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Studies on steroid fever II. Pyrogenic and anti-pyrogenic activity in vitro of some endogenous steroids of man

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Research Article

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Studies on Steroid Fever

II. PYROGENIC AND ANTI-PYROGENIC ACTIVITY IN VITRO OF SOME ENDOGENOUS STEROIDS OF MAN

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ABSTRACT The pyrogenic properties of some C-19 and C-21 steroids were examined by in vitro incubation of human blood leukocytes with serum-buffer solutions of the steroids and injection of the 18-hr supernatants into rabbits. In previous studies this method demonstrated release of leukocyte endogenous pyrogen by etiocholanolone. With two exceptions, steroids known to cause fever in man, such as 11β-OH etiocholanolone and 3α-hydroxy-5β-pregnane-20-one were also pyrogenic in vitro. All steroids tested which are nonpyrogenic in man, such as androsterone, 3β-OH etiocholanolone, and 3α, 17α-dihydroxy-5β-pregnan-20-one were also nonpyrogenic in vitro. Solubility in aqueous solution did not correlate with pyrogenic capacity.

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These studies indicate that a variety of naturally-occurring steroids may alter pyrogen release from leukocytes. Alterations in steroid balance in man may influence normal temperature regulation and contribute to clinical fevers.

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INTRODUCTION

In 1956, Kappas, Hellman, Fukushima, and Gallagher (1) first reported that injection of the naturally occurring steroid etiocholanolone (5β-androstane-3α-ol, 17-one)[1] produced fever in man. This finding has been confirmed in many subsequent reports (2–5). In addition, recent studies have shown that incubation of a serum-buffer solution of etiocholanolone with human blood leukocytes in vitro causes release of an endogenous pyrogen (6, 7). This in vitro reaction has many features which closely resemble those described for the experimental fever caused by injection of etiocholanolone and related steroids into normal subjects (5, 8).

The pyrogenicity of various steroids structurally related to etiocholanolone has been investigated in man and certain features of steroid structure have been correlated with pyrogenic activity (8, 9). Both C-19 (3–5, 10) and C-21 (4, 5, 11) steroids, as well as certain bile acids (12), have been studied. It seemed of interest to examine the action of some of these steroids on leukocytes in vitro, to determine whether similar structural features would also be required for pyrogenic activity in this system.

Inhibition of etiocholanolone fever was observed when subjects had received cortisone injections on preceding days (2), or when cortisol was injected with etiocho-

[1] Systematic nomenclature according to the *Int. Union Pure Appl. Chem.* (1969 Biochemistry. **8**: 2227) for trivial names used in this paper is as follows: etiocholanolone, 3α-hydroxy-5β-androstan-17-one; 11β-OH etiocholanolone, 3α-11β-dihydroxy-5β-androstan-17-one; androsterone, 3α-hydroxy-5α-androstan-17-one; 3β-OH etiocholanolone, 3β-hydroxy-5β-androstan-17-one; hydrocortisone, 11β, 17α,21-trihydroxy-4-pregnene-3,20-dione; estradiol, 1,3,5(10)-estratriene-3,17β-diol; progesterone, 4-pregnene-3,20-dione; lithocholic acid, 3α-hydroxy-5β-cholan-24-oic acid; deoxycholic acid, 3α, 12α-dihydroxy-5β-cholan-24-oic acid; dehydroepiandrosterone (DHEA), 3α-hydroxy-5-androsten-17-one; 3,17-etiocholanedione, 5β-androstane-3,17-dione.

lanolone into the same intramuscular site (13). Etiocholanolone produces less fever in women than in men (14, 15), and in one study, administration of estrogen to women appeared to diminish febrile responses to etiocholanolone, but not to endotoxin (7). In view of these observations, the possible inhibitory effects of hydrocortisone and estradiol on pyrogen release in vitro were investigated.

METHODS

Leukocytes. Preparations of leukocytes and methods of incubation and of injection of supernatants were all as described previously (6). Briefly, a leukocyte-rich fraction was obtained by dextran sedimentation of heparinized blood from male volunteers. The cells were washed with modified Krebs-Ringer phosphate (KRP) buffer, and suspended in a 15% serum-buffer medium. Saturated buffer solutions of steroids were substituted for buffer as required. $8\text{-}10 \times 10^7$ leukocytes were incubated in 8 ml of medium for all experiments with C-19 and C-21 steroids. In experiments with hydrocortisone and estradiol, and heat-killed staphylococci, volumes were 5–6 ml, unless otherwise noted. After being shaken for a few hours at 37°C on a Dubnoff shaker, the flasks were placed overnight in a stationary incubator. Flask contents were then centrifuged at 600–2000 *g* for 20–30 min, and the supernatants injected into rabbits. Preparation of monocytes was as described previously (6). Rabbit blood leukocytes, from female rabbits, were obtained from cardiac blood drawn into a heparinized syringe, and processed as described for human blood.

