Suppression of Prolactin Secretion in Normal Young Women by 2-Hydroxyestrone

Abstract. *The nonuterotropic natural estrogen 2-hydroxyestrone administered to normal young women results in a prompt and profound suppression of serum prolactin in most of the subjects. With the exception of dopamine, this is the only endogenous material known to strongly inhibit prolactin secretion, and its action suggests that the physiological regulation of prolactin by estrogens in the human is dual in nature, consisting of stimulation by estradiol and inhibition by its catechol estrogen metabolite.*

The role of estrogens in the control of prolactin secretion in primates is enigmatic. Prolonged administration of exogenous estrogens has been related to an increase in serum prolactin in both men and women (*1-3*), but there is little evidence that changes in endogenous estradiol concentrations are associated with fluctuations in prolactin secretion (*4*). In the rhesus monkey a paradoxical effect of estrogens on prolactin secretion was reported (*5*), in that endocrine manipulation that either increased or decreased estradiol concentrations produced an augmentation in basal serum prolactin concentrations.

Findings on the role of estradiol metabolites in the expression of the different biological functions of the female sex hormone (*6, 7*) suggest that the complex nature of the estradiol-prolactin relationship may reflect the variable influence of the parent hormone and its metabolites on pituitary prolactin release. The principal metabolite of estradiol in the human is 2-hydroxyestrone (*8, 9*), a catechol estrogen that is inert in the uterotropic assay (*6*), but has effects on the hypothalamic-pituitary axis in several species including humans (*10-12*); it is therefore the first known estrogen whose peripheral and central activities are dissociated. The action of 2-hydroxyestrone on pituitary gonadotropin release in the rodent (*10, 11*) and in the human (*12*) made it of particular interest to study the impact of this metabolite on prolactin secretion in human subjects.

2-Hydroxyestrone administered as a single injection to postmenopausal women, with or without prior priming with estrogens, did not have any effect on concentration of prolactin in the serum (*12*). When 2-hydroxyestrone was administered as a prolonged infusion to postmenopausal women, quite different results were obtained (*13*). In the estrogen-primed subjects, the infused catechol estrogen produced a rapid and profound suppression of serum prolactin, but in the women who had not been treated with estrogen, no discernible effect was observed (*13*). The necessity for prior estrogen treatment to elicit a prolactin response to 2-hydroxyestrone in the postmenopausal women raised a question about the effect of the catechol estrogen in premenopausal females with normal ovarian function. We now report that the infusion of 2-hydroxyestrone to normal untreated young women results in a rapid and large suppression of serum prolactin, a result that constitutes evidence for a physiological mechanism of estrogenic modulation of prolactin secretion.

Twelve women (aged 20 to 37, with normal menstrual cycles), who volunteered for this study, received an hourly intravenous infusion of 80 μg of rigidly purified 2-hydroxyestrone (*6*) in propylene glycol and saline (1:1 by volume). In two of the subjects it was possible to repeat the studies in three successive cycles, providing for a total of 16 studies. Ten of these were in the late follicular and six in the mid-luteal stages of the menstrual cycle. Infusions were started at 9 a.m. and were continued for 3 hours. Blood samples were obtained through an indwelling catheter at several intervals during 2 hours before the start of the infusion and at 30, 60, 120, and 180 minutes thereafter. In several studies single blood samples were obtained on the mornings of days 1 or 2 after the end of the infusion. The samples were analyzed in duplicate for serum prolactin content by radioimmunoassay (*14*); the value for time 0 was taken as the mean of the prolactin concentrations found in the sequential samples drawn before the start of the infusion.

In eight of the studies serum prolactin declined during infusion to < 2 ng, which is the sensitivity limit of the assay. The decrease was rapid, with serum prolactin reaching 59, 26, and 22 percent of control values at 30, 60, and 120 minutes, respectively. In these calculations the value of < 2 ng, which is not distinguishable from 0, was entered as 2 ng so that the actual suppression was probably greater. In five other studies the decrease in serum prolactin was also observed, but to a lesser extent, with a maximum suppression of 50 percent (Fig. 1). Analysis of variance, carried out separately on each of the above groups, showed the prolactin decrease to be highly significant at $P < .004$ and $< .005$, respectively. Scattered values obtained 1 and 2 days after the cessation of fusion indicated a return to control prolactin levels.

