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OESTROGEN-INDUCED ENHANCEMENT OF MYELOPEROXIDASE ACTIVITY IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES – A POSSIBLE CAUSE OF OXIDATIVE STRESS IN INFLAMMATORY CELLS

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Micromolar concentrations of beta-estradiol, estrone, 16-alpha-hydroxyestrone and estriol enhance the oxidative metabolism of activated human PMNL's. The corresponding 2-hydroxylated estrogens 2-OH-estradiol, 2-OH-estrone and 2-OH-estriol act on the contrary as powerful inhibitors of cell activity. Equilenine, a naturally occurring steroid hormone structurally closely related to estrone, removes the estrogen-induced increase in oxidative metabolism of activated PMNL's without diminishing cell activity determined in the absence of enhancing hormone. A number of other female and male sexual hormones were without potentiating effect.

The cell response to hormone treatment was assayed as increase (or decease) in LU-dependent CL of activated PMNL's. When assaying LUC-dependent CL of the cells no stimulatory effects of the estrogens could be detected. This fact may imply that the myeloperoxidase enzyme system of the cells is the target for the hormonal action. Various inhibition experiments using activated PMNL's or purified MPO confirmed this conclusion. The efficicious hormones induced approximately a doubling of CL of activated cells and a tenfold increase of the activity of purified MPO. If cell activity was initiated by the additions of low concentrations of hydrogen peroxide, the presence of estrogens caused a remarkable enhancement of the luminol-dependent chemiluminescence.

PMNL's activated with fMLP release MPO activity into the surrounding cell medium. It has been found here that the presence of estrogens in micromolar concentrations greatly increases such enzyme release. Release of MPO activity from the cells could be achieved by the mere addition of estrogenic hormones. Estrogen-induced release of enzyme activity was abrogated by the simultaneous presence of equilenine in the cell suspension. Released enzyme responded vigorously to estrogens in the presence of chloride ions and its substrate, hydrogen peroxide. About a tenfold increase in enzyme activity could be measured in the presence of 5 μM beta-estradiol or esteriol. The activity of the released enzyme (as well of purified MPO) was effectively inhibited by small amounts of anti-MPO antibodies. This observation together with other inhibition experiments was taken as evidence for the view that the released enzyme was identical with myeloperoxidase.

KEY WORDS: Polymorphonuclear luekocytes, oxidative stress, myeloperoxidase, estrogenic hormones, chemiluminescence.

ABBREVIATIONS: SLE: Systemic Lupus Erythematosus; PMNL's: polymorphonuclear leukocytes; fMLP: formyl-methionyl-leucyl-phenylalanine; CL: chemiluminescence; LU: luminol; LUC: lucigenin; MPO: myeloperoxidase; SOD: superoxide dismutase; PBS: phosphatebuffered saline; HBSS: Hank's Balanced Salt Solution.

INTRODUCTION

Many chronic inflammatory diseases are more frequently encountered in females than in males as e.g. in Systemic Lupus Erthyematosus and Rheumatoid Arthritis.[1] This is particularly true for the autoimmune disease SLE which nearly exclusively afflicts women after puberty.[2] The ratio of diseased females to males is estimated to around 13:1.[2] The corresponding value for Rheumatoid Arthritis is claimed to lie around 4:1. Characteristic for SLE is that its activity varies with the hormonal status of the patient, i.e. flares of disease activity are found at times of high sex hormone level (e.g. during pregnancy and during intake of estrogen-containing contraceptives) and decline in disease activity occurs when sex hormone production is low.[3] Another observation which may be relevant to any importance of sex hormones in SLE is that diseased persons seem to have an altered sex hormone metabolism compared to healthy individuals.[4] Blood from the former group shows elevated concentrations of the estrogenic hormones 16-alpha-hydroxyestrone and estriol and the rate of oxidation of testosterone is higher in persons with SLE than in normal controls.[5]

In an animal model of SLE New Zealand Black × New Zealand White F_1 mice, one finds evidence for a major influence of sex hormones on the onset and development of this inherited disease.[6] Female mice are more severely afflicted than male mice and die prematurely of uremia whilst the male counterparts show a milder progression of the disease and reach nearly normal age.[7] If female mice are ovarectomized or treated with testosterone, the development of the disease is arrested with increased longevity as a consequence.[6] If, however, an estrogen like beta-estradiol is administered to the mice, the disease is remarkably accelerated. This is true also for male mice especially if the animals are castrated before estrogen treatment.[6] It has recently been reported that prolonged administration of beta-estradiol to normal mice induces the formation of autoantibodies.[13] Offspring from mice treated with estrogenic hormones during pregnancy develop a kind of autoimmune disease indistinguishable from the autoimmune disease Sjögren's syndrome in humans.[14]