Pyrogen assay. Techniques for pyrogen testing in rabbits have been described previously (16). Each rabbit received supernatant from 2 to 5×10^7 leukocytes. In all experiments with hydrocortisone and estradiol, and in most other experiments, the same rabbits received all injections from a single experiment. In experiments with pyrogenic steroids, usually two or three different steroids were incubated with aliquots of cells in a single experiment, and experiments were discarded if pyrogen release failed to occur with the pyrogenic steroid, or if control white cells released pyrogen which caused fever of over 0.2°C in rabbits. Usually two or three injections were given to each rabbit each day, in random order, or with control injections preceding pyrogenic ones. Rabbits were not used beyond the 7th day after the first injection of human material.

Steroids. Steroids were autoclaved for 1½ hr, shaken at 37°C for 24 hr with KRP buffer, and the saturated solutions filtered through an ultrafine sintered glass filter. Analysis of these solutions by chromatography, melting points, and infrared spectroscopy indicated that the C-19 steroids were pure, and that the C-21 steroids were greater than 97% pure with two exceptions. These were 5β-pregnan-3α-ol-11,20-dione, which was approximately 90% pure, and 5β-pregnan-3α, 17α-diol-20-one, which was approximately 40% pure. The identity of the impurities was not determined, but from chromatographic mobilities and spectrographic analyses, all components resembled steroids. Samples of all C-19 and C-21 steroids were analyzed before and after autoclaving, and no differences were observed. Solutions were stored at 4°C for up to 8 wk. Longer storage appeared to decrease the concentration of steroid in some solutions. For initial pyrogen testing, in order to detect any possible contaminating endotoxin, 6 ml of each new steroid solution were added to 0.5 ml of normal rabbit serum, incubated at 37°C for 30 min, and injected into one or two rabbits. In addition, control flasks containing steroid solution plus serum were routinely included in each experiment, and tested for sterility and pyrogenicity. C-19 steroid concentrations were determined as follows. Aliquots of buffered steroid solutions were added to flasks which contained known amounts of radioactive steroid. After extraction by chloroform and drying under air, the steroids were analyzed by gas-liquid chromatography, as described previously (6). Appropriate corrections were made for per cent recovery, and the results were compared to assay curves of standards. C-21 steroid concentrations were determined by light absorption of concentrated sulfuric acid solutions (17). Aliquots of both the buffer solutions and of standard solutions of steroids, to which buffer was added, were dried. Concentrated sulfuric acid was added for 2 hr at room temperature, and absorption measured at appropriate peak absorption wavelengths, as determined from known absorption patterns (17), on a Gilford spectrophotometer. Lithocholic and deoxycholic acids were obtained from Mann Research Labs., Inc., New York, and deoxycholic acid was purified by thin-layer chromatography. The acids were determined by gas-liquid chromatography to be $> 97\%$ and $> 99.9\%$ pure, respectively. Lithocholic acid contained 1–2% of a component with the same mobility as 5-β-cholanic acid.[2] Solutions of bile acids were prepared as described for steroids above, but without autoclaving. Estradiol-17β (Mann Research Labs., Inc.) was dissolved in acetone or sterile, absolute alcohol, filtered through a Millipore filter (Millipore Filter Corp., Bedford, Mass.), and aliquots were added to Erlenmeyer flasks. These were thoroughly dried with gentle heating and air blown through a cotton-plugged Pasteur pipette. Usually 200 μg estradiol per flask was used. 5-8 ml of serum-buffer medium, or serum plus a saturated buffer solution of etiocholanolone, were then added, and shaken or allowed to stand for 30 min to 1 hr before leukocytes were added.

