Contraception—A Look Forward, Part III: Inhibin And Brain-Enhanced Estrogen Delivery

Robert J. Woolley, M.D.

Abstract: **The final installment of this review examines two contraceptive methods, inhibin and brain-enhanced estrogen delivery, which are radically different from any currently available.**

Inhibin is a gonadal hormone that specifically inhibits pituitary production of follicle-stimulating hormone. Before it was finally isolated in 1985, inhibin was expected to be an ideal contraceptive. Recent research, however, has shown that the inhibin hormonal system is unexpectedly complex, and hopes for clinical use of inhibin must be suspended for the present.

Although oral contraception is one of the most effective methods ever devised, use is limited by adverse effects of estrogen (or fears of such effects). A system known as brain-enhanced estrogen delivery specifically delivers estrogen to the brain, including the hypothalamus, there inducing suppression of the gonadotropin-releasing hormone. It has been described and tested in animals, and its successful development could replace current oral contraceptives and extend their availability to many more women.
(J Am Board Fam Pract 1991; 4:159-66.)

Inhibin

The earliest clue to the existence of the hormone now known as inhibin was the 1923 observation that radiation damage to the germinal epithelium of rat testes resulted in hypertrophy of specific cells of the anterior pituitary, much as would castration; this hypertrophy was postulated to be due to loss of some unknown testicular secretion. In the early 1930s, a watery extract of bull testes was shown to reverse this pituitary cellular hypertrophy with no apparent effect on the sex organs. "Inhibin" was the name given the unidentified factor responsible for these changes. Its character remained unknown for another 50 years.[1]

During this period, the main features of the luteinizing hormone, follicle-stimulating hormone, and gonadotropin-releasing hormone (LH-FSH-GnRH) system were elucidated. Among other things, release of LH and FSH was found at some periods of the life cycle to be under separate control. This action could not be attributed to GnRH, which consistently stimulates release of both hormones. The explanation seemed to be that LH could be released without FSH, not by selective stimulation of LH, but by selective inhibition of FSH. Inhibin, produced in the male Sertoli cells and female granulosa cells, was believed to be the mechanism responsible. (The inhibition of FSH production was, in fact, the primary means of assaying for the presence or purity of inhibin.)

Based on this model, projections were made that inhibin, once isolated, would make an ideal contraceptive drug. Given to men, it would curtail spermatogenesis by FSH deprivation without depression of LH, testosterone, or libido. In women, it would prevent recruitment and development of ovarian follicles, processes that require FSH. Such optimistic forecasts continued to be made as late as 1986.[2] They would prove to be premature.

Inhibin was finally purified and its amino acid sequence deduced in 1985. (The progression of this work has been reviewed in detail.[1,3,4]) Jock Findlay (World Health Organization Special Programme in Human Reproduction) soon thereafter reviewed what was known and not known about inhibin.[5] He identified, among others, the following defects in our knowledge and the unanswered questions about inhibin's ability to function as a contraceptive agent:

In men:

1. The degree to which spermatogenesis depends upon FSH in normal men is unknown;

From the University of Minnesota, Department of Family Practice and Community Health, St. Joseph's Hospital Residency Program, St. Paul. Address reprint requests to Robert J. Woolley, M.D., c/o Bethesda Family Physicians, 590 Park Street, Suite 310, St. Paul, MN 55103.

at least oligospermic levels can be maintained by LH alone.
2. If FSH is necessary for spermatogenesis, will inhibin suppress its production sufficiently?
3. If oligospermia can, in fact, be induced by inhibin, how is the fertilizing capacity of the remaining sperm affected?
4. Because high doses of inhibin also suppress LH production, will testosterone supplementation be required for treated men?

In women:

1. The effects of pure inhibin (free of other biologically active, gonadally derived agents) on in vivo FSH production and ovulation, luteal function, and cycle length are unknown.
2. In theory, too little FSH suppression would lead to luteal phase deficiency and short cycles; too much suppression could cause amenorrhea, LH suppression, and the problems of unopposed estrogen. Can a correct dosage range be found?
3. What will be the clinical consequences of the known tendency of FSH production to rebound to abnormally high levels after ceasing suppression by inhibin?

For both sexes:

1. Will long-term use of inhibin, a glycoprotein, induce antibody response? (This could theoretically result in eventual hyperfecundity after inhibin treatment ends, because of inactivation of endogenous inhibin.)
2. Can the cost be made competitive with that of other forms of contraception?
3. Inhibin is known to demonstrate some genetic and structural homology with human transforming growth factor-β (TGFβ). TGFβ can either promote or inhibit cell growth, depending on the presence of other growth factors, and is found in some types of neoplastic tissues. Can such potential long-term effects confidently be excluded on the basis of standard clinical trials?