Polymorphonuclear leuckocytes play an important role in inflammatory diseases.[8] The cells accumulate in great numbers at the site of an inflammation and when activated to combat foreign organisms causing the inflammation, they synthesize and release reactive oxygen-centered molecules, hydrolytic enzymes and chemoattractants. The latter substances have the capacity to call for more inflammatory cells to arrive at the inflamed area. The oxygen species and the hydrolytic enzymes are involved in the elimination of invading infectious organisms. There is growing evidence to-day that an excessive production of oxygen-derived radicals and maybe of other types of radicals or reactive compounds as well may play a role in the origin and development of a number of diseases as e.g. chronic inflammatory diseases.[9]

Considering the abnormal metabolism of sex hormones reported to be present in humans with SLE and observed influences by sex hormones in modulating murine SLE, the present studies aimed at finding a relationship between the occurrence of altered levels of certain steroid sex hormones in humans and the rate of formation of oxygen-derived species by activated polymorphonuclear leukocytes.

MATERIALS AND METHODS

The following hormones were purchased from Sigma Chemical Comp., St. Louis, MO, USA and tested for interaction with activated human PMNL's: beta-estradiol;

estrone; 16-alpha-hydroxyestrone; estriol; 2-hydroxyestradiol; 2-hydroxy-esterone; 2-hydroxoy-esteriol; equilenine; progesterone; d-aldosterone; androsterone; 4-androstene-3,-7-dione; testosterone.

Formyl-methionyl-leucyl-phenylalanine, catalase, superoxide dismutase, ferricytochrome C, luminol and lucigenin were all Sigma products. Purified myeloperoxidase and rabbit-anti-human myeloperoxidase antibodies were kindly supplied by Prof. Per Venge, Uppsala, Sweden. For gradient centrifugations LYMPHOPREP™ from Nycomed was used to isolate PMNL's.

Preparation of PMNL's

As a source for the isolation of human PMNL's was used "buffy coats", i.e. a concentrate of leukocytes obtained during the processing of whole blood at a local hospital. The leukocyte concentrate was first freed from red blood cells by lysis with deionized water at +4°C for 30 seconds followed by restoration of tonicity with 0.6 M KCl. After two such treatments the remaining leukocytes were virtually free of red blood cells and the leukocytes were suspended in PBS. The cell suspension was carefully placed on top of a gradient solution (LYMPHOPREP) containing sodium metrizoate (9.6% w/v) and Ficoll (5.6 w/v) with a density of 1.077 g/ml. The gradient was centrifuged at 400 × g for 20 minutes at room temperature. After the centrifugation the PMNL's were recovered from the bottom of the centrifuge tube and washed twice with PBS and suspended in HBSS to the desired cell density, which was determined in a Coulter counter.

Measurement of Chemiluminescence of Activated PMNL's

The chemiluminescence of activated cells was measured with a LKB 1250 luminometer equipped with a thermostated cell holder at 37°C. The light out-put was registered on an ordinary laboratory recorder and the result of an experiment was presented as the millivolt reading at the peak of the chemiluminescence curve obtained. Details of the reaction conditions will be found in the legends for the various figures and tables presented in the text.

Measurement of Myeloperoxidase Activity by Spectrophotometry

Myeloperoxidase activity was assayed spectrophotometrically either by determining chlorination of monochlordimedone[15] or by determining the oxidation of guaiacol.[16]

Measurement of Superoxide Anion Production by Activated PML'S

The production of superoxide anions by activated cells was assayed as the change in absorbance at 550 nm of ferricytochrome C added to the cell suspension. A Beckman ACTA MV1 spectrophotometer was used for continuous monitoring the change in absorbance during the cell reaction after activation. The instrument was equipped with a thermostated cell holder and the reactions were performed at 37°C. Details of reaction conditions are given in the text.

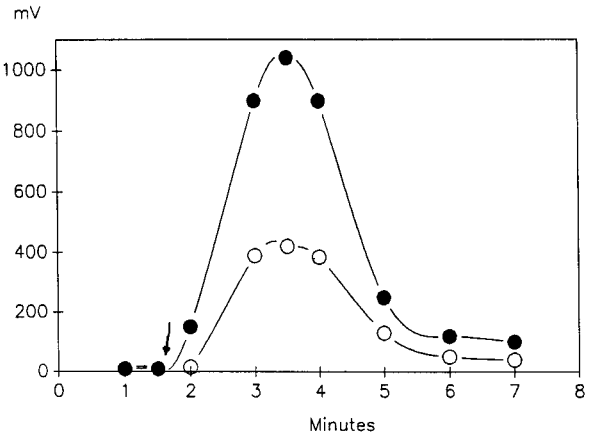

FIGURE 1 Increase in luminol-dependent chemiluminescence of activated human polymorphonuclear leukocytes in the presence of estriol. Reaction mixture at 37°C: 10^6 cells, 0.17 mM luminol and 10^{-7} M fMLP in 1.0 ml HBSS pH 7.4. O-O-O-O no pre-incubation with estriol. ●-●●-● pre-incubation with 10^{-6} M estriol. Arrow indicates the addition of fMLP.

