Hormones, gender and the aging brain
The endocrine basis of geriatric psychiatry

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Estrogens, stress, and psychoneuroimmunology in women over the lifespan

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Stress, sex hormones, and psychoneuroimmunology

Studies of relationships among the central nervous system (CNS), the immune system, and the endocrine system have shown that the systems interact through multiple pathways. The 'stress' hormones have received the greatest attention in the neuroimmunology literature, particularly cortisol and the catecholamines; sex hormones have not been studied as intensively. In this chapter we first briefly review estrogen's immunomodulatory effects. Next we discuss estrogen-related differences in cardiovascular and neuroendocrine reactivity to brief stressors. In the last section we discuss research regarding the effects of estrogen on immune reactivity to stress, and speculate about the possibility that estrogen may moderate hormonal and immunological responses to stress through alterations in sympathetic nervous system reactivity, as well as through regulation of corticotropin-releasing hormone (CRH; Vamvakopoulos & Chrousos, 1993).

Estrogen and immune function

Estrogens, androgens, and progestogens can serve as regulators of immune function; in turn, the circulating levels of these hormones can be modulated by the immune system. Many of these interactions between the immune system and gonadal steroids appear to be mediated through feedback loops in the hypothalamic–pituitary–gonadal–thymic axis, with thymic factors playing a key role (Erbach & Bahr, 1991; Grossman, 1985). In addition, there are at least two types of estrogen receptors in human immune cells: low-affinity high-capacity (Type II) binding sites found in peripheral blood mononuclear cells (Ranelletti et al., 1988), and high-affinity low-capacity (Type I) receptors that appear to be restricted to CD8+ cells both in blood (Cohen et al., 1983; Stimson, 1988; but see Weusten et al., 1986) and in synovial tissue (Cutolo et al., 1993). Receptors for estrogen also have been found in other immune cells and tissues including macrophages (e.g.
Gulshan et al., 1990), cytosol from human thymus (e.g. Nilsson et al., 1984; Weusten et al., 1986), and spleen (e.g. Danel et al., 1983). Gonadectomy typically leads to hypertrophy of the thymus, spleen, and lymph nodes (see Forsberg, 1984; Grossman, 1984).

Estrogen effects on immune function are complex. The nature of the reported effects may depend on many factors, including the concentration of estrogens relative to androgens or progestogens, the hormone dosage and route of administration, the stage of the host being investigated (e.g., age, reproductive status, autoimmune disease condition), and the aspect of immune function being measured. For the most part, however, estrogens appear to enhance humoral immune function (Ansar Ahmed et al., 1985; Grossman, 1984, 1985; Schuurs & Verheul, 1990), although they likely suppress B cell lymphopoiesis during pregnancy in mice (Medina & Kincade, 1994; Smith et al., 1998). Apparent effects on cellular immune function depend on the method of investigation, with both suppression and enhancement reported (Ansar Ahmed et al., 1985; Grossman, 1984, 1985; Schuurs & Verheul, 1990).

Antibody production can be enhanced by estrogens both in vitro and in vivo. For example, the addition of physiological levels of estradiol to pokeweed mitogen-stimulated human peripheral blood lymphocytes (PBL) increased the number of B cells secreting immunoglobulin M (IgM; Paavonen et al., 1981). This effect was apparently mediated by inhibition of CD8+ T-lymphocytes; because these T-cells down-regulate antibody production by B cells, constraints on CD8+ T-cell function can promote antibody production. Illustrating the importance of dosage, enhanced immunoglobulin G (IgG) production was found after stimulation of human PBL with physiological levels of estradiol, whereas supraphysiological levels inhibited IgG production (Weetman et al., 1981). An in vitro study of human tonsillar lymphocytes demonstrated that the presence of intact T-cells was obligatory for the enhancing effect of estradiol on production of both IgG and IgM by B cells (Evagelatou & Farrant, 1994). In vivo studies suggest that estrogen-related amplification of humoral immunity appears to require the presence of thymic factors. For example, Erbach and Bahr (1991) demonstrated in mice that exogenous estradiol led to increases in antibody titers to fluorescein only in mice with intact thymus glands or in thymectomized mice receiving thymosin fraction 5 replacement.

