Environmental Estrogens Induce Mast Cell Degranulation and Enhance IgE-Mediated Release of Allergic Mediators

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BACKGROUND: Prevalence and morbidity of allergic diseases have increased over the last decades. Based on the recently recognized differences in asthma prevalence between the sexes, we have examined the effect of endogenous estrogens on a key element of the allergic response. Some lipophilic pollutants have estrogen-like activities and are termed environmental estrogens. These pollutants tend to degrade slowly in the environment and bioaccumulate and bioconcentrate in the food chain; they also have long biological half-lives.

OBJECTIVES: Our goal in this study was to identify possible pathogenic roles for environmental estrogens in the development of allergic diseases.

METHODS: We screened a number of environmental estrogens for their ability to modulate the release of allergic mediators from mast cells. We incubated a human mast cell line and primary mast cell cultures derived from bone marrow of wild type and estrogen receptor α (ER-α)–deficient mice with environmental estrogens with and without estradiol or IgE and allergens. We assessed degranulation of mast cells by quantifying the release of β-hexosaminidase.

RESULTS: All of the environmental estrogens tested caused rapid, dose-related release of β-hexosaminidase from mast cells and enhanced IgE-mediated release. The combination of physiologic concentrations of 17β-estradiol and several concentrations of environmental estrogens had additive effects on mast cell degranulation. Comparison of bone marrow mast cells from ER-α–sufficient and ER-α–deficient mice indicated that much of the effect of environmental estrogens was mediated by ER-α.

CONCLUSIONS: Our findings suggest that estrogenic environmental pollutants might promote allergic diseases by inducing and enhancing mast cell degranulation by physiologic estrogens and exposure to allergens.

KEY WORDS: allergy, asthma, β-hexosaminidase, environmental estrogen, estradiol, estrogen receptor α, IgE, mast cells. Environ Health Perspect 115:48–52 (2007). doi:10.1289/ehp.9378 available via http://dx.doi.org/ [Online 3 October 2006]
Environmental estrogens enhance mast cell degranulation

Estrogens. We obtained 17β-estradiol from Sigma-Aldrich Corporation (St. Louis, MO). We used the following environmental estrogens in our studies: organochloride pesticides or their metabolites (endosulfan, dieldrin, and DDE); a by-product of plastics manufacturing (nonylphenol); and the PCBs Aroclor 1242 and Aroclor 1254. We obtained DDE and endosulfan from Ultra Scientific (North Kingstown, RI) and nonylphenol, dieldrin, Aroclor 1242, and Aroclor 1254 from Sigma.

*Effects of environmental estrogens (EEs) on IgE-dependent degranulation from HMC-1 cells and basophil granule contents (Dastych et al. 1999). We stimulated cells (2 × 10^4) in Tyrode’s buffer (Dastych et al. 1999) containing various concentrations of E2. We measured β-hex release as previously described (Dastych et al. 1999), using p-nitrophenyl-N-acetyl-β-D-glucopyranoside (8 mM; Sigma-Aldrich) as the substrate. We expressed the amount of β-hex release into media as the percentage of the total amount of β-hex originally in the cells [% release = 100 × (experimental β-hex release – spontaneous β-hex release + total cellular β-hex)].

Statistical analyses. Data were expressed as the mean ± SE. Statistical analysis was performed by one-way analysis of variance. Where differences between groups were present, they were further analyzed by the multiple comparisons (Bonferroni) for Figure 1 and Student t-test for Figures 2–5. A p-value of <0.05 was defined as statistically significant.

Results

Environmental estrogens induce degranulation of HMC-1 cells. We performed a series of experiments to screen for the effects of various concentrations (1 × 10^{-12}–10^{-8} M) of E2 and six different environmental estrogens on mast

Figure 1. Release of β-hex from HMC-1 after incubation with six different environmental estrogens (EEs): E2, dieldrin, endosulfan, DDE, nonylphenol, Aroclor 1254, and Aroclor 1242. Experiments were conducted in triplicate and expressed as mean ± SE. *p < 0.05 vs. phosphate buffered saline control.

Figure 2. Additive effects of environmental estrogens (EEs) and E2 on β-hex release from HMC-1 cells incubated with 10^{-11} M E2, dieldrin, endosulfan, DDE, nonylphenol, Aroclor 1254, or Aroclor 1242 alone; or each EE plus E2. Experiments were conducted in triplicate and expressed as mean ± SE. *p < 0.05 compared with EE alone.

