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# Neuroendocrine Response to Estrogen and Sexual Orientation

Abstract. *A neuroendocrine component, the positive estrogen feedback effect, thought to be related to sexual orientation and, indirectly, to sexual differentiation, was evaluated in healthy, noninstitutionalized research volunteers. Men and women with a lifelong heterosexual orientation and men with a lifelong homosexual orientation were administered an estrogen preparation known to enhance the concentration of luteinizing hormone in women but not in men. The secretory pattern of luteinizing hormone in the homosexuals in response to estrogen was intermediate between that of the heterosexual men and that of the women. Furthermore, testosterone was depressed for a significantly longer period in the homosexual men than in the heterosexual men. These findings suggest that biological markers for sexual orientation may exist.*

A fundamental question of human psychosexuality is whether there are biological influences on sexual behavior and sexual orientation. Of particular interest is whether hormonal correlates exist for the development and expression of patterns of sexual behavior. The results of previous efforts to relate the plasma concentration of androgen (primarily testosterone) or estrogen (chiefly estradiol) to homosexual or heterosexual orientation were not convincing (*1*). Currently, a biological explanation of homosexuality is being pursued most vigorously in the hypothalamic-pituitary-gonadal axis, a neuroendocrine system. Presumably the endocrine responsiveness of this system is sexually dimorphic, perhaps reflecting organizing influences of hormones during critical periods of sexual differentiation (*2*). It has been suggested that such differences in neuroendocrine responsiveness are linked to sexual orientation (*3, 4*). Because of the importance of these controversial findings, we measured the neuroendocrine response to estrogen administration in men and women who differ in their sexual orientation. We found that men declaring a lifelong homosexual orientation had patterns of luteinizing hormone (LH) and testosterone secretion in response to estrogen that were intermediate between those of men and women declaring lifelong heterosexual orientation.

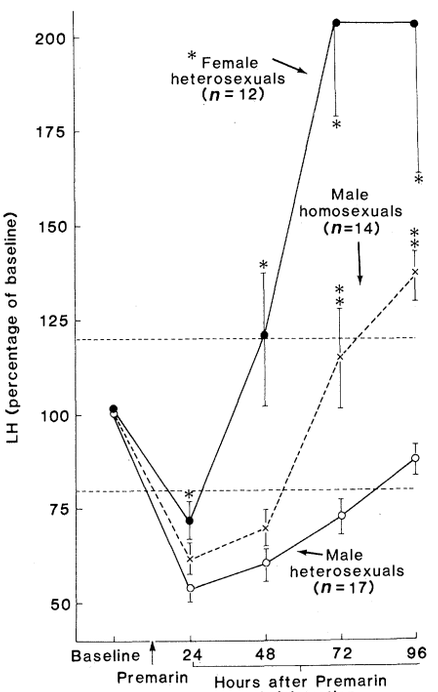


Fig. 1. Changes in LH in response to a single injection of Premarin. Values are means ± standard errors (vertical bars). Dashed lines indicate the 95 percent confidence interval for baseline values for all groups. Group comparisons: (*) female heterosexuals significantly different from male heterosexuals and homosexuals at all times ($P < 0.05$, Newman-Keuls multiple comparison test) and (**) male homosexuals significantly different from male heterosexuals at 72 and 96 hours ($P < 0.05$, SNK). All groups show a decrease from baseline at 24 hours.

During the follicular phase of the menstrual cycle, increasing concentrations of estrogen initiate a transient decrease in LH (negative feedback) that is soon followed by a sharp increase in LH (*5, 6*). The ability of increased estrogen to enhance the release of LH (positive feedback) is thought to reflect the adult consequences of hormone-mediated sexual differentiation. Such an LH response pattern, typically seen in females, presumably reflects the "female" differentiation of the brain; the typical absence of this response in males presumably reflects "male" brain differentiation (*7*). Exposure to high concentrations of androgen during a critical period of development in males results in a relatively steady LH secretion pattern. Females, not ordinarily exposed to such levels of androgen during this period, secrete LH in the cyclical pattern related to the ovulatory cycle. A brief negative feedback response of LH to estrogen followed by a late rebounding of LH above pretreatment levels (positive feedback) was recently used to explore, indirectly, human sexual differentiation and interrelationships of brain function, hormone responsiveness, and sexual orientation (*3, 4*).