The effects of estrogens on cellular immune responses appear primarily to be inhibitory, although stimulatory effects also have been reported (for review see Ansar Ahmed et al., 1985; Grossman, 1984, 1985; Schuurs & Verheul, 1990). Estrogens have well-established down-regulatory effects on the thymus (see Grossman, 1985), and estradiol appears to regulate the production of thymic factors that influence cell-mediated immunity. For example, serum from castrates

Enhanced rat thymocyte blastogenesis in response to the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A), but serum from castrated rats that were treated with physiological levels of estradiol depressed the blastogenic response back to pre-castrate levels (Grossman et al., 1982). Estradiol added in vitro also can inhibit the stimulation of human PBL by PHA and Con A (Herrera et al., 1992; Mendelsohn et al., 1977; Neifeld & Tormey, 1979; Wyle & Kent, 1977). The suppression of CD8+ lymphocyte function described above (Paavonen et al., 1981) is another example of a down-regulatory effect of estradiol on one aspect of cellular immune function. On the other hand, enhancement may be illustrated by the effects of in vitro culture of tonsillar T-lymphocytes with a wide range of estradiol concentrations. Without mitogenic stimulation, all levels of estradiol led to increased proliferation, whereas when cells were stimulated with PHA, only the higher concentrations of estradiol enhanced proliferation (Evagelatou & Farrant, 1994).

Estrogens have potent regulatory effects on both developmental and effector functions in macrophages and monocytes (for review see Miller & Hunt, 1996). For example, depending on the dose, estradiol can either enhance or suppress cytokine production. Interleukin-1 (IL-1) was increased in rat peritoneal macrophages incubated with low concentrations of estradiol (Hu et al., 1988), whereas the addition of increasing concentrations of estradiol led to a graded reduction of both IL-1 (Polan et al., 1988) and IL-1β mRNA (Polan et al., 1989) production in lipopolysaccharide (LPS)-activated cultured human peripheral monocytes. Estradiol treatment in vitro also inhibited the LPS-stimulated expression of mRNA for monocyte chemoattractant protein-1 by murine peritoneal macrophages, an effect that was reversed by the addition of tamoxifen (Frazier-Jessen & Kovacs, 1995).

Numerous animal studies have documented enhancement of cellular immunity resulting from gonadectomy, including suppression of tumor development and more rapid tissue rejection responses (see Grossman, 1984). In these models, sex steroid replacement typically restores presurgical function. For example, both male and female mice infected with the parasitic protozoan Toxoplasma gondii showed decreased mortality following gonadectomy; in contrast, implantation of a hestrol (a synthetic estrogen) pellet in half the animals led to increased mortality (Kittas & Henry, 1980). Similar enhancement of cellular immune function has been found in women undergoing surgical menopause. For example, Pacifi et al. (1991) showed that surgically induced menopause in 15 healthy premenopausal women led to increases in phytohemagglutinin (PHA)-induced secretion of granulocyte-macrophage colony-stimulating factor, as well as elevations in spontaneous secretion of IL-1 and tumor necrosis factor α (TNF-α). Nine women in their study subsequently began estrogen replacement therapy 4 weeks after surgery; cytokine secretion returned to presurgical levels among these women following estrogen replacement.
Modulation of immune function by estrogen has health consequences. In general, immune function in females tends to be higher than that in males. Females have higher levels of immunoglobulins, a stronger in vitro response to mitogens, and better resistance to the induction of immune tolerance (for review see Ansar Ahmed et al., 1985; Lahita, 1997; Schuurs & Verheul, 1990). This higher level of immune function can be beneficial, as in the case of better resistance in females to a variety of infections. On the other hand, females are more prone to autoimmune diseases, and sex hormones appear to be a factor in this susceptibility (Ansar Ahmed et al., 1985; Beesoon, 1994). For example, estrogens accelerate murine lupus erythematosus, while androgens decelerate the disease process (Roubianian et al., 1979). In humans, postmenopausal estrogen replacement therapy has been associated with a slightly higher relative risk for systemic lupus erythematosus (Sanchez-Guerrero et al., 1995). Oral contraceptives containing estrogens can exacerbate the symptoms of lupus (Garovich et al., 1980). On the other hand, estrogen-containing oral contraceptives reduce, rather than enhance, the characteristic joint inflammation of rheumatoid arthritis in many patients (Vandenbroucke et al., 1982), illustrating the complexity of sex hormone-immune interactions.