To determine the solubility of estradiol, a 0.025 ml aliquot of 6,7-H^3-estradiol, 1.0 mCi/ml, 6.8 μg/ml, was added to 200 μg of unlabeled estradiol in absolute alcohol, and the mixture allowed to dry in 25-ml Erlenmeyer flasks, as in the normal procedure for preparing coated flasks. To duplicate flasks was then added 6 ml of KRP buffer, 6 ml of etiocholanolone-saturated KRP buffer, or 5.2 ml of buffer or etiocholanolone-buffer with 0.8 ml of human serum. Samples were removed from each flask after 2 or 6 hr of shaking, and after dilution in alcohol, counted in a Packard liquid scintillation counter. Solubility was determined by comparison of counts in solution with the original tritiated estradiol solution. Hydrocortisone 21-phosphate for injection (Merck, Sharp & Dohme, West Point, Pa.) was diluted in saline as required.

Bacteria. Heat-killed staphylococci were prepared as described previously (18), except that the final suspension was autoclaved for 20 min. Ratios of 10–30 bacteria per leukocyte were used.

RESULTS

Pyrogenic properties of C-19 steroids. Saturated buffer solutions of a number of closely related C-19 steroids were incubated with leukocytes, and their ability to release endogenous pyrogen determined. For comparison

[2] These procedures and studies were kindly performed by Dr. Marc Taylor.

TABLE I

Pyrogenic Activity of C-19 Steroids and Bile Acids

Steroid	Concentration	Human WBC ($\times 10^7$)	Maximum fever (rabbit)	i.m. injection (man)	References
	μg/ml		*°C*		
Etiocholanolone (3α-hydroxy-5β-androstane-17-one)	14–29	4.5	0.67 (14)* ±0.27‡	Pyrogenic	1–5
11β-OH Etiocholanolone (3α, 11β-Dihydroxy-5β-androstane-17-one)	11, 8	4.6	0.85 (4) ±0.08	Pyrogenic	4, 5
11-Keto etiocholanolone (3α-hydroxy-5β-androstane-11, 17-dione)	16, 105	4.7	0.49 (11) ±0.51	?	
3, 17 Etiocholanedione (5β-androstane-3, 17-dione)	18, 110	4.4	0.29 (5) ±0.15	Weakly pyrogenic	4
3β-OH etiocholanolone (3β-Hydroxy-5β-androstane-17-one)	27, 24	4.8	0.21 (8) ±0.16	Nonpyrogenic	3
Androsterone (3α-hydroxy-5α-androstan-17-one)	9–30	5.0	0.18 (11) ±0.23	Nonpyrogenic§	3, 10
Dehydroepiandrosterone (3α-hydroxy-5-androsten-17-one)	30, 34	4.0	0.05 (4) ±0.09	Nonpyrogenic	19
Testosterone (17β-hydroxy-1-androsten-3-one)	<5–24	4.2	0.07 (5) ±0.12	Nonpyrogenic	20
Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid)	—	4.3	0.51 (4) ±0.28	Pyrogenic	12
Deoxycholic acid (3α, 12α-dihydroxy-5β-cholan-24-oic acid)	30, >75	3.9	0.16 (4) ±0.12	Nonpyrogenic	12

* No. of rabbits injected.

‡ ±SD.

§ Occasionally pyrogenic after repeated injections.

steroids are listed in roughly descending order of pyrogenicity (see Table I, steroids), as determined by prior injection studies in man (see Table I, column 6). The fourth column, maximum fever, contains the average febrile responses of a group of rabbits to supernatants of human leukocytes incubated with each of these steroids. As shown by the decreasing heights of maximum fever, the capacity of these agents to release pyrogen in vitro correlates quite closely with their ability to produce fever in man.

A concentration of etiocholanolone over 10 μg/ml is essential for pyrogenic activity in vitro (6). Since variations in solubility might also influence the pyrogenicity of these different steroids, we determined their concentration in saturated buffer solution. The second column in Table I lists the concentration of the different steroids in the incubation flasks during these experiments. Although different steroids varied widely in their solubility, pyrogenic activity did not appear to be directly related to solubility in aqueous solution.

Pyrogenic properties of bile acids. Certain bile acids, which have structures similar to those of the steroids discussed above, have been reported to be pyrogenic in man (12). A saturated buffer solution of lithocholic acid was clearly effective in stimulating release of leukocyte pyrogen, whereas deoxycholic acid did not induce pyrogen release (see Table I). Similar pyrogenic properties of these compounds have been reported in vivo (12).

Pyrogenic properties of C-21 steroids. In Table II are presented data on the pyrogenic activity in vitro of a group of C-21 steroids. As in Table I, steroids are listed in roughly descending order of pyrogenicity as determined by previous injection studies in man (see last column for references). The fourth column again shows the average maximum responses of recipient rabbits to supernatants of leukocytes incubated with these steroids. It is clear that correlation with in vivo studies is not as close as with the C-19 steroids. For example, 3α-hydroxy-5β-pregnane-11,20-dione and 5β-pregnane-3α, 20α-diol were not pyrogenic in our system although they have been reported to induce fever in man. Also, 5β-pregnane-3,20-dione, which is only weakly pyrogenic in man was the only potent member of this group in vitro. None of the steroids which have failed to produce fever in man was active in our system.

