Effects of sex hormones on basophil histamine release in recurrent idiopathic anaphylaxis

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A subset of patients with recurrent anaphylaxis experience ovarian hormone–related exacerbations. Symptoms in several of these women may be provoked by gonadotropins or progesterone (P) and improved by ovarian suppression, with long-term remissions noted in several patients after oophorectomy. Since adverse reactions to P might explain this association, the effects of P and estrogen on basophil histamine release from these patients were studied. Eight patients and 10 control subjects were examined. Neither estrogen nor P caused histamine release from the basophils of patients or control subjects. Moreover, anti-IgE–induced histamine release was not influenced by P or estrogen. Attempts to culture basophils for 24 hours revealed that basophil preparations from eight of 10 normal subjects but only three of eight patients retained the capacity to respond to anti-IgE after 24 hours (p = 0.088). Culture with dexamethasone reduced anti-IgE–induced histamine release in all subjects, and the possibility that P might interfere with the effect of dexamethasone was also studied. P failed to affect dexamethasone-induced reduction of basophil histamine release. Therefore, P and estradiol appear to have no effect on basophils from either patients with hormone-related exacerbations of anaphylaxis or from control subjects. (J ALLERGY CLIN IMMUNOL 1987;80: 285-90.)

The normal human ovarian cycle is driven by a complex series of hormonal interactions involving the hypothalamus, pituitary, and ovaries. LHRH, which is produced in the hypothalamus, stimulates the synthesis and release of the gonadotropins, luteinizing hormone and follicle-stimulating hormone by the anterior pituitary. The ovary, in turn, responds to these pituitary hormones with follicle maturation, ovulation, and E2 production. A short-lived endocrine organ, the corpus luteum, appears on the site of the ruptured follicle and produces P. The cycle is thus divided into two phases, the preovulatory or follicular phase and the postovulatory or luteal phase, each approximately 14 days in duration.

Recurrent idiopathic anaphylaxis is an illness characterized by recurring anaphylactic or anaphylactoid attacks without evidence of an external inciting cause.1-3 Meggs et al.4 have described a patient in whom the attacks appeared to be caused by P secretion. This patient was successfully treated by ovarian suppression with LHRH analogues and, subsequently, by oophorectomy. We have since studied several more female patients with recurrent idiopathic anaphylaxis in a clinical trial of the efficacy of ovarian suppression in reducing the number and severity of attacks. Preliminary results indicate that those subjects whose attacks may be provoked by the intradermal injection of medroxyprogesterone or the infusion of LHRH responded dramatically to LHRH analogue and subsequent oophorectomy.5

Luteal phase exacerbations of allergic diseases have been published for many years.6-10 In addition, studies

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**Abbreviations used**

E2: Estradiol
EDTA: Ethylenediaminetetraacetic acid
HEPES: N-2-hydroxyethylpiperezine-N'-2-ethanesulfonic acid
HEPES-CM: HEPES buffer with calcium and magnesium
LHRH: Luteinizing hormone-releasing hormone
LHRH analogue
P: Progesterone

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TABLE I. Peak histamine release: Immediate and 24 hours

<table>
<thead>
<tr>
<th>Releasers</th>
<th>n</th>
<th>Immediate</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control subjects</td>
<td>+</td>
<td>8</td>
<td>56 ± 4</td>
</tr>
<tr>
<td>-</td>
<td>2</td>
<td>40 ± 10</td>
<td>11 ± 6</td>
</tr>
<tr>
<td>Patients</td>
<td>+</td>
<td>3</td>
<td>59 ± 9</td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>35 ± 6</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

Releaser status indicates whether the subject’s basophil preparations released sufficient histamine after 24 hours to be studied in these experiments (at least 25% of initial release). Release is percent total ± SEM in response to anti-IgE.

of “autoimmune P dermatitis”11,12 and “endocrine allergies”13 have raised the possibility that patients may experience immediate hypersensitivity responses to endogenous P or other hormones associated with the menstrual cycle. An alternative explanation of these phenomena is that these hormones may modulate mast cell reactivity to either antigens or to nonimmunologic mast cell secretagogues such as neuropeptides. Thus, patients with evidence of increased allergic symptoms in response to ovarian hormones may be experiencing mediator release from tissue mast cells as a result of a hormone-induced mast cell degranulation (either direct or possibly IgE mediated) or because of increased responsiveness of the hormone-treated mast cells to other degranulating agents. The absence of immediate skin test reactions to ovarian hormones in these patients reduces the possibility that IgE is directed against these hormones.