Table 1. Plasma concentrations of LH and testosterone in female heterosexuals (FHT), male heterosexuals (MHT), and male homosexuals (MHS) before and various intervals after administration of Premarin. Values are means ± standard errors. Values with different letter superscripts are significantly different at $P < 0.05$ (Newman-Keuls multiple comparison test).

| Group | $n$ | Baseline | 24 hours | 48 hours | 72 hours | 96 hours |
|---|---|---|---|---|---|---|
| | | | *LH (mIU/ml)* | | | |
| FHT | 12 | 8.41 ± 1.15[a] | 5.88 ± 0.72[a] | 9.51 ± 1.45[a] | 16.00 ± 2.31[a] | 16.87 ± 3.60[a] |
| MHT | 17 | 7.18 ± 0.50[a] | 4.00 ± 0.48[b] | 4.34 ± 0.46[b] | 5.28 ± 0.54[b] | 6.34 ± 0.59[b] |
| MHS | 14 | 7.28 ± 0.61[a] | 4.40 ± 0.35[b] | 5.07 ± 0.56[b] | 8.03 ± 0.91[c] | 10.03 ± 0.99[c] |
| | | | *Testosterone (ng/ml)* | | | |
| FHT | 12 | 0.57 ± 0.03[a] | 0.54 ± 0.03[a] | 0.56 ± 0.03[a] | 0.61 ± 0.03[a] | 0.61 ± 0.03[a] |
| MHT | 17 | 5.40 ± 0.30[b] | 3.21 ± 0.30[b] | 3.38 ± 0.30[b] | 4.00 ± 0.29[b] | 4.33 ± 0.26[b] |
| MHS | 14 | 5.48 ± 0.33[b] | 3.22 ± 0.37[b] | 2.95 ± 0.32[b] | 3.31 ± 0.26[c] | 3.83 ± 0.27[b] |

We selected subjects from volunteers who met the criteria of good health, no use of any medications or hormonal preparations (including oral contraceptives) within the past 90 days, and no use of drugs that affect neuroendocrine factors (marijuana, excessive alcohol consumption, narcotics, amphetamines, or barbiturates) (*8*). Women participated during the early follicular phase of their menstrual cycle. All subjects were objectively classified a priori into behaviorally distinct categories of sexual orientation (*9*). We monitored the subjects for changes in plasma LH, testosterone, and estrone in response to a single injection of the estrogen preparation Premarin (*4, 5, 10*).

Baseline blood samples were taken twice daily (1 hour apart between 0800 and 1000 hours) on Friday and the following Monday. After taking the second sample on Monday, we gave the subjects intravenous injections of Premarin (25 mg). Additional samples were taken on the same schedule on each of the next 4 days. The sampling schedule was designed to minimize circadian and ultradian fluctuations in hormone concentrations (*11*). Baseline samples from the first two sampling days (Friday and Monday) were assayed for LH, testosterone, and estrone (*12*) and the values were averaged to yield mean baseline values for that subject. Similarly, the samples taken after the Premarin injections were assayed and both daily values for each hormone were averaged. Samples were numbered and were assayed without knowledge of their origin. All values for each subject were statistically analyzed as absolute values or as percentages of baseline. Hence, each subject served as his or her own control. The baseline values for each hormone were well within clinically normal limits.

We observed a clear sex difference in the LH response to Premarin, with females having an earlier peak of greater amplitude than all males (Fig. 1). This sex difference in the LH response to a neuroendocrine challenge is a critical feature in any evaluation of hormone responsiveness and sexual orientation: to our knowledge, this is the first simultaneous direct comparison of heterosexual and homosexual men with heterosexual women. Mean plasma LH concentrations in the females ($n = 12$) decreased to 72 percent of baseline 24 hours after Premarin treatment and increased sharply to 122 and 204 percent of baseline by 48 and 72 hours, respectively, leveling off at 208 percent by 96 hours. This response pattern is comparable to that reported in earlier studies of women receiving similar estrogen treatment (*5, 6*).