The concentration of these steroids in buffer solution is shown in the second column of Table II. In the "pyrogenic" group, the first and fourth were poorly soluble and only moderately pyrogenic in our system, whereas the "weakly pyrogenic" 3,20-dione, which was

TABLE II

Pyrogenic Activity of C-21 Steroids

Steroid	Concentration	Human WBC ($\times 10^7$)	Maximum Fever (rabbit)	i.m. injection (man)	References
	($\mu g/ml$)		(°C)		
3α-Hydroxy-5β-pregnan-20-one	8	4.5	0.42 (4) ±0.06*	Pyrogenic	4
3α-Hydroxy-5β-pregnane-11, 20-dione	61	4.5	0.03 (4) ±0.04	Pyrogenic	4, 5
5β-Pregnane-3α, 20α-diol	78	4.5	0.07 (4) ±0.03	Pyrogenic	4, 5
5β-Pregnane-3α, 20β-diol	13	4.8	0.25 (6) ±0.22	?	
5β-Pregnane-3, 20-dione	70	4.6	0.93 (6) ±0.18	Weakly pyrogenic	4
Progesterone‡	26	4.5	0.06 (4) ±0.04	Nonpyrogenic§	8
3α, 17α-Dihydroxy-5β-pregnan-20-one	64	4.7	0.09 (4) ±0.10	Nonpyrogenic	4
3β-Hydroxy-5β-pregnan-20-one	29	4.5	0.14 (6) ±0.10	?	
5α-Pregnane-3, 20-dione	5	4.5	0.05 (4) ±0.12	?	

* ±SD.

‡ 4-Pregnene-3, 20-dione.

§ Sustained small elevations of body temperature with repeated injections.

quite soluble, was very active. On the other hand, the 3α-ol-11,20-dione, 3α,20α-diol, and the 3α, 17α-diol-20-one were all present in good concentration although they were nonpyrogenic. Thus, pyrogenic activity did not correlate with solubility in aqueous solution.

Effect of a nonpyrogenic androgen on pyrogen release. Experiments were done to investigate the possibility that a nonpyrogenic androgen would interfere with the pyrogenic action of etiocholanolone. Dehydroepiandrosterone (DHEA) was chosen because of its good aqueous solubility and lack of pyrogenicity (see Table I). A mixed solution of etiocholanolone and DHEA was therefore prepared by autoclaving a mixture of both steroids, and preparing a buffer solution as usual. In three experiments, this solution caused as much pyrogen release as did etiocholanolone alone. Furthermore, attempts to alter release of pyrogen with DHEA alone were unsuccessful when leukocytes were stimulated by phagocytosis regardless of whether the leukocytes were incubated with DHEA before or after addition of staphylococci.

Inhibition of pyrogen release by hydrocortisone. When cortisone was given to volunteers before an injection of etiocholanolone, experimental steroid fever was suppressed (2). Cortisone also suppresses fevers due to other causes (21, 22). It seemed possible that corticosteroids might suppress pyrogen release in vitro. Experiments were accordingly set up to determine the effect of hydrocortisone on release of pyrogen from human blood leukocytes stimulated by etiocholanolone or by phagocytosis of heat-killed staphylococci. As shown in Fig. 1, a partial suppression of pyrogen release by hydrocortisone was observed in both systems ($P = < 0.01$ in both experimental groups). Concentrations of hydrocortisone of both 12 and 120 μg/ml gave similar results in the etiocholanolone experiments. Since in some early studies (23, 24), but not other subsequent ones (25, 26), corticosteroids were reported to alter phagocytosis, this possibility was considered. Repeated examination of coverslip preparations showed no effect of hydrocortisone on numbers of ingested bacteria.