RESULTS

Effects of Steroid Sex Hormones on Chemiluminescence of Activated PMNL's

Since increased oxidative stress to tissues, cells and various macromolecules is believed to be a factor in autoimmune diseases,[10] it seemed appropriate to investigate if sex hormomes contribute to increased oxidative stress by influencing the production of oxidants in inflammatory cells such as PMNL's. It was found that 4 estrogenic hormones were capable of enhancing the LU-dependent CL of activated PMNL's namely beta-estradiol, esterone, 16-alpha-hydroxyestrone and estriol. From Figure 1 it is clear that the addition of 1 μM estriol markedly enhances the chemiluminescence of activated cells.

The corresponding 2-hydroxylated estrogens (2-OH-beta-estradiol, 2-OH-estrone and 2-OH-estriol) were found to act as powerful inhibitors of the respiratory burst of PMNL's when added in concentrations roughly equal to those of the enhancing hormones (Table I).

Male sex hormones did not cause any effect on the CL of activated PMNL's when used in concentrations $< 10^{-5}$ M. When applying concentrations of 10^{-5} M or higher they caused inhibition of cell activity.

TABLE I
Inhibition of luminol-dependent chemiluminescence of activated polymorphonuclear leukocytes in the presence of 2-hydroxylated estrogenic hormones

Hormone added to cell suspension	Concentration of hormone/% Inhibition of cell activity (mean ± SD) 10^{-6} M	5.10^{-7} M	10^{-7} M	10^{-8} M
2-hydroxyestradiol	69.3 ± 1.7	53.6 ± 1.3	30.6 ± 1.5	0.9 ± 1.9
2-hydroxyestrone	71.5 ± 1.4	52.1 ± 1.4	33.0 ± 1.7	0.0 ± 2.1
2-hydroxyestriol	65.2 ± 1.7	45.3 ± 1.0	20.2 ± 1.3	1.5 ± 1.8

Reaction mixture: 10^6 cells; 0.17 mM luminol; 10^{-7} M fMLP and hormone as indicated in table. Total reaction volume 1.0 ml HBSS. Results are mean ± SD, $n = 5$

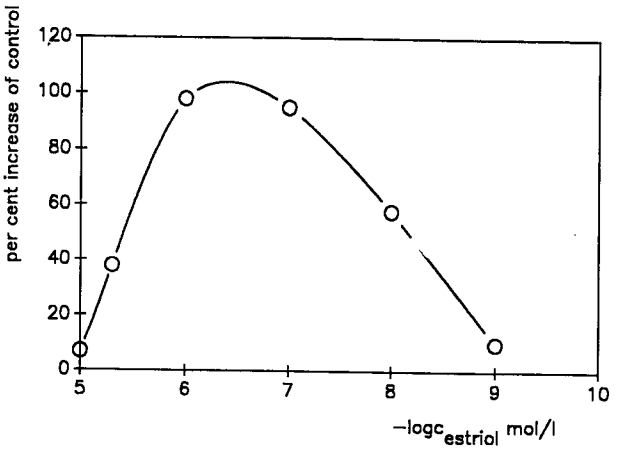

FIGURE 2 Dose-response curve for luminol-dependent chemiluminescence of activated human polymorphonuclear leukocytes in the presence of various concentrations of estriol. Reaction conditions as in Figure 1.

Estriol was the most effective in enhancing the LU-dependent CL of activated PMNL's and gave, on average, a doubling of the chemiluminescent yield at a concentration of approximately 1 μM. The increase in CL at a certain hormone concentration varied somewhat between different cell preparations. It is not known whether these variations were due to the fact that each preparation was obtained from blood from different donors or if the preparation procedure gave rise to damage to the cells varying from one preparation to another. Figure 2 shows the dose-dependent increase in CL of PMNL's pre-incubated with various concentrations of estriol and then activated with fMLP. Some enhancing effect is still measureable at hormone concentrations as flow as 0.01 μM.

Myeloperoxidase as the Target for the Hormonal Actions

The chemiluminescent probe lucigenin (bis-methylacridinium nitrate) is sometimes used instead of luminol to amplify CL formed during the respiratory burst of activated PMNL's.[11] LUC is claimed to monitor CL caused by superoxide anions formed during cell metabolism whilst LU is believed to measure CL originating from cell reactions of peroxidative nature such as the formation of hypochlorous acid by myeloperoxidase. When using LUC as chemiluminescent probe, no enhancement of PMNL chemiluminescence could be detected in the presence of estrogens (data not shown). This observation suggests that myeloperoxidase is the target for the estrogen effect on CL.