P affects responsive cells by binding to a specific intracytoplasmic receptor, stimulating messenger ribonucleic acid synthesis and new protein production.14 P may also, however, bind to the glucocorticoid receptor and has been demonstrated to behave as a glucocorticoid antagonist in some steroid-responsive systems.15,16 Lichtenstein and Osler17 have developed a simple method, based on the observations of Noah and Brand18 and Middleton et al.,19 of preparing and testing blood basophils for histamine release in the presence of allergens or anti-IgE. This technique can be used to study the mechanisms of immediate hypersensitivity.17,20 This article describes our examination of the effects of P and estrogen on basophil histamine release in patients with recurrent idiopathic anaphylaxis.

MATERIAL AND METHODS

Subjects

We studied a total of eight patients with idiopathic anaphylaxis and 10 normal control subjects at the National Institutes of Health Clinical Center. The patients were women aged 15 to 43 years, with recurrent episodes characterized by some or all of the following: flushing, diffuse pruritus, urticaria, laryngeal edema, syncope, abdominal bloating, and wheezing with elevated urinary histamines during attacks.21 After exhaustive evaluation, no external inciting cause for the attacks was found. In each of the patients, symptoms appeared to worsen during the luteal phase of the menstrual cycles. All were skin tested with 40 to 2000 μg of medroxyprogesterone, and three of the eight patients developed positive systemic reactions consistent with anaphylaxis. One patient was being treated with LHRHa at the time that her blood was drawn, but none of the other patients was receiving hormonal therapy of any kind. None of the patients had a positive immediate skin test reaction to medroxyprogesterone, and systemic symptoms developed in the three positive provocations after 20 to 60 minutes. The normal control subjects, aged 22 to 41 years, were male and female employees of the Laboratory of Clinical Investigation and the National Institutes of Health Clinical Center. They reported no medical problems and were not receiving any hormonal medications at the time of study.

Material

P, β-E2, dexamethasone, and HEPES were obtained from Sigma Chemical Co., St. Louis, Mo. Dextran 75 in 0.9% NaCl was obtained from Abbott Laboratories, North Chicago, Ill. RPMI 1640 containing 25 mmol/L of HEPES was purchased from Biofluids, Rockville, Md. Dextrose was the product of J. T. Baker Chemical Co., Phillipsburg, N.J. Ethylenediaminetetraacetic acid was obtained from Fisher Scientific Co., Fair Lawn, N.J. Fetal bovine serum was heat inactivated, mycoplasma tested, virus screened, and purchased from Gibco Laboratories, Chagrin Falls, Ohio. HEPES buffer contained HEPES, 20 mmol/L; NaCl, 137 mmol/L; KCl, 2.7 mmol/L; and glucose, 10 mmol/L; and was titrated to pH 7.2 with concentrated NaOH. HEPES-CM contained, in addition to the above, CaCl2, 1.8 mmol/L, and MgCl2, 1 mmol/L. Polyclonal rabbit antihuman IgE was prepared in our laboratory.

Leukocyte preparation

Leukocytes were prepared according to the method described by Lichtenstein and Osler. They were prepared by the method described by Lichtenstein and Osler. Venous blood was drawn and added immediately to a filtered solution containing dextran, ethylenediaminetetraacetic acid, and dextrose. Erythrocytes were allowed to sediment between 60
TABLE II. Age and sex of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Average age ± SEM</th>
<th>Range</th>
<th>M/F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>30 ± 1.5</td>
<td>22-41</td>
<td>4/6</td>
</tr>
<tr>
<td>Releasers</td>
<td>31 ± 1.5</td>
<td>26-41</td>
<td>4/4</td>
</tr>
<tr>
<td>Nonreleasers</td>
<td>28 ± 3.9</td>
<td>22-33</td>
<td>0/2</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>34 ± 3.1</td>
<td>15-43</td>
<td>0/8</td>
</tr>
<tr>
<td>Releasers</td>
<td>30 ± 6.1</td>
<td>15-39</td>
<td>0/3</td>
</tr>
<tr>
<td>Nonreleasers</td>
<td>37 ± 2.7</td>
<td>26-43</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Releaser status refers to basophil histamine release at 24 hours, as in Table I.