In another group of experiments, hydrocortisone was added to leukocytes either an hour before the bacteria, or 1 or 2 hr afterwards. As shown in Fig. 2, a significant suppression of pyrogen release was observed only when the leukocytes were incubated with hydrocortisone

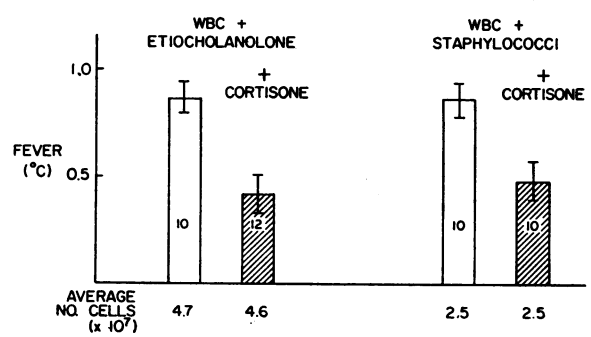

FIGURE 1 Effect of hydrocortisone on release of pyrogen from human blood leukocytes stimulated by etiocholanolone or by phagocytosis of heat-killed staphylococci. In this and following figures, the average maximum height of fever in rabbits after injection of supernatants from 18-hr incubations of leukocytes (WBC) is plotted on the ordinate, numbers of rabbits are shown within bars, and SEM is indicated. In all figures the same rabbits were injected with both control and experimental supernatants. The concentration of hydrocortisone was 120 μg/ml except in four experiments in the left-hand group, when it was 12 μg/ml. Incubation volumes were 7.5–8.2 ml. Hydrocortisone was added at the same time as staphylococci or etiocholanolone.

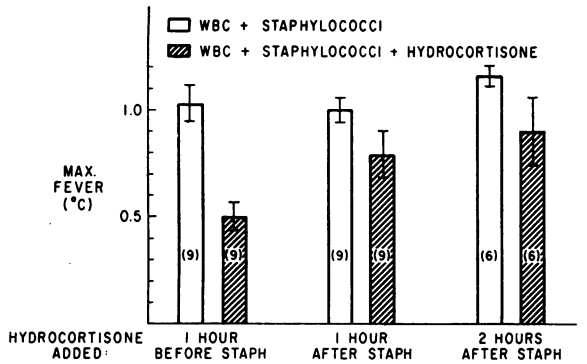

FIGURE 2 Effect of time at which hydrocortisone was added to leukocytes on release of pyrogen after stimulation by phagocytosis of heat-killed staphylococci. The concentration of hydrocortisone was 30 μg/ml.

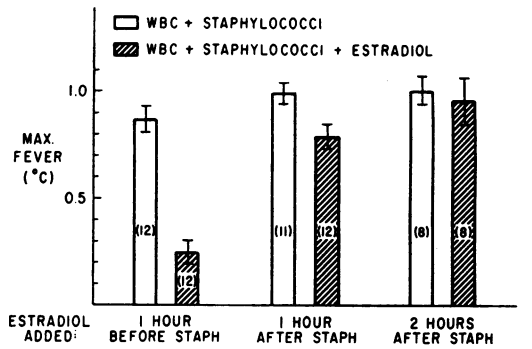

FIGURE 4 Effect of the time at which estradiol was added to leukocytes on release of pyrogen after stimulation by phagocytosis of heat-killed staphylococci. 200 μg of estradiol was added to each flask.

before the initiation of phagocytosis ($P = < 0.001$). The differences were not significant ($P = > 0.1$) in the other two groups.

Effect of estradiol on release of pyrogen. Estrogens have been noted to cause depression of body temperature, as occurs in the preovulatory phase of the menstrual cycle, or after administration of the hormone to subjects (27, 28). Several experiments were carried out to investigate a possible effect of estrogen on pyrogen release from leukocytes in vitro.

Flasks coated with estradiol were prepared as described in Methods. To these were added buffer, with or without etiocholanolone, usually with 15% serum and leukocytes. In such flasks estradiol concentration in buffer averaged 7.6 μg/ml, and in serum-buffer 28.7 μg/ml, irrespective of the presence of etiocholanolone. Leukocytes were incubated with estrogen for 1 hr, and either etiocholanolone or heat-killed staphylococci were then used to stimulate pyrogen release.[3] The results of a group of experiments are presented in Fig. 3. Significant suppression of pyrogen release occurred when estrogen-treated leukocytes were stimulated by phagocytosis, $P = < 0.01$ (see right-hand bars, Fig. 3), whereas only a small suppression was noted when etiocholanolone was used as stimulus (see left-hand bars, Fig. 3).

