women using OC agents (5). Furthermore, van Royen et al. (18) demonstrated spontaneous augmentation of plasma renin activity in certain cold-stored plasmas that also manifest spontaneous shortening of the Thrombotest time. Recently, we reported that a high titer of Hageman factor, such as that observed in women using OC agents, is required for cold activation of Factor VII and shortening of the prothrombin time (19). This observation stimulated us to investigate the role of high Hageman factor titer in the cryoactivation of plasma prorenin.

The present study confirms that cold activation of plasma prorenin is markedly enhanced in OC users compared with nonusers. The change in plasma renin activity during cold storage was strongly related to the titer of Hageman factor in the frozen control plasmas of 55 individuals. Furthermore, the addition of sufficient purified Hageman factor to normal plasma, to raise its clot-promoting activity to a level observed in OC users, increased plasma renin activity at least twofold as compared with the untreated plasma. In contrast, the reduction of the HF coagulant titer in the plasma of OC users correspondingly reduced the rise in the plasma renin activity. The reduction in PRA after cold activation of plasma was not due to depletion of prorenin, as measured by trypsin activation.

The measurement of PRA depends upon conversion of renin substrate to angiotensin I by renin. Conceivably, cryoactivation might bring about this conversion by generation of an enzyme that acted directly or indirectly upon renin substrate without the intermediation of plasma renin. Experiments demonstrate that the formation of angiotensin I was not a direct effect of activated HF upon renin substrate; it was not blocked by a potent HF inhibitor derived from corn (25), which was added to plasma after cold activation. These experiments do not preclude the possibility that enzymes activated by HF during cold activation, such as plasma kallikrein, activated plasma thromboplastin

antecedent (Factor XIa), or plasmin, might generate angiotensin I by mechanisms not dependent upon the activation of prorenin.

$C\bar{1}$-INH is a potent plasma inhibitor of the activated form of C1 ($C\bar{1}$) as well as of activated Hageman factor, plasma kallikrein, and activated plasma thromboplastin antecedent (Factor XI) (32, 33). Previously, we reported a simultaneous diminution in both functional and antigenic titers of $C\bar{1}$-INH among OC users (30). In the present study, we observed a further decrease in the $C\bar{1}$-INH titer from its initially low level during cold storage of OC users' plasma. This decrease was associated with a reduction of the Hageman factor coagulant titer. The mechanisms involved in these phenomena are not clear. Armstrong and Dias da Silva (34) provided evidence that HF is activated upon incubation of normal human serum at 0°C. Furthermore, Cochrane and Wuepper (35) demonstrated partial activation of purified human HF at cold temperatures. An appealing concept, then, is that the evolution of angiotensin I-generating properties during cold storage of plasma is dependent upon the activation of HF. The low titer of $C\bar{1}$-INH, a major plasma inhibitor of activated HF, in OC users allows the generation of sufficient amounts of activated HF to bring about angiotensin I formation. How this comes about is uncertain, but the angiotensin I generation may be mediated through activation of other proenzymes, such as plasminogen or prekallikrein. In this regard, the reduced titer of $C\bar{1}$-INH in the plasmas of OC users would foster the accumulation of plasma kallikrein, which was activated by HF. Whether plasmin, plasma kallikrein, or some as yet unrecognized HF-activated plasma enzyme then cleaves renin substrate directly, or through the activation of plasma prorenin is conjectural. In any event, the elaboration of activated HF and kallikrein may explain the reduction of $C\bar{1}$-INH titers during cold storage of the plasmas of OC users, as this inhibitor binds to these activated enzymes.

No evidence presently available links the in vitro changes described in the present study to the development of thrombosis and hypertension in women using OC agents. Nevertheless, two in vitro phenomena, the cold activation of Factor VII and of plasma prorenin, appear to be direct consequences of the high titer of Hageman factor in the plasmas of women using OC agents.

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