Estrogen increases the severity of anaphylaxis in female mice through enhanced endothelial nitric oxide synthase expression and nitric oxide production

Valerie Hox, MD, PhD, Avanti Desai, MSc, Geethani Bandara, PhD, Alasdair M. Gilfillan, PhD, Dean D. Metcalfe, MD,* and Ana Olivera, PhD* *Bethesda, Md*

Background: Clinical observations suggest that anaphylaxis is more common in adult women compared with adult men, although the mechanistic basis for this sex bias is not well understood.
Objectives: We sought to document sex-dependent differences in a mouse model of anaphylaxis and explore the role of female sex hormones and the mechanisms responsible.
Methods: Passive systemic anaphylaxis was induced in female and male mice by using histamine, as well as IgE or IgG receptor aggregation. Anaphylaxis was assessed by monitoring body temperature, release of mast cell mediators and/or hematocrit, and lung weight as a measure of vascular permeability. A combination of ovariectomy, estrogen receptor antagonism, and estrogen administration techniques were used to establish estrogen involvement.
Results: Anaphylactic responses were more pronounced in female than male mice. The enhanced severity of anaphylaxis in female mice was eliminated after pretreatment with an estrogen receptor antagonist or ovariectomy but restored after administration of estradiol in ovariectomized mice, demonstrating that the sex-specific differences are due to the female steroid estradiol. Estrogen did not affect mast cell responsiveness or anaphylaxis onset. Instead, it increased tissue expression of endothelial nitric oxide synthase (eNOS). Blockage of NOS activity with the inhibitor L-NG-nitroarginine methyl ester or genetic eNOS deficiency abolished the sex-related differences.
Conclusion: Our study defines a contribution of estrogen through its regulation of eNOS expression and nitric oxide production to vascular hyperpermeability and intensified anaphylactic responses in female mice, providing additional mechanistic insights into risk factors and possible implications for clinical management in the further exploration of human anaphylaxis. (J Allergy Clin Immunol 2015;135:729-36.)

Key words: *Anaphylaxis, estrogen, nitric oxide synthase, vascular permeability, mast cells*

A number of clinical studies have indicated sex differences in the incidence of systemic anaphylaxis,[1,2] showing that adult women have more frequent anaphylaxis induced by food,[3,4] drugs,[2,3,5] and radiocontrast agents,[6] as well as idiopathic anaphylaxis,[7] compared with adult men. The fact that this sex difference is observed during the reproductive but not the prepubertal years[3] suggests that sex hormones might be involved in this sexual dimorphism. Additionally, clinical reports of catamenial (or cyclical) anaphylaxis, which is characterized by recurrent episodes of anaphylactic reactions occurring around the time of menstruation,[8] suggest that female sex hormones, such as estrogens or progesterone, might be involved in susceptibility to anaphylaxis. However, other than in selected cases of suggested autoimmune progesterone sensitivity,[9,10] there is little insight into how sex hormones influence anaphylaxis.

Anaphylaxis is usually triggered by an antigen that recognizes and aggregates antigen-specific IgE bound to FcεRI, the high-affinity receptor of IgE, in tissue-resident mast cells of sensitized subjects. Binding of antigen to FcεRI leads to the release and generation of various mediators, including proteases, lipid-derived molecules, cytokines, and histamine.[11] These mediators act on the surrounding tissues, leading to a number of biologic effects, including vasodilation, plasma exudation, and edema, causing anaphylaxis in the most severe cases. In experimental mouse models anaphylaxis can also be elicited through IgE-independent mechanisms, including activation of IgG receptors (FcγR) present on mast cells, basophils, neutrophils, and macrophages.[12-14] Anaphylaxis in human subjects can also follow the administration of drugs or radiocontrast agents, the mechanisms of which are not fully understood but are believed to be independent from FcεR.

Here we explore sex differences in the severity of anaphylaxis using a well-established mouse model of passive systemic anaphylaxis (PSA) and demonstrate an enhanced severity and duration of anaphylaxis in female compared with male mice. The advantage of using this anaphylaxis model in male and female mice is that the differences in severity and duration at given concentrations of IgE and antigen can be attributed to sex and not

From the Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health.
*These authors contributed equally to this work.
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Corresponding author: Valerie Hox, MD, PhD, Mast Cell Biology Section, Laboratory of Allergic Diseases, NIAID, NIH, Bldg 10, Rm 11C209, 10 Center Dr MSC-1881, Bethesda, MD 20892-1881. E-mail: valeriehox@gmail.com.
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Abbreviations used
BMMC: Bone marrow–derived mast cell
DMSO: Dimethyl sulfoxide
DNP: Dinitrophenyl
E2: 17β-Estradiol
eNOS: Endothelial nitric oxide synthase
ER: Estrogen receptor
HSA: Human serum albumin
L-NAME: L-NG-nitroarginine methyl ester
MCPT-1: Mast cell protease 1
MLEC: Mouse lung endothelial cell
NIH: National Institutes of Health
NO: Nitric oxide
PAEC: Pulmonary artery endothelial cell
PAF: Platelet-activating factor
PSA: Passive systemic anaphylaxis

to confounding factors, including previous antigen exposure, type of antigen, antigen-specific IgE levels, underlying disease, and treatments, that might affect severity and lethality in human subjects.[15] Differences in anaphylaxis severity found in this experimental model relate to incidence data only in that more severe reactions are more likely to be recognized and thus disproportionately contribute to the calculation of incidence. We find that increased severity of anaphylaxis in female mice is dependent on estradiol-mediated upregulation of endothelial nitric oxide synthase (eNOS), the enzyme responsible for production of nitric oxide (NO). The mechanisms that underlie human anaphylactic shock are not well understood in part because of the difficulties in designing appropriate clinical studies. However, we are hopeful that the insights from the mouse studies reported in this work will stimulate further research into sex differences and in the role of NO as a biologic mediator in patients with severe systemic allergic reactions.