**Estrogen-related differences in physiological reactivity to brief stressors**

Individual differences in cardiovascular and neuroendocrine reactivity to brief stressors, such as verbal subtraction and speech preparation and delivery, have been studied extensively because they appear to be risk factors for coronary heart disease. These stressors typically lead to activation of the sympathetic-adrenomedullary (SAM) axis, as indicated by increases in heart rate, blood pressure, and catecholamine levels, and less consistently to activation of the hypothalamic-pituitary-adrenal (HPA) axis, as indexed by increases in adrenocorticotropic hormone (ACTH) and cortisol (Cacioppo et al., 1995). However, relatively little research has assessed cardiovascular and endocrine reactivity in women, while a broad literature exists on men's stress responses (Manuck & Polefrone, 1987; Saab, 1989). Limited evidence suggests that women typically show smaller blood pressure and epinephrine stress responses than men, indicating lower SAM activation (Frankenhaeuser, 1983; Matthews & Stoney, 1988; Stoney et al., 1987), and female reproductive hormones, especially estrogens, are thought to be possible contributors to these gender differences (Matthews, 1989; Saab et al., 1989).

Studies investigating the effects of the menstrual cycle on SAM reactivity to psychological stress are contradictory; overall, the weight of the evidence suggests that hormonal fluctuations during a normal menstrual cycle may be insufficient in either magnitude or duration to cause detectable estrogen effects on SAM reactivity (e.g. Stoney et al., 1996; Litschauer et al., 1998; for review see Light et al., 1998). Studies that include both pre- and postmenopausal women are thus of particular interest in exploring the influence of estrogens on cardiovascular and neuroendocrine responses. For example, Saab et al. (1989) compared the responses of 15 premenopausal and 16 postmenopausal women to two stressors, mental arithmetic and a speech stressor. Premenopausal women showed greater heart rate increases than premenopausal women on both tasks, with the largest differences during the speech stressor. In addition, postmenopausal women also showed greater increases in epinephrine and systolic blood pressure (SBP) than premenopausal women during the speech stressor only. Similarly, Blumenthal et al. (1991) documented greater epinephrine reactivity for postmenopausal compared to premenopausal women in response to a speech task. In a third study, Owens et al. (1993) compared the cardiovascular and neuroendocrine function of pre- and postmenopausal women and of men in response to brief psychological stressors. Postmenopausal women had higher stress-reactive increases in both SBP and diastolic blood pressure (DBP) than did either premenopausal women or men. Finally, Stoney et al. (1997) prospectively compared premenopausal women who had either hysterectomy or bilateral salpingo oophorectomy (BSO). Before surgery, there were no differences in stress responses; after surgery, marginally higher stress-induced increases in SBP and DBP were found in the BSO group. These results support the idea that estrogens ameliorate SAM responses to brief stressors, but are not conclusive because of their non-experimental nature.