The heterosexual and homosexual men as a group ($n = 31$) showed a strikingly different response to Premarin. With LH initially decreasing to 60 percent of baseline at 24 hours, the men did not exceed baseline until 96 hours (106 percent). Thus, while baseline values did not differ according to sex [$F(1, 41) = 1.63$, $P = 0.21$], significant sex differences appeared 24 hours ($F = 6.62$, $P = 0.02$), 48 hours ($F = 20.9$, $P = 0.001$), 72 hours ($F = 32.1$, $P = 0.001$), and 96 hours ($F = 13.3$, $P = 0.005$) after Premarin injection. Table 1 lists the hormone statistics for all groups.

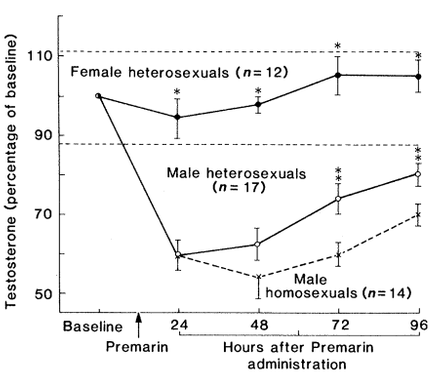


Fig. 2. Changes in testosterone in response to Premarin. Asterisks indicate significant differences ($P < 0.05$, Newman-Keuls multiple comparison test): (*) female heterosexuals different from both male groups indicating no change in testosterone among females and (**) male heterosexuals different from male homosexuals at 72 and 96 hours. Values are means ± standard errors. Dashed lines indicate 95 percent confidence interval for baseline values. Note that only the male groups show a decrease in testosterone in response to Premarin.

A 3 × 5 analysis of variance for LH revealed significant effects of group ($F = 10.94$, $P = 0.002$) and day of sample ($F = 29.84$, $P = 0.001$) and a significant interaction between them ($F = 8.21$, $P = 0.001$). As a result, pairwise multiple comparisons were performed and probabilities were corrected for repeated testing by using a Student-Newman-Keuls (SNK) procedure. These comparisons showed that the females had significantly higher LH values than either group of men at all time points after treatment ($P < 0.05$).

Post hoc comparisons between the homosexual and heterosexual men revealed significant differences in their LH response patterns (Fig. 1 and Table 1). While these groups did not differ at baseline or soon after estrogen injection, significant differences appeared at 72 and 96 hours ($P < 0.05$). In addition, the homosexuals at those times had LH values that were intermediate between those of the heterosexual men and women ($P < 0.05$).

Examination of individual response patterns showed a greater variability among the homosexual men than among the heterosexual men. If we consider a positive estrogen feedback response of LH to be a change in LH at 72 or 96 hours that exceeds mean baseline values for all men by more than 2 standard deviations, then 9 of the 14 homosexuals exceeded this criterion, compared to 0 of the 17 heterosexual men ($P = 0.0001$, Fisher's Exact Test) and 11 of the 12 heterosexual women (89 percent) ($P = 0.104$). Note that, while the number of homosexual men responding is similar to that of women, the magnitude of the response is clearly dissimilar (Fig. 1). Comparing a response only 1 standard deviation above baseline, 11 of the

homosexual men exceeded this criterion and only one heterosexual man ($P = 0.000044$). Hence, although not all the homosexual men studied showed an enhanced response of LH to estrogen compared to the heterosexual men, significantly more did.