METHODS

Further details can be found in the Methods section in this article's Online Repository at www.jacionline.org.

Mice

Wild-type C57Bl/6, BALB/c, and *NOS3*$^{-/-}$ mice, as well as ovariectomized and sham-operated mice, were obtained from the Jackson Laboratory (Bar Harbor, Me). Mice were used in accordance with National Institutes of Health (NIH) guidelines and an animal study proposal (LAD 2E) approved by the NIH/National Institute of Allergy and Infectious Diseases Institutional Animal Care and Use Committee.

Systemic anaphylaxis

For IgE-induced PSA, mice were sensitized with 3 μg of dinitrophenyl (DNP)–specific IgE and challenged 24 hours later with 200 μg of antigen administered intravenously (DNP–human serum albumin [DNP-HSA]; Sigma-Aldrich, St Louis, Mo) in PBS. Anaphylactic responses were determined by measuring core body temperature. Alternatively, anaphylaxis was induced by a bolus of histamine dihydrochloride (5 μmol, Sigma Aldrich) or by 80 μg of anti-mouse CD16/CD32 2.4G2 clone in PBS administered intravenously. Mice were injected intravenously with 2.5 mg of L-NG-nitroarginine methyl ester (L-NAME; Sigma-Aldrich) in 100 μL of PBS 1 hour before DNP challenge to investigate the involvement of NOS.

In some experiments mice were killed 90 seconds after the induction of anaphylaxis, and blood was collected. Plasma histamine and mast cell protease 1 (MCPT-1) levels were measured with specific ELISA kits (Beckman Coulter, Fullerton, Calif, and eBioscience, San Diego, Calif, respectively).

To determine hematocrit levels, blood was collected before and 2 hours after anaphylaxis with microhematocrit tubes (Jorvet, Loveland, Colo), and the ratio of red blood cell volume compared with total volume of blood was determined. For determination of lung fluid, left lung lobes were excised 2 hours after antigen injection. Excised lungs were weighed immediately (wet weight), as well as after drying at 55°C overnight (dry weight), and the wet/dry weight ratio was calculated. The right lung lobes were excised, fixed, and embedded in paraffin. Histologic sections of the paraffin-embedded lungs were prepared and stained with hematoxylin and eosin (American Histolabs, Gaithersburg, Md).

Estrogen receptor blockage and estradiol administration in female mice

Mice received 5 injections with 20 mg/kg of the estrogen receptor (ER) antagonist ICI182,780 (Tocris, Ellisville, Mo) to block estrogen binding to the ER.

To restore estrogen levels in ovariectomized female mice, slow-releasing pellets (Innovative Research of America, Sarasota, Fla) containing either 17β-estradiol (E2; 0.1 mg per pellet, 21-day release) or placebo (0.1 mg per pellet, 21-day release) were implanted, and PSA was performed 2 weeks after implantation.

Statistics

All data are presented as means with SEMs or as box plots with minimum/maximum ranges. Comparisons of temperature changes between groups were performed by using 2-way ANOVA. Differences between 2 variables were compared with the 2-tailed Student *t* test and between multiple variables with 1-way ANOVA (GraphPad Prism 4.01; GraphPad Software, San Diego, Calif). A *P* value of less than .05 was considered significant.

RESULTS

Sex differences in systemic anaphylaxis are estrogen dependent

Resembling epidemiologic studies on systemic anaphylaxis, IgE-mediated PSA was more severe in female C57Bl/6 mice than in male mice (Fig 1, *A*), as determined by a decrease in core body temperature, which correlates with increases in plasma histamine and hematocrit values, hypotension, and visible behavioral changes.[16] The differential severity between age-matched male and female mice was also observed in BALB/c mice (see Fig E1, *A*, left panel, in this article's Online Repository at www.jacionline.org) and was unrelated to differences in body weight because a similar difference in PSA was found in weight-matched instead of age-matched female and male mice (see Fig E1, *B*).

We next explored the basis for the sex disparity. Ablation of the major source of sex hormones in female mice by using ovariectomy resulted in a reduced PSA response compared with that seen in sham-operated female mice and appeared similar to that seen in male mice (Fig 1, *B*, and see Fig E1, *B*, right panel). We then focused on E2, which is the predominant circulating estrogen in females the reproductive years.[17] Subcutaneous implantation of estradiol-releasing pellets into ovariectomized mice restored the presence of the hormone in circulation (see Fig E1, *C*), as well as the severity of the anaphylactic response (Fig 1, *C*), demonstrating a direct link between the presence of the female hormone estradiol and the sex disparities in anaphylaxis. Furthermore, there was a linear correlation between the circulating levels of estradiol and anaphylaxis severity and duration in female mice (see Fig E1, *D*).