A specific assay to measure the formation of superoxide anions during the respiratory burst of activated PMNL's is to include ferricytochrome C in the cell suspension and to monitor the SOD-inhibitable reduction of ferricytochrome C at 550 nm. Determination of the formation of superoxide anions of activated PMNL's in this way showed that estrogens had no effect on radical synthesis (Figure 3). This experiment shows that the estrogenic hormones do not influence the superoxide anion

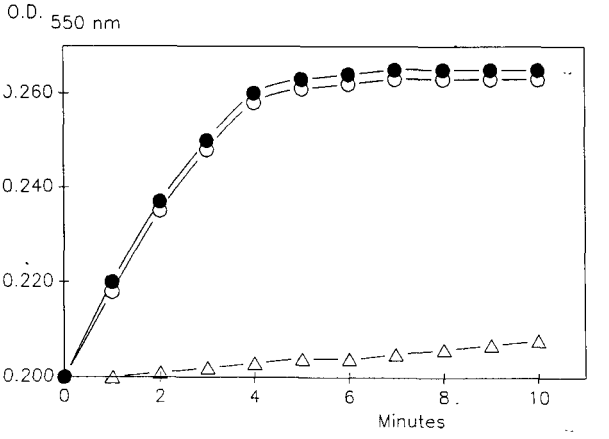

FIGURE 3 Superoxide anion production in activated human polymorphonuclear leukocytes in the absence of presence of estriol. Reaction mixture at 37°C: 10^6 cells, 100 μM ferricytochrome C and 10^{-6} M fMLP in a total volume of 3.0 ml HBSS pH 7.4. O-O-O-O no pre-incubation with estriol. ●-●-●-● pre-incubation with 10^{-6} M estriol. Δ-Δ-Δ-Δ in the presence of 100 ug SOD. fMLP added at zero time.

forming enzyme system (NADPH-oxidase) of the cells, consistent with the view that myeloperoxidase is the enzyme affected when PMNL's are treated with the hormones.

A third argument in favour of the opinion that MPO is the system that is affected by hormone treatment of PMNL's, is the effect of sodium azide. As is shown in Table II, the inhibition of CL by azide is proportionally greater at a certain concentration of the inhibitor when CL is enhanced by the presence of estrogen. This would be expected if the enhancing effect of the hormone on CL is due to an effect on MPO.

If MPO is the key enzyme system for the hormonal action on activated PMNL's, it should be possible to abrogate this effect by removing the peroxidase substrate (hydrogen peroxide) from the cell system. This can be done by including the enzyme catalase in the reaction mixture. The data presented in Table III clearly shows that the enhancement of cell metabolism due to the presence of estrogen is completely suppressed if catalase is included in the reaction mixture. The remaining CL of the cell suspension, whether containing hormone or not, may be due to superoxide anions.

It seemed necessary to make sure that the estrogens are affecting the enzyme and not just enhancing the reaction between the formed hypochlorous acid and luminol in the reaction mixture. Therefore HOCl was added to the standard reaction mixture,

TABLE II

Inhibition of luminol-dependent chemiluminescence of activated polymorphonuclear leukocytes with sodium azide in the absence or presence of estriol

Addition of estriol (10^{-6}M) to cell suspension	Concentration of azide/% Inhibition of cell activity		
	10^{-4}M	10^{-5}M	10^{-6}M
None	80.2 ± 1.5	67.5 ± 1.7	20.2 ± 1.3
Yes	90.8 ± 1.6	80.4 ± 1.1	57.3 ± 1.6

Reaction mixture: 10^6 cells, 0.17 mM luminol, 10^{-7} M fMLP and hormone and azide in concentrations as indicated in table. Total Reaction volume 1.0 ml HBSS. Results are mean ± SD, $n = 5$

TABLE III

Effect of catalase on luminol-dependent chemiluminesence of activated polymorphonuclear leukocytes in the absence or presence of estriol

Addition to reaction mixture Estriol 10^{-6} M	Catalase 200 μg	Peak value of CL. mV	Percent Inhibition
–	–	875 ± 18.4	–
+	–	1560 ± 14.2	–
–	+	160 ± 7.1	80.4 ± 1.5
+	+	180 ± 7.5	89.1 ± 1.4

Reaction mixture: 10^6 cells, 0.17 mM luminol, 10^{-7} fMLP and estriol and catalase as indicated in table. Total reaction volume 1.0 ml HBSS. Results are mean ± SD, $n = 5$