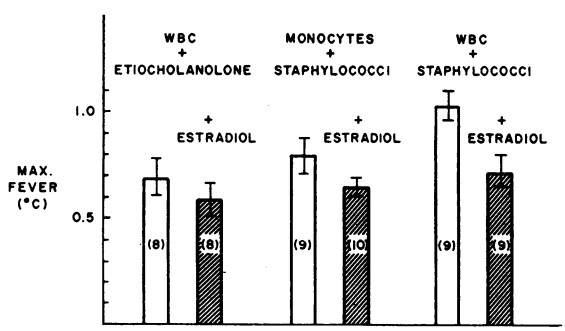

FIGURE 3 Effect of estradiol on release of pyrogen from human blood leukocytes (WBC) or monocytes, stimulated by etiocholanolone or by phagocytosis of heat-killed staphylococci. 200 μg of estradiol was added to each flask, and leukocytes were incubated for 1 hr in volumes of 6.5–8.2 ml before addition of staphylococci or transfer to a serum-buffer solution of etiocholanolone.

Since etiocholanolone stimulates monocytes to release pyrogen (6), it seemed possible that this smaller effect could be explained by a less effective suppression of pyrogen release from monocytes under these experimental conditions. As shown in the third group of experiments in Fig. 3 (see middle bars), suppression of pyrogen release by estradiol from monocytes stimulated by phagocytosis was also slight ($P = > 0.1$) and corresponded closely to values observed with blood leukocytes and etiocholanolone.

In order to investigate the importance of the time of estradiol administration, estradiol was added to leukocytes 1 hr before bacteria, or 1 or 2 hr afterwards. As seen in Fig. 4, significant suppression of pyrogen release occurred only when leukocytes were treated with estradiol before the pyrogenic stimulus was added. These results correspond closely to those obtained with hydrocortisone (see Fig. 2). In other experiments, in which leukocytes were incubated with estradiol for 1 hr, and then washed before addition of bacteria, pyrogen release was not suppressed.

In order to determine what concentration of estrogen was effective, a group of experiments were carried out with 9, 18, and 36 μg/ml estradiol. Increasing suppression of 8, 21, and 30% of control values, respectively, was observed. Thus, only concentrations of estradiol

[3] In studies of phagocytosis not reported here, measurement of disappearance of live staphylococci from flask supernatants indicated that estradiol-treated leukocytes phagocytized the same number of bacteria as normal cells.

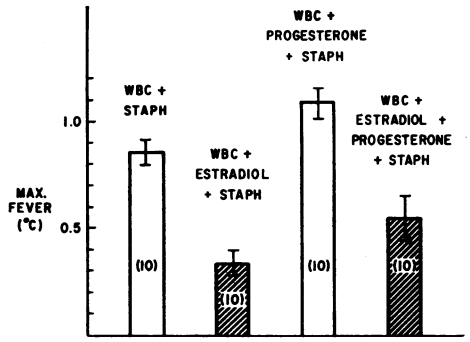

FIGURE 5 Effect of estradiol and progesterone on release of pyrogen from human blood leukocytes after stimulation by phagocytosis of heat-killed staphylococci. 200 μg of each steroid was added to the flasks. Each rabbit received all four injections from one experiment.

equal to or greater than 18 μg/ml effectively suppressed pyrogen release.

Effect of progesterone on release of pyrogen. Progesterone causes slight elevation of basal body temperature (8), but does not stimulate pyrogen release from blood leukocytes in vitro (see Table II). Because progesterone is thought to cause the normal cyclic elevations in body temperature after ovulation in women, it seemed of interest to determine whether progesterone could reverse the suppresion of pyrogen release caused by estradiol in vitro. A group of experiments were carried out in which aliquots of leukocytes were preincubated with estradiol, progesterone, or estradiol and progesterone for 1 hr before heat-killed bacteria were added. Control cells were preincubated in serum-buffer alone, and in some experiments with serum-buffer plus each of the hormones. The results of these experiments are shown in Fig. 5. It is apparent that progesterone partially reversed the suppression of pyrogen release by estradiol ($P = < 0.01$).[4] Progesterone also augmented pyrogen release caused by staphylococci ($P = < 0.001$),[4] although leukocytes incubated with progesterone alone (not shown) did not release pyrogen (see also Table II).

Effect of estrogens and hydrocortisone on pyrogen release from rabbit cells in vivo and in vitro. Since etiocholanolone has not been shown to have pyrogenic effects in animals (29), and does not cause release of pyrogen from rabbit cells in vitro (6), it was of interest to determine whether estrogens would suppress febrile responses in rabbits. Six female rabbits were injected with three different pyrogenic stimuli on 3 successive days, and their temperatures recorded. Endogenous pyrogen, prepared from rabbit blood leukocytes stimulated by phagocytosis, a purified endotoxin derived from *Proteus vulgaris*,[5] and 5×10^8 heat-killed

[4] Calculated by paired *t* test.