It has also been suggested that an increase in the ratio of circulating LH to FSH (an effect that would be expected after inhibin administration) could contribute to the genesis of polycystic ovarian disease.[1]

As though these challenges were not enough, subsequent research has shown the model of a simple inhibin-FSH feedback loop to be woefully inadequate.

Inhibin is found in two forms, A and B, each consisting of an α subunit and a β subunit. The α subunit has only one known form, an 18-kilodalton (kD) polypeptide. Two known forms of the β subunit are the similar but distinct polypeptides of 14-kD molecular weight, known as β_A and β_B. Inhibin A is a dimer of α and β_A, joined by disulfide bonds; inhibin B is formed by α and β_B (Figure 1). Inhibins A and B appear to have similar activities in vitro, and the physiological purpose of the existence of separate forms, if any, is not known. It has been demonstrated, however, that these forms of inhibin are produced in several extragonadal sites (such as bone marrow, placenta, and pituitary and adrenal glands), as well as in the ovary and testis, and that RNA expression of the inhibin subunits varies among different tissues, implying that the ratios of the dimers may cause tissue-specific differences in inhibin function.[6]

In 1986, it was reported that dimers of the β subunits, with no α component, also possessed biological activity, but their effects *oppose* those of the inhibins.[7-9] The β_A-β_A homodimer and the β_A-β_B heterodimer are potent stimulators of pituitary FSH synthesis and secretion in vitro. "Activin" is produced by the Leydig cells in men; the ovarian site of synthesis has yet to be identified.[10] Whether activin is actually circulated outside the gonads in vivo is unknown. The two

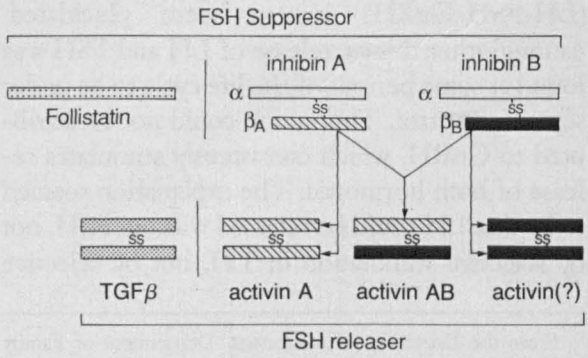


Figure 1. Gonadal protein hormones modulating the secretion of FSH. Redrawn and used with permission from Ying.[4]

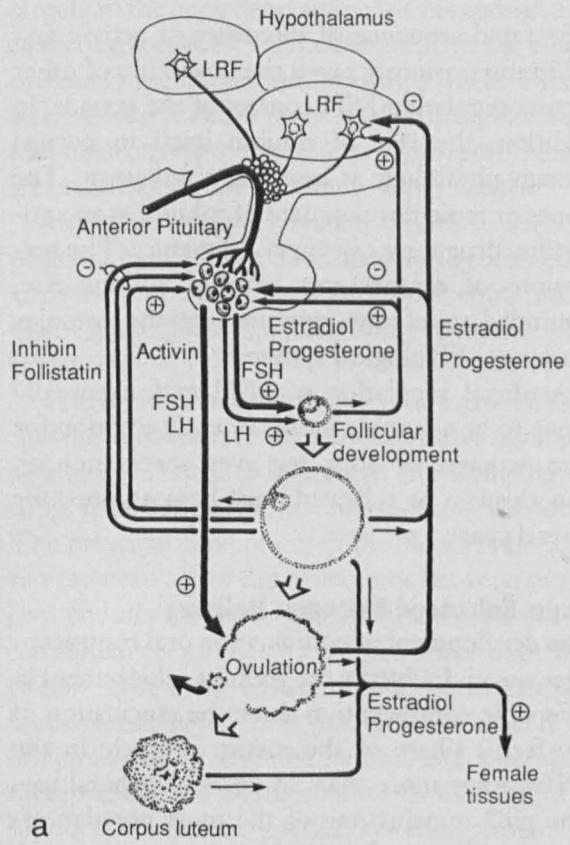
forms are being called activin A and activin A-B, respectively.[11] (The existence of a β_B-β_B homodimeric form would produce a logical completeness but has not yet been verified) (Figures 1–3).