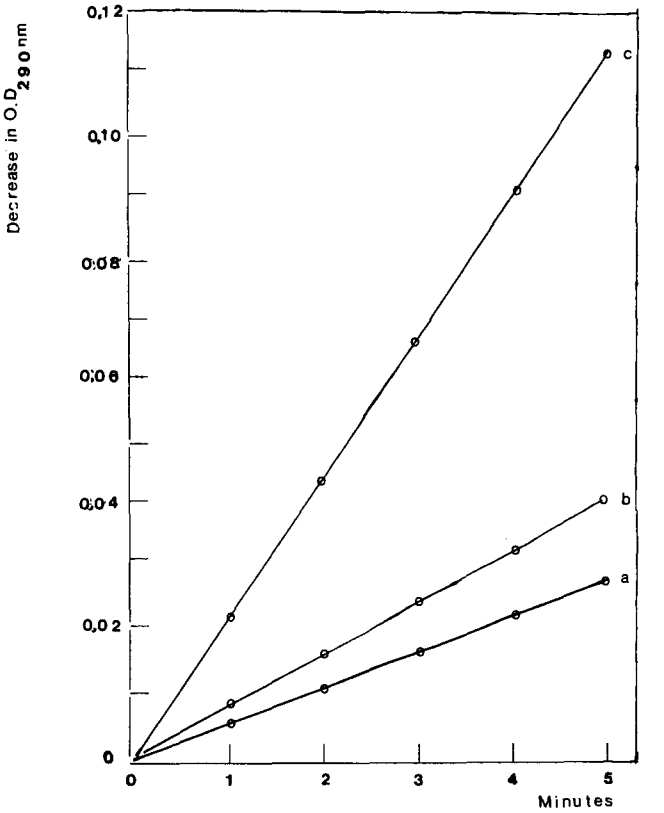

FIGURE 4 Chlorination of monochlordimedone by myeloperoxidase enzyme in the absence or presence of estriol measured as decrease in absorbancy at 290 nm. Reaction conditions at 37°C: enzyme, monchlordimedone 100 μM and Cl^- 137 mM in a total volume of 3.0 ml PBS. Curve a: no addition of estriol; curve b: addition of 10^{-6} M estriol; curve c: addition of 5×10^{-6} M estriol.

TABLE IV
Direct activation of the oxidative metabolism of polymorphonuclear leukocytes by hydrogen peroxide in the presence of estriol

Exp. no	Addition to reaction mixture			Peak value*
	H_2O_2 10^{-4} M	Estriol 10^{-6} M	Catalase 200 μg	
1.	–	+	–	0
2.	+	–	–	50
3.	+	+	–	1160
4.	+	+	+	0

Reaction mixture: 10^6 cells, 0.17 mM luminol in a total volume of 1.0 ml HBSS.
*Average value of 3 determinations.

omitting PMNL's. The CL formed under this condition did not increase in the presence of hormone (data not shown), which implies that the estrogens act on the enzyme itself. This statement is supported by assaying MPO activity spectrophotometrically using either the monochlordimedone method[15] or the guaiacol method[16] to measure the enzyme activity. It is clear from Figure 4 that 5.10^{-6} M estriol substantially enhances the capacity of the enzyme to chlorinate monochlordimedone. The oxidation of guaiacol by the enzymatically formed HOCl was also enhanced in the presence of estrogen (data not shown).

Effect of Hydrogen Peroxide on Estrogen-treated PMNL's

The formation of superoxide anions by actived PMNL's is a pre-requisite for generation of hydrogen peroxide and subsequent triggering of myeloperoxidase activity. External addition of low concentrations of hydrogen peroxide to resting cells has only a marginal effect on luminol-amplified CL. If however the resting cells were preincubated with estrogen, the addition of hydrogen peroxide caused a remarkable

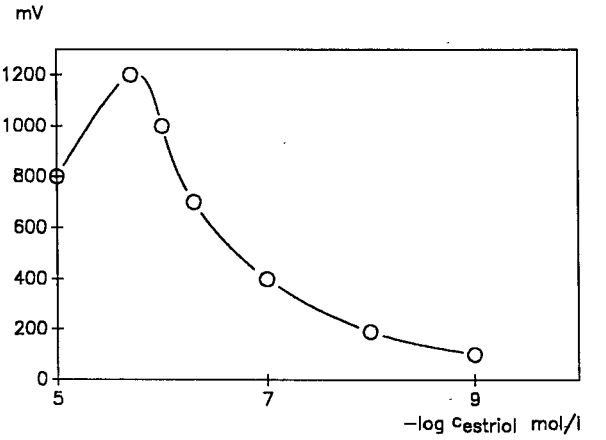

FIGURE 5 Dose-response curve for the stimulatory effect of estriol on luminol-dependent chemiluminescence of human polymorphonuclear leukocytes activated by the addition of hydrogen peroxide. Reaction mixture at 37°C: 10^6 cells, 0.17 mM luminol, 10^{-4} m hydrogen peroxide and various concentrations of estriol in a total volume of 1.0 ml HBSS pH 7.4.

increase of the chemiluminescence. As can be seen from Table IV, a 20-fold increase increase in CL occurred as a result of treating the non-activated cells with 10^{-6} M estriol. This influence of estrogenic hormones on non-activated PMNL's may have a pathological importance. In individuals with increased blood levels of these hormones, circulating cells may become incorrectly triggered to produce the strong oxidant hypochlorous acid provided the myeloperoxidase substrate is also available.