[5] E pyrogen, Organon Laboratories, Ltd., England.

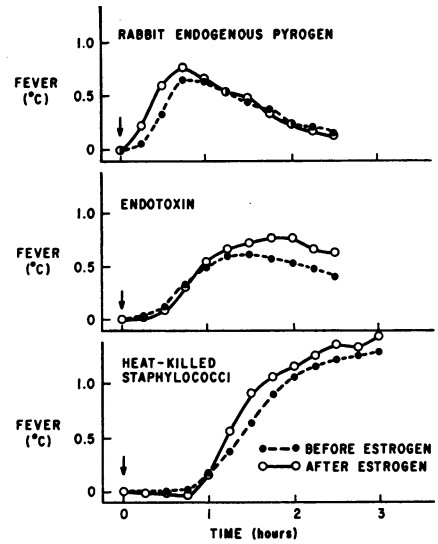

FIGURE 6 Effect of estrogen treatment on the febrile responses of rabbits to intravenous injection of endogenous pyrogen, endotoxin, and heat-killed staphylococci (see text). Average responses of the same six rabbits before and after 6–8 days of injection of premarin, 2 mg/day, are shown for each pyrogenic stimulus.

staphylococci suspended in saline were used as pyrogenic stimuli. Following this initial testing, the rabbits received 8–10 daily intramuscular injections of Premarin,[6] 2 mg per injection. During the last few days of treatment they were challenged again on successive days with the same pyrogens. As shown in Fig. 6, the average temperature responses were not significantly different after the series of injections of estrogens.

Similarly, female rabbit blood leukocytes activated by phagocytosis did not show suppression of release of pyrogen after pretreatment with estradiol in vitro. On the contrary, slightly greater release of pyrogen was con-

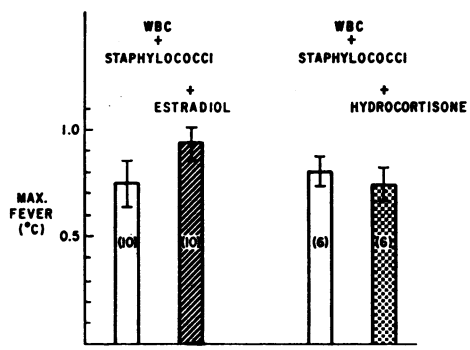

FIGURE 7 Effect of estradiol and hydrocortisone on release of pyrogen from rabbit blood leukocytes after stimulation by phagocytosis of heat-killed staphylococci. 200 μg of estradiol, or hydrocortisone 200 μg/ml, was added to flasks 1 hr before the bacteria.

[6] Conjugated Estrogens, Ayerst Laboratories, New York.

sistently observed in the presence of estradiol, as shown in Fig. 7, left-hand bars. Experiments with blood cells from male rabbits also showed no suppression of pyrogen release in the presence of estradiol. Hydrocortisone also did not have a significant effect on pyrogen release from rabbit blood cells incubated with staphylococci in vitro, although high concentrations of steroid were used (see Fig. 7, right-hand bars). In other experiments with hydrocortisone at 10–30 μg/ml, the concentrations shown to be effective for human cells, again no effect was observed.

Thus, estrogen treatment did not modify febrile responses in rabbits, and no suppression of pyrogen release was observed when estradiol or cortisone-treated rabbit cells were stimulated by phagocytosis in vitro.

DISCUSSION

The discovery that etiocholanolone and some related endogenous steroids produced fever in normal volunteers (1, 2) suggested that certain clinical fevers might be caused by abnormalities of steroid metabolism (30). Recently, incubation of human blood leukocytes with etiocholanolone in vitro was shown to result in release of pyrogen that could be tested in rabbits (6, 7), and this model appeared to have a number of features similar to those reported for experimental steroid fever in man. The results reported here extend these initial observations with etiocholanolone to an examination of the action of other endogenous steroids in this in vitro model.

Previous reports have related structural alterations of steroid configuration with pyrogenic activity in man (8, 10). Our in vitro studies with a group of C-19 steroids showed a close correlation with the earlier in vivo data in man. Substitution of a hydroxy group at the 11 position of etiocholanolone did not diminish pyrogenicity, whereas conversion of the 3α-hydroxy group to a ketone (as in 3,17-etiocholanedione) or a β-hydroxy (as in 3β-OH etiocholanolone) markedly reduced pyrogenic activity. Androsterone, the 5α-analogue of etiocholanolone, was nonpyrogenic. Similarly, studies in man have indicated that the 5β-steric configuration of the A and B rings is essential for pyrogenicity. Results of studies with two bile acids, lithocholic and deoxycholic acid, also correlated well with reports of their pyrogenic activity in vivo.