Testosterone concentrations also differed in response to Premarin. Women did not show any significant change across the test period, whereas both homosexual and heterosexual men showed a marked decrease in testosterone immediately after treatment. Testosterone gradually returned to baseline by 96 hours (Fig. 2 and Table 1). However, there were significant differences between the heterosexual and homosexual men in their response to estrogen. While baseline testosterone concentrations were virtually identical for the two groups (5.40 and 5.48 ng/ml, respectively) and concentrations at 24 and 48 hours were similarly depressed, at 72 hours the homosexuals had significantly lower testosterone levels (60 percent of baseline) than the heterosexual men (74 percent of baseline) ($P < 0.05$, SNK) (Fig. 2 and Table 1). This difference was also found at 96 hours ($P < 0.05$, SNK).

Testosterone concentrations were slower in returning to baseline in the homosexual men than in the heterosexual men. Since LH was initially depressed equally in both groups of men and yet later was higher in the homosexual group, one might expect a corresponding increase in testosterone because of LH stimulation of testosterone by the testes (*13*). That testosterone concentrations were depressed in the presence of elevated LH may reflect "down-regulation" of testicular receptors: increased concentrations of LH or human chorionic gonadotropin may decrease available LH receptor sites and paradoxically temporarily depress testosterone production (*14*). Hence, decreased testosterone may be a secondary effect of enhanced LH in these homosexual men.

Estrone concentrations did not differ in accordance with sexual orientation among the men at any point in the study. There was a sex difference: in the females estrone was significantly higher than in the men 24 and 48 hours after injection of Premarin ($P < 0.01$, SNK). Presumably this was due to the higher endogenous level and slower metabolism of estrone in women. Assays for estrone confirmed the delivery of estrogens in the Premarin injections: after 24 hours plasma estrone was approximately 250 percent of baseline for all men and 350 percent of baseline for women.

None of the males showed a typically "female" response pattern. Responses in the women were earlier and of greater magnitude than those in the men. However, a differential response pattern was found among the homosexual men that suggests a neuroendocrine responsiveness intermediate between that of the heterosexual men and that of the women. This invites the idea that there may be physiological developmental components in the sexual orientation of some homosexual men. In addition, an intermediate level of neuroendocrine responsiveness to estrogen in some male homosexuals would, in these individuals, constitute a biological marker of their sexual orientation.

Possible biochemical factors might have accounted for the different neuroendocrine responses of the heterosexual men and the homosexual men. They may have differed in their use of drugs known to affect central nervous system activity or they may have been differentially stressed with elevated cortisol in the homosexual men contributing to depressed testosterone and thus indirectly elevating LH. However, it is unlikely that either of these factors influenced our results. Groups did not differ in reported substance use (caffeine, nicotine, or alcohol). No subject reported use of any drug known to affect neuroendocrine factors. As for stress, psychiatric interview of all subjects precluded study of anyone with significant life stress circumstances. Furthermore, post hoc assay of plasma cortisol did not reveal any significant differences in baseline values among groups.

These findings are based on a particular subset of homosexual men and may not apply to all male homosexuals. While recent surveys show that 75 percent of the male homosexual population claims to be exclusively homosexual (a Kinsey rating of 6), the respondents were asked to describe their orientation and activities for only the preceding 12 months (*15*). According to Kinsey (*9*), 4 percent of the male homosexual population meet criteria for lifelong homosexual orientation. In our study the two groups of men were selected such that they represented the opposite ends of the spectrum of sexual orientation, in order that any subtle differences in neuroendocrine responsiveness might be detected. The homosexual men in this study reported a long-term pattern of sexual behavior and fantasies involving male partners. Whether a differential neuroendocrine response is present in men of less exclusive homosexual orientation is an open question.

Since we may have measured an adult hormonal correlate of sexual orientation that is causally independent of sexual differentiation, a causal relation should not be inferred. Unknown physiological factors in the adult may account for the differential responses of LH and testosterone reported here. However, even though a developmental relation between neuroendocrine response and sexual orientation is not certain, our findings are not inconsistent with such an interpretation.

Future investigations should include larger samples of homosexual and heterosexual men and women and transsexuals who have not been receiving contrasex hormones. Other forms of estrogen, including long- and short-acting preparations, may further clarify this neuroendocrine response pattern and its relation to sexual orientation.