Figure 5 shows the dose-response curve for the stimulatory capacity of estriol on LU-dependent CL of PMNL's activated with hydrogen peroxide instead of with fMLP. The chemiluminescent yield is of the same magnitude as that found when fMLP-activated cells are stimulated with optimal concentration of estrogens.

Release of Myeloperoxidase from Non-activated PMNL's

It is well known that activated PMNL's secrete myelperoxidase activitiy into the surrounding medium.[12] Experiments accounted for here show however, that resting (non-activated) cells can be made to release substantial amounts of MPO activity by treatment with estrogenic hormones. Resting PMNL's were pre-incubated with various concentrations of estriol for 15 minutes at 37°C and then the cell suspensions were centrifuged so that cell-free supernatants were obtained. Portions of the supernatants were mixed with luminol in HBSS and the induced CL was recorded after addition of hydrogen peroxide. Strong chemiluminescence was observed for all supernatants except the one obtained when incubation was carried out in the absence of estrogen. Figure 6 shows that CL of the various supernatants from estriol-treated cells varied linearly with the concentration of estriol in the incubation medium.

From these experiments it was not possible to know how much of the measured MPO activity was due to actual release of the enzyme and how much was due to the enhancing effect of the hormone on the released enzyme. Preliminary experiments had

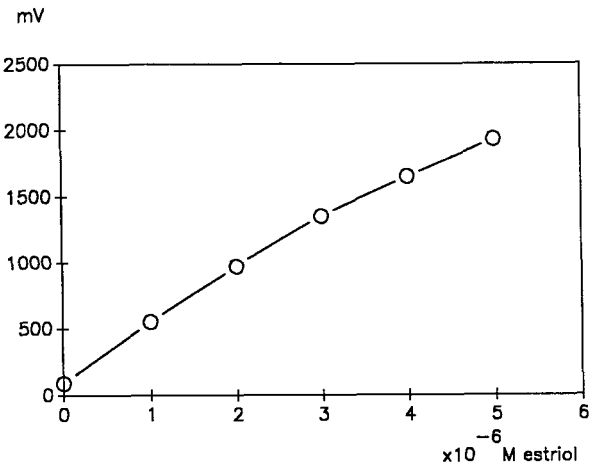

FIGURE 6 Luminol-dependent chemiluminescence of cell-free supernatants from human polymorphonuclear leukocytes activated with 10^{-6} M fMLP and incubated at 37°C for 15 minutes with various concentrations of estriol. Reaction mixture at 37°C: 0.5 ml cell-free supernatant, 0.17 mM luminol, 10^{-4} M hydrogen peroxide in a total volume of 1.0 ml HBSS pH 7.4.

TABLE V
Release of myeloperoxidase activity from PMNL's incubated at 37°C with fMLP and/or estriol

Experiment No.	Additions to incubated cells			Peak value of CL of supernatant in mV.*
	fMLP	Estriol		
	10^{-6}M	10^{-6}M	10^{-7}M	
1	−	−	−	45
2	+	−	−	400
3	−	+	−	350
4	−	−	+	200
5	+	+	−	1050
6	+	−	+	640

Cell density for release of enzyme: $5 \times 1-^{6}$cells/ml HBSS. Reaction mixture for assaying released enzyme activity: 0.5ml cell-free supernatant, 0.17mM luminol, 5×10^{-6}M estriol, 10^{-4}M hydrogen peroxide in a total volume of 1.0ml HBSS.
*Average value of 3 determinations

shown that purified MPO responded like intact cells on treatment with estrogens in that the enzymatic activity was dramatically increased (data not shown). In order to separate the two effects from each other, the following experiment was designed. 2.5 ml of the supernatant above was filtered through a small Sephadex-G25M column. After this amount of liquid had passed through the column, the high molecular weight fraction containing the released enzyme could be eluted with 3.5ml of HBSS. The low molecular weight components of the supernatant among them the estrogen initially added to the cell suspension would be retained by the column material but could subsequently be eluted with buffer. Portions of the high molecular weight fraction freed from the initially added estrogen were then analyzed for LU-dependent CL in the presence of newly added estriol and after supplying the enzyme substrate, hydrogen peroxide. Table V shows that cells incubated without estrogen or fMLP released only insignificant amounts of enzyme activity. With 1 μM fMLP in the incubation medium there is a ten-fold increase in release and about the same extent of release is found when the cells are incubated with 1 μM estriol. If these two substances are present simultaneously in the cell suspension, a dramatic increase in enzyme release was observed.