To address this issue, Lindheim et al. (1992) conducted a two-part investigation of cardiovascular and neuroendocrine responses to brief speech and math stressors. First, a cross-sectional comparison between 13 premenopausal and 36 postmenopausal women showed that postmenopausal women produced larger stress-reactive elevations in SBP and smaller increases in cortisol than premenopausal women. After this baseline testing, the postmenopausal women were randomly assigned to either a placebo or a transdermal estradiol treatment condition. Six weeks later, stress reactivity was reassessed and the researchers compared the maximum percent change in women who received estradiol with those who received placebo. Although there were no pre-existing differences between the groups, the estradiol treatment eliminated significant cardiovascular (SBP and DBP) and neuroendocrine (ACTH, cortisol, norepinephrine, and androstenedione) responses to laboratory stressors. In a later study, Lindheim et al. (1994) tested the effects on stress reactivity of adding a progestin to estradiol treatment. Stress-induced increases in DBP, ACTH, and cortisol did not differ between the two treatment groups, although both were lower than in the placebo group. However, stress effects on SBP and norepinephrine were similar in the placebo group and the estradiol plus progestin group, indicating that progestin may reverse some of the ameliorating effects of estradiol on the stress response.

In an experimental test of acute effects of transdermal estradiol on stress
responses in menopausal women, Del Rio et al. (1998) demonstrated higher stress-induced SBP and epinephrine increases in the placebo group, suggesting that some of the effects of estradiol may occur through non-genomic pathways. A randomized, double blind, placebo-controlled, cross-over experiment carried out in young men also found reduced heart rate, SBP, epinephrine, and norepinephrine reactivity to brief psychological stressors after transdermal treatment with estradiol (Del Rio et al., 1994).

These data strongly support a role for estrogens in modulating both SAM and HPA reactivity to stress. However, one recent report provided somewhat contradictory evidence (Matthews et al., 1998). In this study, a sample of young women had their ovarian hormones temporarily suppressed using an agonist to gonadotropin releasing hormone. Although the suppression of estradiol was accompanied by typical menopause symptoms, cardiovascular and neuroendocrine responses did not differ from their pre-suppression levels. Thus, factors in addition to estrogen level may contribute to the reduction in physiological effects of stress seen in postmenopausal women after estradiol treatment.

Estrogen-related differences in immune reactivity to stress: possible pathways

Recent studies of the immunological consequences of brief experimental stressors have provided preliminary evidence that individuals who exhibited the largest sympathetically mediated increases in cardiovascular reactivity also showed the largest catecholaminergic increases and immune changes (Cacioppo et al., 1995; Kiecolt-Glaser et al., 1992; Manuck et al., 1991; Sgoutas-Emch et al., 1994). These same individuals also may show the highest increases in ACTH and cortisol (Cacioppo et al., 1995), and very preliminary work in our laboratory suggests that individuals who demonstrated the greatest cortisol changes in response to brief stressors also showed poorer responses to influenza vaccine, as measured by the IL-2 response of T-lymphocytes to virus-specific antigens in vitro (Cacioppo, 1994). In addition, Mills et al. (1995a) showed that cellular immune responses were best predicted by lower basal norepinephrine, higher stress-induced norepinephrine, and higher sensitivity of β2-adrenergic receptor on lymphocytes. Thus, if estrogen dampens SAM activity or reactivity, it could have implications for immune reactivity to stress.

Alterations in SAM function could be particularly important in older adults because SAM can inhibit antigen processing and presentation (Helligt et al., 1993). Infectious diseases exact a high toll in morbidity and mortality among the elderly; together, pneumonia and influenza are the fourth leading cause of death in individuals over the age of 75 (Yoshikawa, 1983).