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9. All the subjects were obtained through advertisements and referrals by friends who knew of or were participating in this study. When volun-

teers telephoned they were told the rationale and plan of the experiment and asked about their age, medical history, and any recent drug use. Women were also asked to give detailed information on their menstrual cycle. Interested volunteers were scheduled for a clinical interview to establish sexual orientation, past and current sexual history and attitudes, and general psychological state. Volunteers who gave their informed consent became subjects and were scheduled for a series of visits for blood sampling. Appropriate Institutional Review Board guidelines for human research subjects were followed and confidentiality was guaranteed. Subjects were paid for their participation in the 6-day sampling regime. Subjects in all groups came from a variety of occupations: health care professionals, educators, paralegal assistants, clerical and technical personnel, laborers, homemakers, sales people, and students. They appeared to be in good health and to be free of psychiatric and drug problems at the time of their participation. All subjects were Caucasians 21 to 37 years of age. To be included in the homosexual sample, men had to have had erotic fantasies exclusively or primarily involving male partners since puberty (a rating of 5 to 6 on the Kinsey scale of sexual orientation) [A. C. Kinsey, W. B. Pomeroy, C. E. Martin, *Sexual Behavior in the Human Male* (Saunders, Philadelphia, 1948)]. Similarly, genital experiences had to have exclusively or primarily involved a male since puberty. Individuals included in the heterosexual sample reported having had erotic fantasies about and genital sexual contact with persons of only the opposite sex since puberty (Kinsey rating of 0). Thus, there was no overlap in fantasies or behavior between the heterosexual and homosexual samples. Groups were matched for comparable age distributions (range, 21 to 37; median age, 26). A clinical interview was used to classify all volunteers, the records being reviewed under blind conditions by all three authors. Subjects were included for analysis only if consensus was obtained as to their Kinsey ratings. This was especially critical for the integrity of the homosexual sample. Homosexuality is a term that covers a wide range of childhood and adult experiences. To establish a group of fairly exclusively homosexual men, it was essential to distinguish volunteers having exclusively homosexual desires, interests, and experiences lifelong from men having such characteristics intermittently, periodically, or only recently. Of 150 volunteers, 90 met age, health, and substance use criteria. Of these, 70 eventually met criteria for inclusion in a group and agreed to participate as subjects. Several withdrew from the study because of scheduling conflicts, problems in giving blood samples, irregularities in menstrual cycling, illness, or undisclosed reasons. Of the 55 subjects participating throughout the study, data on several were excluded from statistical analysis because of abnormal baseline hormone concentrations, leaving a final sample size of 43 subjects.

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12. Blood was allowed to clot at room temperature for 30 minutes, then refrigerated (4°C) for 1 hour. Serum was stored frozen (−20°C) for later analysis. All samples from a given subject were assayed for a given hormone within a single run to minimize interassay variability. Radioimmunoassay for LH, in a preparation calibrated against appropriate reference preparations, was determined by a modified radioimmunoassay method [W. D. Odell *et al.*, *J. Clin. Med.* **70**, 873 (1967)]. Intra- and interassay variance for this method were 6.3 and 9.5 percent, respectively. Estrone was determined with a direct coated-charcoal radioimmunoassay by using a modified method of N. Jiang, R. J. Ryan, and A. Albert [*Clin. Chem.* **19**, 470 (1973)] with an antibody specific for estrone (antibody to estrone-6-thyroglobulin; Miles-Yeda). In addition to binding estrone, this antibody binds constituent steroids of Premarin, notably equilin (4.6 percent) and equilenin (14.5 percent), while not cross-reacting with any androgens or other estrogens. Plasma concentrations of testosterone were determined by coated charcoal radioimmunoassay with an antibody highly specific for testosterone (anti-T-3-oxime-BSA; a gift of G. Bolleli), which cross-reacts with androstenedione (<0.05 percent; all other, <0.01 percent). This method, a modification of that reported by F. Kohen, S. Bauminger, and H. R. Lindner [in *Steroid Immunoassay*, E. H. D. Cameron, S. G. Hillier, K. Griffiths, Eds. (Alpha Omega, Cardiff, Wales, 1975), pp. 11–32] requires no chromatography, a simple extraction with petroleum ether:ethyl acetate (5:2 by volume), and is routinely used in our laboratory. Intra- and interassay variances were 7.4 and 9.5 percent, respectively.
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# Plasticity of Substance P in Mature and Aged Sympathetic Neurons in Culture