TABLE VI
Effect of anti-human MPO-antibodies on luminol-dependent chemiluminescence of estriol-treated activated polymorphonuclear leukocytes, of enzyme released from cells and of purified human myeloperoxidase

Exp. No.	Measurement on			Addition of antibodies 5 μg	Peak value of CL in mV.*
	intact cells	released enzyme	purified enzyme		
1.	+	−	−	−	720
2.	+	−	−	+	130
3.	−	+	−	−	1500
4.	−	+	−	+	0
5.	−	−	+	−	2100
6.	−	−	+	+	0

Conditions for enzyme release: see legend in Table V. Reaction mixture for assaying CL in intact cells: see legend in Table I. Reaction of mixture for assaying activity of released enzyme: see legend in Table V. Reaction mixture for assaying purified enzyme: 87ng enzyme, 0.17mM luminol, 5×10^{-6}M estriol, 10^{-4}M hydrogen peroxide, total volume 1.0ml in HBSS.
*Average value of 3 determinations

Inhibition of Estrogen-induced Chemiluminescence with Antibodies to Human Myeloperoxidase

The correctness of the assumption that the enzyme that is stimulated by estrogens in PMNL's and also is released into the surrounding medium when the cells are incubated in the presence of the hormone, is MPO could be proved by examining the effect of antibodies to human MPO. These antibodies are considered to a specific inhibitor of the enzyme.[17] The data presented in Table VI clearly shows that addition of the antibodies effectively suppressed the LU-dependent CL of estriol-treated activated PMNL's. The remaining CL after antibody treatment may be due to the formation of superoxide anions, the synthesis of which by the activated cells is not affected by the inhibitor. It is also evident from Table VI that purified MPO and the enzyme released from metabolizing cells are effectively inhibited by the presence of anti-MPO antibodies in the reaction mixture.

Effects of Equilenine on Estrogen-induced Enhancement of Chemiluminescence of Activated PMNL's and Purified Myeloperoxidase

During the testing of various steroid hormones for effects on the oxygen burst of PMNL's, it was observed that the estrogenic hormone equilenine had a remarkable effect. The hormone is found in urine from pregnant mares and is structurally very similar to estrone. At concentrations higher than 5 μM the hormone inhibits cell metabolism assayed as LU-dependent CL irrespective of whether the cells were pre-incubated with estrogen or not, but the inhibition was proportionally larger in cells pre-incubated with estrogen. At concentrations below 5 μM equilenine did not inhibit the chemiluminescence of cells not pre-incubated with estriol, but removed the enhancement in chemiluminescence caused by the hormone (Table VII). For comparison the effect of 2-hydroxyestriol in the presence or absence of estriol is included in the Table. 2-Hydroxyestriol acts differently from equilenine in that the former hormone inhibits cell metabolism to the same extent whether the cells had been treated with estriol or not.

Cell preparations from ten different buffy coats were each activated with fMLP in the absence or in the presence of estriol or equileneine or in a mixture of the two hormones and LU-dependent CL was recorded. For each cell preparation nearly the same counteracting effect by equilenine on the enhancing capacity of estriol on CL

TABLE VII

Effects of the hormone equilenine on estrogen induced potentiation of luminol-dependent chemiluminescence of activated polymorphonuclear leukocytes

Experiment No.	Addition to cell suspension			Peak value of CLin mV*
	Estriol 10^{-6}	Equilenine 5.10^{-7} M	2-OH-estriol 5.10^{-7} M	
1.	–	–	–	600
2.	+	–	–	1300
3.	–	+	–	570
4.	–	–	+	230
5.	+	+	–	560
6.	+	–	+	330

Reaction mixture: 10^6 cells, 0.17 mM luminol and 10^{-7} m fMLP in a total volume of 1.0 ml HBSS.
*Average value of 3 determinations

TABLE VIII

Effects of estriol and equilenine on luminol-dependent chemiluminescence of activated polymorphonuclear leukocytes

Prep. No.	Control peak value, mV*	Addition to reaction mixture/Peak value/Change in peak value					
		10^{-6} M estriol		10^{-6} M equilenine		10^{-6} M estriol + 10^{-6} M equilenine	
		mV*	% change	mV*	% change	mV*	% change
1	550	1200	+118	400	−27	470	−14
2	840	1450	+ 72	540	−36	580	−30
3	790	1950	+146	560	−29	580	−27
4	940	2350	+150	860	− 9	1200	+27
5	780	1500	+ 92	560	−28	640	−18
6	740	2000	+170	800	+ 8	900	+21
7	950	1900	+100	800	−16	800	−16
8	590	1300	+120	500	−15	560	− 5
9	600	1150	+ 92	570	− 5	700	+16
10	1000	2100	+110	900	−10	1000	0
Mean value	778	1690	+117	649	−17	743	−5
+ S.D.	151	398	29	165	13	218	19

Reaction mixtrure: 10^6 cells, 0.17mM luminol and 10^{-7} M fMLP in a total volume of 1.0ml HBSS. Concentrations of added hormones as indicated in the table.