In four studies (three subjects) the 2-

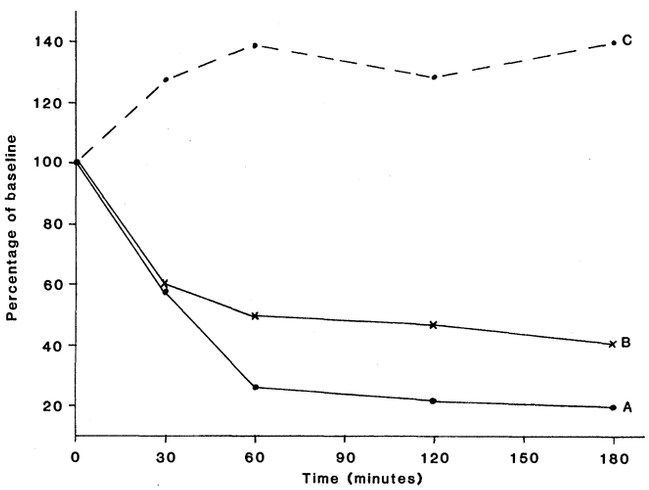

Fig. 1. Changes from baseline serum prolactin during an infusion of 2-hydroxyestrone (80 μg/hour) to normal young women. All means were computed with prolactin values of less than 2 ng/ml being considered as 2 ng/ml. (Curve A) Means of eight studies in which complete suppression of prolactin to < 2 ng/ml was observed. The mean (± standard error) prolactin values, in nanograms per milliliter, were: at 0 minute, 9.8 ± 1.9; 30 minutes, 5.3 ± 2.0; 60 minutes, 5.0 ± 2.3; 120 minutes, 2.2 ± 0.1; 180 minutes, 2.0 ± 0. Analysis of variance of the changes (carried out by the BMD p2v program developed at the Health Sciences Computing Facility, University of California, Los Angeles) showed them to be highly significant at $P < .004$. (Curve B) Means of five studies in which serum prolactin did not reach the < 2 ng/ml level. The mean (± S.E.) prolactin values were: at 0 minute, 21.6 ± 3.2; 30 minutes, 12.8 ± 1.4; 60 minutes, 10.8 ± 2.5; 120 minutes, 10.4 ± 2.7; 180 minutes, 8.9 ± 2.1. The analysis of variance was significant at $P < .005$. (Curve C) Means of four studies in which no suppression of serum prolactin was observed. The mean (± S.E.) prolactin values were: 0 minute, 10.1 ± 1.5; 30 minutes, 13.1 ± 2.8; 60 minutes, 15.0 ± 4.0; 120 minutes, 13.4 ± 2.9; 180 minutes, 14.6 ± 3.2. These changes were not significant by analysis of variance.

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hydroxyestrone infusion produced no decrease in serum prolactin, with the apparent increase being not significant by analysis of variance. We do not know why we failed to obtain a response in these four studies, but it is noteworthy that the two responding subjects who were studied repeatedly exhibited either a total or partial prolactin suppression in each study, whereas the one nonresponding subject twice failed to respond in both studies, suggesting that the prolactin response to 2-hydroxyestrone is subject and not time dependent.

The profound suppression by 2-hydroxyestrone of prolactin secretion in young women has significant physiological and clinical implications. The infusion of 2-hydroxyestrone resulted in blood concentrations of 2-hydroxyestrone that were six times that at time 0 (90 versus 15 pg/ml) as determined by radioimmunoassay (*15*), indicating a very rapid metabolic clearance of the administered material. The physiological relevance of this observation must be considered in light of the demonstrated capacity of neuronal tissue to convert estrogens to 2-hydroxyestrone (*16–18*). The material biosynthesized in situ would then be expected to affect the neuronal target tissue at physiological concentrations.