Abstract. *The effect of age on the plasticity of the putative peptide neurotransmitter substance P (SP) was examined in the rat superior cervical sympathetic ganglion. Explantation of ganglia from 6-month-old rats to serum-supplemented culture resulted in a tenfold increase in SP concentration, reproducing results previously obtained for ganglia from neonatal rats. Veratridine prevented the increase in SP concentration in adult ganglia, and tetrodotoxin blocked the veratridine effect, suggesting that membrane depolarization and sodium influx prevented the rise in the SP content of adult ganglia as well as of neonatal ganglia. However, the time courses of the increase in the amount of the peptide differed in neonatal and mature ganglia, suggesting that some aspects of regulation may differ in the two. The effects of aging on neural plasticity were further analyzed by explanting ganglia from 2-year-old rats. No significant increase in SP concentration was observed in these ganglia. Remarkable plasticity thus seems to persist in mature neurons but may be deficient in aged sympathetic neurons.*

Evidence indicates that developing neurons are remarkably plastic, altering transmitter metabolism and even phenotypic expression in response to extracellular stimuli (*1–3*). Sympathetic neurons, long regarded as noradrenergic, may express cholinergic characters or the putative peptide transmitter substance P (SP) in vivo and in vitro during the neonatal period (*2–5*). However, whether transmitter plasticity is restricted to the developing neuron or whether it persists during maturity is unclear (*6*). Transmitter plasticity may play a role in the function of the adult nervous system.

To investigate this issue, we examined sympathetic neurons in the adult rat superior cervical ganglion (SCG). Details of the preparation and characteristics of antiserum to SP as well as the radioimmunoassay procedure have been described (*7*); the authenticity of immunoreactive SP was documented by high-performance liquid chromatography (*3*). Earlier studies have indicated that denervation (decentralization) of the neonatal rat SCG in vivo or its explantation to culture (with consequent denervation) markedly increased the amount of SP in principal sympathetic neurons (*3*). To determine whether explantation also increased SP in the adult rat SCG, we grew ganglia from 6-month-old rats under a variety of conditions for 1 week (Fig. 1). All groups of adult ganglia exhibited at least an eightfold increase in SP concentration, whether they were cultured in fully defined or serum-supplemented medium on buffer- or ammonia-reconstituted collagen (Fig. 1).

Depolarization of neonatal ganglia in culture is known to prevent the increase in SP concentration upon explantation (*3*). To determine whether depolarization similarly affects adult ganglia, we exposed explants to the depolarizing agent veratridine (*8*). Veratridine exposure completely blocked the increase of SP concentration in adult ganglia, reproducing the effect observed in neonatal ganglia (Fig. 2). Moreover, tetrodotoxin, which prevents the transmembrane sodium influx elicited by veratridine (*9, 10*), completely blocked the effect of veratridine (Fig. 2). Thus depolarization with attendant sodium influx prevented the increase in SP concentration in mature ganglia as it did in developing ganglia.

To characterize more fully the regulation of SP concentration in adult ganglia, we compared the time courses of the increases of the SP content of adult and neonatal ganglia. Although both adult and neonatal ganglia exhibited approximately tenfold increases in SP concentration after 7 days in culture, the temporal profiles of these increases differed markedly. In the neonatal ganglia the rise in SP concentration began virtually immediately, attaining a plateau after only 48 hours in culture (Fig. 3). In contrast, the amount of SP in adult ganglia increased relatively gradually, reaching only 18 percent of its final con-