Figure 3. Effects of environmental estrogens (EEs) on IgE-dependent degranulation from HMC-1 cells and BMMC. (A) Release of β-hex from HMC-1 cells by 0.75 AU/DM alone; 10 pM of dieldrin, endosulfan, DDE, nonylphenol, Aroclor 1254, or Aroclor 1242 alone; or each EE plus DM. (B) Release of β-hex from BMMC by anti-DNP IgE/DNP-BSA alone; 10^{-12} M dieldrin, 10^{-12} M endosulfan, 10^{-11} M DDE, 10^{-11} M nonylphenol, 10^{-9} M Aroclor 1254, or 10^{-9} M Aroclor1242 alone; or each EE plus anti-DNP IgE/DNP-BSA. Experiments were conducted in triplicate and expressed as mean ± SE. *p < 0.05 compared with EE alone.
cell degranulation, using release of β-hex from
HMC-1 cells as a marker for degranulation and
release of allergic mediators. Figure 1 shows that all of the environmental estrogens tested except Aroclor 1254 caused the release of a significant portion of intracellular β-hex at concentrations ranging from $10^{-11}$ to $10^{-8}$ M after 30 min of stimulation. For comparison, a Ca$^{2+}$ ionophore induced approximately 30%
release of intracellular β-hex (data not shown), presumably because not all β-hex resides in releasable granules. Therefore the environmental estrogens alone released up to 50% of the releasable granular contents.

**Combined effects of E$_2$ and environmental estrogens on degranulation of HMC-1 cells.** To analyze the effect of combinations of endogenous estrogen with environmental estrogens, we incubated HMC-1 cells with combinations of suboptimal concentrations of E$_2$ ($1 \times 10^{-11}$ M) and varying concentrations of all six estrogenic compounds. We used suboptimal concentrations to test for additive effects, because the release of β-hex from cells incubated with an optimal dose of the estrogenic compounds was not significantly increased by other estrogens (data not shown).

Figure 2 shows that these combinations of estrogenic compounds induced degranulation more effectively than either of the compounds alone at these concentrations. The resulting stimulations were approximately additive and again were fairly rapid (< 30 min).

Environmental estrogens enhance IgE-mediated degranulation of HMC-1 cells and BMMC. We found that, like E$_2$, low concentrations of environmental estrogens caused a rapid, partial
degranulation using our responsive cell systems, which were sensitized with IgE antibodies from the appropriate species. When HMC-1 cells sensitized with human IgE were subsequently exposed to combination of DM allergen and $10^{-13}$–$10^{-9}$ M environmental estrogens, the release of β-hex was significantly enhanced compared to cells exposed to the same concentration of DM allergen alone (Figure 3A). This was the case for all of the environmental estrogens tested.

We also tested the effects of environmental estrogens on IgE-induced degranulation of primary cultures of BMMCs. We sensitized BMMCs with monoclonal IgE anti-DNP antibodies and stimulated them with DNP-BSA in the presence of $10^{-12}$–$10^{-9}$ M concentrations of our six test environmental estrogens. Each of these environmental estrogens, except nonylphenol, significantly enhanced the β-hex release induced by DM (Figure 3B). We assessed the dose–response relationship for one of these environmental estrogens (Aroclor 1242) to define the concentrations that had the strongest additive effects on IgE-mediated degranulation and the shape of the dose–response curve. Concentrations of Aroclor 1242 of $10^{-14}$–$10^{-12}$ M significantly enhanced the effect of IgE cross-linking, whereas higher concentrations of Aroclor 1242 also appeared to increase the response, but not to significant levels (Figure 4).

**ERα is required for β-hex release induced by some concentrations of environmental estrogens.** To determine which types of ERs were involved in the degranulation of mast cells by environmental estrogens, we performed a dose–response analysis on BMMCs derived from WT versus ER-α KO mice. Figure 5 indicates that some concentrations of environmental estrogens induce significantly more degranulation of mast cells from the WT compared with the ER-α KO mice (Figure 5).

However, the degranulation response to some concentrations of environmental estrogens was not significantly reduced by the absence of ER-α expression. In fact, many of the concentrations of environmental estrogens alone cause significant degranulation of ER-α-deficient mast cells. This is in contrast to the effects of E$_2$, which seems to require ER-α, because E$_2$ did not induce significant degranulation from BMMC derived from ER-α KO mice (Zaitsu et al. 2006).