The experiments with the C-21 steroids showed a partial correlation with in vivo data. The steroid 5β-pregnane-3,20-dione, reported to be weakly pyrogenic after injection, was the most pyrogenic in our in vitro system. Two steroids, 5β-pregnane-3α, 20α-diol and 3α-hydroxy-5β-pregnane-11,20-dione did not release pyrogen in the in vitro system, although they are reportedly pyrogenic when injected. Since both these steroids were present in good concentration, inadequate aqueous solubility does not explain these results. It is possible that conversion of some steroids to a pyrogenic metabolite could normally occur in vivo (31). Leukocytes may lack metabolic pathways required for such conversions. Also, since mononuclear and reticuloendothelial cells are now known to release pyrogen (32–35), blood leucocytes may not be the cells responsible for some "steroid" fevers.

The in vitro system used in these studies requires incubation of blood leukocytes for several hours with high, unphysiologic concentrations of steroids.[7] The mechanism by which certain of these steroids produce cell "activation" under these conditions is unknown, as is the number and type of cells which become stimulated to release pyrogen. Steroids which appeared to be nonpyrogenic could have induced release of small amounts of pyrogen which were not detected by our assay method. Until the factors necessary for initiating and maintaining pyrogen production by cells in vitro and in vivo are better understood, the relation of in vitro studies to clinical fever will remain unclear.

Several studies of the effect of cortisone on experimental fever in rabbits have suggested that the steroid inhibits the usual response of the hypothalamus to endogenous pyrogen (36–38). Another study, however, has reported a diminution in the amount of circulating endogenous pyrogen in cortisone-treated rabbits following administration of endotoxin or virus (39). Blood leukocytes of cortisone-treated rabbits have also been reported to release less pyrogen when incubated in vitro with endotoxin than do the leukocytes of normal rabbits (40). Our inability to demonstrate in vitro effects of hydrocortisone on pyrogen release from normal rabbit cells after a phagocytic stimulus, even when high concentrations of steroid were used, indicate that rabbit and human leukocytes differ in their response to corticosteroids, as they do to pyrogenic steroids such as etiocholanolone (6) and to estrogens (see above).

Estrogens have commonly been associated with lowered body temperature (27, 28), as in the preovulatory phase of the menstrual cycle, although the mechanism of this action is unknown. Etiocholanolone produces less fever in women than in men (15), and estradiol has been reported to diminish fevers in women volunteers caused by injection of etiocholanolone, but not endotoxin (7). The results of in vitro studies reported here, in which estradiol significantly suppressed pyrogen release from human leukocytes activated by phagocytosis, but not etiocholanolone, may be due to differing factors in the incubation system for each activator, such as the cell type activated or the necessary length of exposure to estradiol.

[7] Normal plasma concentration of unconjugated etiocholanolone is about 0.04 μg/100 ml (7).

Repeated progesterone injections result in small, sustained elevations of body temperature in volunteers (8). In our studies, progesterone did not cause spontaneous release of pyrogen from leukocytes. However, progesterone-treated cells released significantly greater amounts of pyrogen than did normal cells after stimulation by phagocytosis. Similar pretreatment of leukocytes with a nonpyrogenic androgen, DHEA, did not alter the release of pyrogen after phagocytosis. When leukocytes were incubated with both progesterone and estradiol before phagocytosis, the inhibition of pyrogen release by estradiol was partially reversed. Competing influences of estrogen and progesterone on body temperature have been noted after administration of hormones in vivo (41).

Hydrocortisone and estradiol were only found to have clear-cut effects on pyrogen suppression when leukocytes were exposed to the steroid before the pyrogenic stimulus was added. Since new RNA and protein synthesis must occur soon after stimulation in order for normal subsequent pyrogen release to occur (42), these hormones may conceivably influence this process. A similar role has been defined for estrogen in other systems (43–46).

Further studies are needed to determine to what extent naturally occurring steroids affect both normal temperature regulation and fever in man. Since various steroids have pyrogen-augmenting and pyrogen-suppressing activity in vitro, however, investigations of the possible contribution of steroids to clinical problems of recurrent fever should in the future include study of a wide variety of steroids.

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