Regulation of prolactin secretion by estrogens in the human can be construed as one of dual nature, with estradiol acting in a positive feedback mode and its 2-hydroxylated metabolite acting in an inhibitory fashion. The control of prolactin secretion by estrogens would then be dependent on the activity of the estradiol-2-hydroxylase enzyme in the appropriate central or pituitary sites. Evidence has been obtained that opiate agonists that stimulate prolactin release (*19*) are effective inhibitors of estradiol-2-hydroxylase in the rat brain, whereas the opiate antagonist naloxone, which suppresses prolactin release, increases estradiol-2-hydroxylase activity in the brain (*20*). The action of opiate agonists and antagonists on prolactin release is therefore in concert with their impact on estradiol-2-hydroxylase in the brain, which suggests that the action of the opiates on prolactin release is modulated by their effect on estrogen metabolism in neuronal tissue.

The suppression of prolactin secretion by 2-hydroxyestrone may have significant clinical applications. Hyperprolactinemia has now been associated with reproductive disorders in both men and women (*21*), and the suppression of pituitary prolactin secretion has become an important treatment. The suppression of prolactin secretion by a nonuterotropic natural estrogen may offer an alternative and possibly superior treatment of hyperprolactinemia.

It is not known whether the inhibition of prolactin secretion by 2-hydroxyestrone occurs at the pituitary or at the hypothalamic level, nor is it known whether dopaminergic mechanisms are involved. Resolution of some of these questions may make it possible to use the effect of 2-hydroxyestrone on serum prolactin to differentiate between functional hyperprolactinemia and that resulting from the presence of pituitary tumors, a goal which has thus far proved elusive (*22*).

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23. This research was supported by grant CA 22795 from the National Cancer Institute.

11 March 1980; revised 28 May 1980

Erythroid Differentiation of Clonal Rauscher Erythroleukemia Cells in Response to Erythropoietin or Dimethyl Sulfoxide

Abstract. *Clonal lines of Rauscher erythroleukemia cells exhibited selective responses to two inducers of differentiation, erythropoietin and dimethyl sulfoxide. There were substantial quantitative differences between clones that responded to both inducers. Several clones differentiated only in response to erythropoietin. Erythropoietin stimulated cell proliferation and differentiation whereas dimethyl sulfoxide inhibited proliferation, suggesting dissimilar modes of action.*

A definitive understanding of the control of erythroid differentiation requires that the mechanism of action of erythropoietin (*1*) be clarified. Advances in our knowledge of erythropoiesis have been made with Friend erythroleukemia cells—a murine viral leukemia line, grown in continuous culture, that exhibits erythroid differentiation in response to various chemical inducers (*2*). However, this cell line does not respond to erythropoietin. Hence, studies of the hormone's mechanism of action have been limited to examination of its effects on unfractionated bone marrow (*3*) or mouse fetal liver (*4*), both of which are heterogeneous (although the fetal liver is much less so). Clearly, a cell line that could be maintained continuously in vitro and that differentiated selectively in response to erythropoietin would provide an ideal system for studying the hormone's biochemistry.

Such a system is provided by the recently described Rauscher murine erythroleukemia line (*5*). The parent, uncloned cell line undergoes erythroid differentiation in response to either erythropoietin or dimethyl sulfoxide (DMSO). We maintained the cells in plastic culture flasks or petri dishes in continuous suspension culture in a mixture of Eagle's minimum essential medium (Dulbecco's modification) and fetal calf serum (10 percent) in a humidified atmosphere of 95 percent air and 5 percent CO_2 (37°C). Individual cells gave rise to discrete colonies when grown in plasma-clot culture (*6*), a semisolid medium (100 cells per 0.1-ml clot). The clots were fixed on glass slides with glutaraldehyde, stained with dimethoxybenzidine and H_2O_2 to detect hemoglobin, and counterstained with hematoxylin. The cells in each colony were then examined microscopically for erythroid

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