**Discussion**

In this study, we examined the effects of environmental estrogens—alone and in combination with physiologic concentrations of E$_2$—on the activation of a human mast cell line and primary cultures of murine mast cells. We found that, like E$_2$, low concentrations of environmental estrogens caused a rapid, partial
degranulation of mast cells. The range of en-
vironmental estrogen concentrations that
induced β-hex release was somewhat broader
for environmental estrogens (10⁻⁸–10⁻¹²) com-
pared to that of E₂ (10⁻¹⁰–10⁻¹¹) (Zaitsu et al.
2006). However, the dose–response curves for
the environmental estrogens were similar to
that for E₂, in that they are biphasic (inverted
U-shaped) curves. This type of response is also
typical for other steroid-induced responses
(Watson et al. 1999; Welshons et al. 2003).
Exposing HMC-1 cells to a combination of
suboptimal concentrations of E₂ and an en-
vironmental estrogen had an additive effect on
degranulation. Environmental estrogens also
enhanced the release of β-hex induced by aller-
gen cross-linking of IgE on the surface of these
cells. However, when these mast cells were
incubated with an optimal dose of environ-
mental estrogens, the addition of E₂ did not
enhance the effects of the environmentalestro-
gen alone (data not shown). Finally, BMMCs
deficient in ER-α expression had significantly
reduced responses to some concentrations of
environmental estrogens, suggesting that at
least part of the degranulating activity of en-
vironmental estrogens on mast cells is mediated
through ER-α.

These findings taken together suggest that
the mechanisms of activation of mast cells by
environmental estrogens are similar to those
of the endogenous estrogen E₂. Key charac-
teristics of that response are high sensitivity
and rapid onset (minutes), partial degranula-
tion, biphasic dose response, requirements for
ER-α and extracellular Ca²⁺, and additivity or
synergy with IgE cross-linking (Zaitsu et al.
2006). Many of these characteristics are also
consistent with those described for activation of
the nongenomic (membrane) form of ER-α (Watson et al. 1999; Watson and Gametchu 2003). However, some of the en-
vironmental estrogens had residual activity at
some concentrations in ER-α KO mast cells.
These might be due to compound-specific
binding to truncated ER-α in the KO cells
(Kos et al. 2002) or to nonclassical ERs, such as
the newly described estrogen-binding protein
GPR30 (Bologa et al. 2006; Thomas et al.
2005), or other unrecognized receptors.

For instance, we previously described low-
dose and rapid effects of environmentalestro-
gen via a membrane-resident ER-α in
pituitary tumor cells (Bulayeva and Watson
2004; Wozniak et al. 2005). In that model,
environmental estrogens in nanomolar (parts
per billion) to picomolar (parts per trillion)
concentrations induced extracellular-regulated
kinase-1 (ERK-1) and ERK-2 activation via
ER-α and Ca²⁺ elevations, leading to rapid
prolactin secretion. We have not studied the
effects of environmental estrogens on these
specific signaling pathways of mast cells, but
our recent data suggests that intracellular
Ca²⁺ levels rise within 1 min of exposure to
E₂ (Zaitsu et al. 2006).

In the present study, we chose endogenous
and environmental estrogen concentrations
that would mimic tissue levels that occur in
individuals after typical environmental expo-
sures (Ayotte et al. 2003; Ibarlueza et al. 2004;
Metcalfe et al. 2001; Solomon and Weiss
2002; Vartiainen et al. 1997; Wang et al.
2004). However, an additional concern is that
most environmental estrogens are present in
the environment and in tissues and fat stores in
combinations, because of their long half-lives
and co-prevalence in the environment. Our
demonstrations of additive effects between
environmental and endogenous estrogens are
the first steps toward understanding exposure
to complex mixtures of estrogenic compounds.
The results of these experiments are consistent
with the hypothesis that the effects of both
xenestrogens and physiologic estrogens together will determine the estrogenic impact
on an individual. This estrogenic impact is
likely to be important both for rapid disease-
promoting responses, such as mast cell activa-
tion, and for more long-term pathogenesis,
such as estrogen-induced cancers.

Some chemicals that accumulate in
women’s tissues are also transferred to their
infants during breast-feeding. This is espe-
cially true for environmental lipid-soluble
pollutants such as polyhalogenated com-
pounds, because these chemicals tend to
degrade slowly in the environment, to bio-
accumulate and bioconcentrate in the food
chain, and to have long half-lives in humans.
Although the World Health Organization
(WHO) strongly supports breast-feeding,

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