*Average value of 3 determinations.

was observed according to Table VIII. On the average a slight inhibition of CL was found when equilenine only was present in the reaction mixture. Using equimolecular concentrations of both hormones in the cell suspension, the mean value of enhancing effect of estriol was very near zero, indicating that equilenine could abrogate the effect of estriol on the oxidative metabolism of the cells.

As mentioned before (cf. Table IV) the CL of resting PMNL's can be initiated by external addition of low concentrations of hydrogen peroxide provided the cells are pre-treated with estriol. Using 1 mM hydrogen peroxide to initiate activity of PMNL's pre-incubated with 5 μM estriol, an increase of 1200% in chemiluminescence could be noted (Table IX). Addition of the same concentration of equilenine to the cell suspension reduced the enhancement of chemiluminescence by approximately 90%.

TABLE IX

Inhibition by equilenine of estriol-induced stimulation of luminol-dependent chemiluminescence, initiated with hydrogen peroxide, of polymorphonuclear leukocytes and of purified myeloperoxidase enzyme

H_2O_2 mM		Addition to reaction mixture		Peak value of Cl*
PMNL	MPO	Estriol μM	Equilenine μM	mV
1.0	–	–	–	205
1.0	–	5	–	2610
1.0	–	–	5	235
1.0	–	5	5	490
–	1.0	–	–	230
–	1.0	5	–	2040
–	1.0	–	5	235
–	1.0	5		440

Reaction conditions: 10^6 cells or 87 ng myeloperoxidase enzyme, 0.17 mM luminol, substrate and hormone concentrations as indicated in table. Total reaction volume 1.0 ml HBSS.

*Average value from 3 determinations

If released MPO from activated cells was used instead of intact cells, addition of hydrogen peroxide to the enzyme solution gave rise to moderate CL. The reaction rate was dramatically increased by including 5 μM estriol in the reaction mixture according to Table IX. The simultaneous presence of equilenine in equimolar concentration virtually abrogated the increase in enzymatic activity due to the presence of estriol. This concentration of equilenine had no inhibitory effect on the enzymic activity found in the absence of estriol (Table IX).

DISCUSSION

The results presented here show that in the presence of estrogen: (i) the oxidative metabolism of activated PMNL's is noticeably enhanced due to the stimulation of myeloperoxidase activity; (ii) release of myeloperoxidase occurs from non-activated cells; (iii) cell metabolism is triggered by low concentrations of hydrogen peroxide and (iv) 2-hydroxylated estrogens act as powerful inhibitors of cell function and of activity of purified myeloperoxidase. The influence of the estrogens on PMNL's might therefore *in vivo* lead to increased oxidative stress on tissues, cells and various macromolecules due to abundance of the strong oxidant hypochlorous acid, the initial product of myeloperoxidase activity, in female sex-related chronic inflammatory, autoimmune diseases like Systemic Lupus Erythematosus. The frequency of this disease is significantly higher in females than in males. This is considered to be due to a more active immune system in the former sex possibly caused by influence of female sex hormones.[13] It has been shown that the metabolism of estrogenic hormones in SLE is aberrant leading to elevated concentrations of 16-alpha-hydroxylated-and lowered concentrations of 2-hydroxylated estrogens.[4] In murine SLE the severity of the disease can be modulated by administrations of sex hormones.[7] The crucial point if adverse oxidative stress will appear in certain autoimmune disease may therefore be the concentration ratio between the two forms of estrogens. Reports give at hand that this ratio is approximately doubled in human SLE.[3] The observation reported here that PMNL's can be brought into metabolic activity by relatively low concentrations of the myeloperoxidase substrate, hydrogen peroxide, in the presence of estrogens may be of particular significance for the increased oxidative stress *in vivo*. A scenario in the vicinity of an inflamed site of an increased ratio between 16-alpha-hydroxylated and 2-hydroxylated estrogens together with availability of the myeloperoxidase substrate (not necessarily hydrogen peroxide) may constitute conditions for continuous metabolic activation of inflammatory cells. The consequence would be an adverse production and secretion of harmful oxidants, i.e. the prerequisite for elevated oxidative stress.

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