TABLE III. Effect of incubating basophils with P, E2, or dexamethasone for 24 hours on histamine content

<table>
<thead>
<tr>
<th></th>
<th>P (%) reduction histamine content ± SEM</th>
<th>E2 (%) reduction histamine content ± SEM</th>
<th>Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>-6 ± 5 (7)</td>
<td>-9 ± 3 (2)</td>
<td>-5 ± 2 (7)</td>
</tr>
<tr>
<td>Patients</td>
<td>-2 ± 2 (7)</td>
<td>-27 ± 11 (2)</td>
<td>-5 ± 6 (7)</td>
</tr>
<tr>
<td>All</td>
<td>-4 ± 3 (14)</td>
<td>-18 ± 7 (4)</td>
<td>-5 ± 3 (14)</td>
</tr>
</tbody>
</table>

Number for each group is in parentheses.

and 90 minutes, the supernatant was collected and centrifuged at 100 × g at room temperature for 8 minutes, and the pellet was washed twice in HEPES buffer.

Histamine release experiments

Immediate release experiments were performed by suspending the pelleted cells in HEPES-CM buffer and adding 0.8 ml of the cell suspension to 0.2 ml of HEPES-CM or buffer containing between 1:200 and 1:6000 dilutions of anti-IgE (final concentrations, 1:1000 to 1:30,000). Cells were then incubated for 40 minutes at 37°C and the cells were then centrifuged. The supernatants were decanted and added to 0.2 ml 18% perchloric acid (final concentration, 3%). Total histamine content was determined by addition of 0.2 ml of 18% perchloric acid to the cell pellet.

Steroid incubations

Six- or 24-hour incubations were performed by suspending the washed cells in HEPES-buffered RPMI 1640 containing 7% fetal bovine serum, L-glutamine, 2 mmol/L; penicillin, 100 U/ml; streptomycin, 100 µg/ml; and amphotericin B, 0.25 µg/ml. P, β-E2, or dexamethasone was dissolved in ethanol and added to the cell suspension with a final ethanol concentration of 0.2% (v/v). Control cells were exposed to 0.2% ethanol as well. Incubations were performed at 37°C in 5% CO₂. After the incubations, the suspensions were centrifuged at 100 × g for 8 minutes, and the cells were washed twice in HEPES and resuspended in HEPES-CM for challenge as indicated above.

Histamine assay

Histamine release was measured according to the automated fluorometric method described by Siraganian.22 Results are expressed as percent of total histamine content as determined by perchlorate lysis.

Statistics

Student’s t test or Fisher’s exact test was used where it was appropriate.

RESULTS

Basophil histamine release

The basophil preparations of all the patients and normal subjects included in this study released between 21% and 81% (mean ± SEM = 49 ± 4%) of the total histamine content when these were incubated with 1:30,000 to 1:1000 dilutions of anti-IgE. There was no significant difference between release in the patient and in normal subjects. After 24 hours of incubation, eight of the 10 normal subjects, but only three of eight patients, still released histamine at acceptable levels for inclusion in this study, at least 25% of the initial release. The difference between the two groups is not statistically significant (p = 0.088) by Fisher’s exact test (Table I), nor is there any obvious relationship between release after 24 hours and the ages of the subjects tested (Table II). The only dis-
TABLE IV. The effect of 6- or 24-hour incubations with E2 and progesterone

<table>
<thead>
<tr>
<th>Control (6 hr, n = 5)</th>
<th>E2 (24 hr, n = 4)</th>
<th>Control (24 hr, n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-IgE (dilution)</td>
<td>Control E2 (24 hr, n = 9)</td>
<td></td>
</tr>
<tr>
<td>1:1,000</td>
<td>30 ± 8</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>1:3,000</td>
<td>45 ± 9</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>1:10,000</td>
<td>42 ± 9</td>
<td>26 ± 9</td>
</tr>
<tr>
<td>1:30,000</td>
<td>—</td>
<td>21 ± 2</td>
</tr>
</tbody>
</table>

The effect of 6- or 24-hour incubations with E2, 2 × 10⁻⁸ mol/L, and 24-hour incubations with P, 10⁻⁷ mol/L, on histamine release. Data are pooled from patients and normal subjects.

The distinguishing characteristic of those subjects with loss of histamine-releasing capacity after 24 hours was a trend toward lower initial histamine release. Although none of the three patients whose basophils could be studied at 24 hours had a systemic reaction to injected medroxyprogesterone, all had experienced worsening of their symptoms in the luteal phase of the menstrual cycle, and one had a systemic reaction after challenge with LHRH.

Changes in histamine content

The total histamine content of the basophil preparations after 24-hour incubations with P, 10⁻⁷ mol/L; E2, 2 × 10⁻⁸ mol/L; and dexamethasone, 10⁻⁷ mol/L; was examined in patients and normal subjects. Although the general trend was to a small decrease in histamine content, no statistically significant differences between patients and normal subjects were observed (Table III).