The question of estrogen effects on immune reactivity to brief stressors has been addressed in past research by investigating the possible effects of menstrual cycle stage on stress-related changes in leukocyte subsets (Mills et al., 1995a) and in the proliferative response of PBLs to PHA (Caggiula et al., 1990). Neither study found any differences in immune reactions that could be attributed to menstrual cycle phase. Given that menstrual phase effects on SAM reactivity are negligible (Stoney et al., 1990; Litschauer et al., 1998), this lack of findings is not surprising, and may be explained by the fact that the duration of action of estrogens can be maintained for up to 13 weeks (Gangar et al., 1991). Thus, the time window afforded by changes in menstrual cycle phase may be too short to detect estrogen effects (Mills et al., 1995b). Postmenopausal women provide a population in which estrogen effects can be more closely controlled, through the comparison of women using estrogen replacement therapy (ERT) with women not using any hormone treatment.

Two studies from our laboratory provide information relevant to the issue of ERT effects on stress reactivity in older women. In the first, 22 postmenopausal women carried out brief math and speech stressors (Cacioppo et al., 1995). As in other populations, the stressors heightened SAM activity, as indexed by cardiac activation and elevated plasma catecholamine concentrations, and reduced cellular immune function, as indexed by mitogen-stimulated blastogenesis of PBLs. The subset of women on ERT had lower plasma epinephrine, consistent with other studies, but we found no significant interactions between use of estrogen supplements and physiological changes induced by the stressors. However, only 6 of the 22 women in the study were taking estrogen supplements, severely limiting power for detecting estrogen differences.

The second study was designed specifically to evaluate possible effects of long-term ERT (longer than two years) on stress reactivity. Three groups of postmenopausal women carried out laboratory speech and math tasks: women who did not use ERT; women who were taking estrogen alone; and women who were taking both estrogen and progesterin. Cardiovascular, neuroendocrine, and immune variables were measured prior to and after the stress protocols, providing us with estimates of baseline function and stress reactivity (Burleson et al., 1998).

In contrast to many previous studies of brief stress effects on immune function in which mitogen-stimulated blastogenesis declined post-stress (e.g. Cacioppo et al., 1993; Sgoutas-Emch et al., 1994), we did not find significant main effects of the stress tasks on Con A- or PHA-stimulated lymphocyte proliferation. Closer examination of our data, however, revealed that among the women who were not using ERT, the expected stress-related declines in blastogenesis did occur. Only in the two groups of women using ERT was mitogen-stimulated blastogenesis unaffected by the stress tasks. If stress-related changes in cellular immune function result from activation of the SAM system, as proposed by Manuck et al. (1991), then these findings would be consistent with reduced sympathetic reactivity in the women using ERT. Although our data provide no direct evidence of reduced SAM responsiveness with ERT, previous research described above has found both
premenopausal status and exogenous estrogen treatment to be associated with reduced stress reactivity in the SAM system.

Along with the difference in immune reactivity, the women using ERT had significantly higher baseline and overall levels of both Con A- and PHA-induced blastogenesis than the women who did not use hormones. Because the level of mitogen-stimulated blastogenesis is considered an index of the ability of lymphocytes to respond to a pathogen, this finding supports a tonic stimulatory effect of long-term ERT on cellular immune function in this sample. Previous studies of estrogen effects on mitogen-stimulated blastogenesis typically have shown either no effect (e.g. Grossman et al., 1982) or a suppressive effect (e.g. Morishima & Henrich, 1974); however, ours is the first reported study in this population using long-duration estrogen treatment.

In addition to modulating SAM or HPA stress reactivity, recent research suggests another pathway through which estrogen may influence immune reactivity to stress. Vanvakopoulos and Chrousos (1993) provided evidence for direct estrogenic enhancement of human CRH gene expression. Through its central role in the activity of the HPA axis, CRH plays an important role in the regulation of both stress responses and immune and inflammatory reactions. Thus, these findings also may help to explain sexual dimorphism in the stress response and in immune regulation.

Our understanding of the roles that the sex hormones play in the complex interactions among the central nervous system, the SAM and HPA axes, and the immune system is still in its infancy. The limited evidence available to date suggests the effects could be very important.

REFERENCES