Incubation with E2 and P

The short-term exposure of human basophils to P, 10⁻⁷ mol/L, or E2, 2 × 10⁻⁸ mol/L, alone for up to 4 hours, failed to cause histamine release, and the presence of these agents failed to affect anti-IgE-induced histamine release as well (data not presented). Incubation of leukocytes for 24 hours in the presence of P, or for 6 and 24 hours in the presence of E2, had no significant effect on histamine release in either patients or normal subjects (Table IV).

Incubation with dexamethasone

As reported by Schleimer et al., the incubation of basophils with 10⁻⁸ or 10⁻⁷ mol/L of dexamethasone for 24 hours leads to a significant decrease in histamine release to all concentrations of anti-IgE (Fig. 1). This observation was equally true for both patients and normal subjects. The addition of P, 10⁻⁷ mol/L, to dexamethasone, 10⁻⁷ mol/L, had no statistically significant effect on the reduction of histamine release by dexamethasone at 24 hours in either patients or normal subjects (Fig. 2).

DISCUSSION

Recurrent “idiopathic” anaphylaxis may be associated with luteal phase P secretion in some women and in addition, there are multiple studies of luteal phase exacerbations of several allergic diseases. One of the possible mechanisms by which sex hormones might affect allergic diseases is by modulating normal regulatory influences that control mediator release. There is evidence along these lines that P may bind to the glucocorticoid receptor and thereby inhibit glucocorticoid action in some systems. The observation that basophil histamine release may be inhibited by prolonged in vitro exposure to glucocorticoids suggested the possibility that P might increase basophil histamine release from patients with suspected P sensitivity by this mechanism. We therefore studied the effect of dexamethasone, E2, and P incubations on basophil histamine release in patients with recurrent idiopathic anaphylaxis and normal control subjects.

Basophil histamine release after 24 hours in culture could only be studied in three of eight patients with idiopathic anaphylaxis compared to eight of 10 normal subjects. Although this difference is not statistically significant (p = 0.088 by Fisher’s exact test), it suggests the possibility that the patients may have some underlying differences in the basophils, perhaps causing a more rapid turnover rate or a shorter half-life in vivo. Neither the age of the donor nor sex differences between the groups can account for this disparity. The only obvious association was a lower initial histamine release in the seven subjects whose basophils did not release after 24 hours.

Exposure of basophils to P, E2, or dexamethasone
Effects of sex hormones on basophil histamine release

alone for up to 24 hours failed to either cause histamine release or affect the histamine content of the cells. Neither P nor E2 affected anti-IgE-stimulated histamine release in patients or control subjects. Therefore, there was no evidence for either sex hormone to have a direct or indirect influence on basophil histamine release.

Incubation with dexamethasone reduced histamine release in basophil preparations from both patients and normal subjects; both groups of subjects were affected equally. Culture of basophils with dexamethasone in the presence of P had no effect on the dexamethasone-induced inhibition of histamine release in either the normal subjects or patients with idiopathic anaphylaxis. Therefore, it appears that P does not compete for binding to the corticosteroid receptor in human basophils, and it is unlikely that P elicits anaphylaxis in these patients by this mechanism.

We conclude that there appears to be no abnormality of histamine release in P-treated basophils from patients with recurrent idiopathic anaphylaxis whose symptoms exacerbate in the luteal phase of their menstrual cycles. Unfortunately, the three patients whose cells retained the ability to release histamine after 24 hours of culture did not develop systemic reactions in response to injected medroxyprogesterone. However, the anaphylactoid reactions elicited by medroxyprogesterone occur within 30 to 45 minutes and are therefore more immediate reactions to the hormone than the modulatory effect sought by the experiments involving 24-hour incubations. Shorter exposures of these patients' basophils to P failed to elicit any effect on histamine release.

The failure to demonstrate any effect of P or E2 on basophil histamine release, histamine content, and dexamethasone inhibition may indicate that other hormones are involved or that basophils may be an inappropriate target tissue. The tissue mast cell is more likely to be the responding cell type in patients with recurrent anaphylaxis but unfortunately is not readily accessible for in vitro examination. Skin testing of subjects with medroxyprogesterone fails to cause the classic wheal-and-flare response, suggesting that skin mast cells in these patients do not have IgE anti-P antibodies. Systemic reactions elicited by skin testing do not involve the local test site. However, clinical observations suggest that sex hormones do influence mast cell–related diseases such as asthma and urticaria.6-10 Notwithstanding these limitations, it appears that neither P nor E2 triggers histamine release from basophils obtained from subjects with idiopathic anaphylaxis, nor do they affect dexamethasone suppression of basophil histamine release.

REFERENCES

3. Sonin L, Grammer LC, Greenberger PA, Paterson R. Idio-