Although both GnRH and activin can provoke release of FSH, activin differs in having no effect on LH, no interference from synthetic GnRH analogues, and a slower onset of action. (Both inhibin and activin appear to influence FSH production at the level of protein synthesis, rather than the level of hormonal release, as with GnRH.) A hypothalamic-releasing hormone more specific for FSH than for LH has long been postulated, with some supporting experimental evidence,[3] and has even been provisionally named by its proponents "follicle-stimulating hormone releasing hormone" (FSHRH); activin appears not to be the same as this supposed factor.[7]

The model of the hypothalamic-pituitary-gonadal axis was further complicated the year after the discovery of the activins by the announcement of follistatin.[12,13] Follistatin is a protein derived from follicular fluid. (Actually, it is two somewhat different proteins; the molecular weight of one is 32 kD, the other is 35 kD.) It specifically suppresses the secretion of FSH by pituitary cells in vitro but is not homologous with inhibin or activin (Figures 2 and 3). Its action is distinguished from that of inhibin by lower potency (on an equimolar basis) and by suppression of FSH release rather than FSH synthesis. The effects of follistatin and inhibin are additive, and antibodies raised against one are not cross-reactive with the other. As with the activins, whether follistatin is released outside the gonads in vivo is not yet known.

Surprises about this endocrinologic axis continue to be announced at a perplexing rate. The following are samples of recent discoveries:

1. Inhibin is secreted by selected areas of rat brain, including neurons that termi-



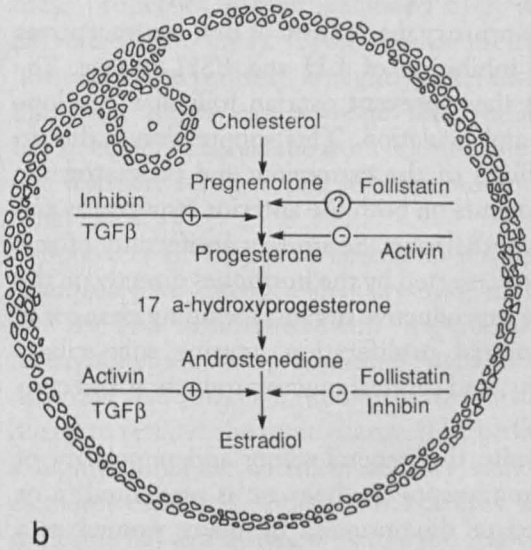


Figure 2. (a) Summary of hormonal control of ovarian function. The hypothalamus produces GnRH (LRF), which acts on the pituitary cells to secrete FSH and LH. A secretion of FSH, together with tonic LH, stimulates the follicular development. The developed follicles secrete estradiol, progesterone, inhibins, activins, and follistatins. Estradiol and progesterone, at different concentrations or ratios, either positively or negatively feed back to the hypothalamic hypophyseal axis in regulating the secretion of FSH and LH. Inhibins and follistatins specifically suppress, whereas activin and TGFβ enhance the secretion of FSH by the pituitary. (b) Schematic representation of intragonadal function of inhibins, activins, TGFβ, and follistatin. Redrawn and used with permission from Ying, 1988.[4]

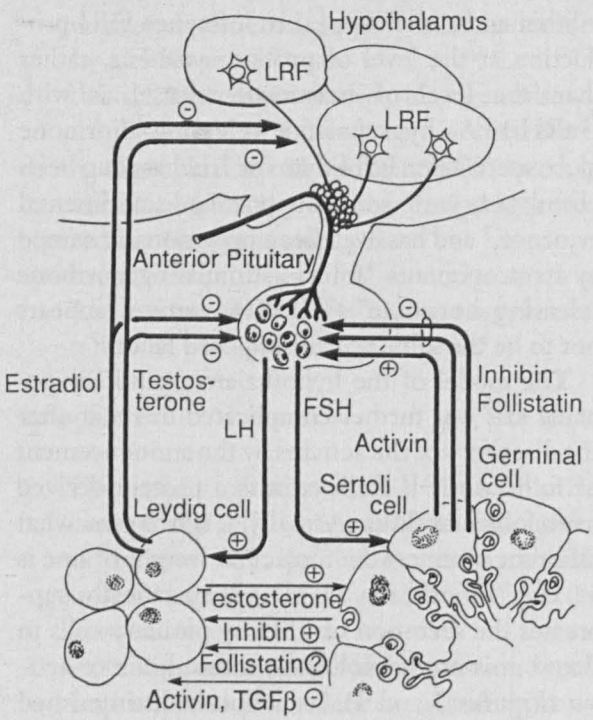


Figure 3. Summary of hormonal control of testicular function. GnRH (LRF) secreted by hypothalamic neurons transported by axons to hypophyseal portal vessels to the anterior pituitary to release LH and FSH. LH acts on Leydig cells to secrete testosterone, which stimulates the germinal cells for spermatogenesis, whereas FSH stimulates gonadal protein production by Sertoli cells (inhibin, activins, or follistatin). Testicular steroids negatively feed back to hypothalamic-hypophyseal axis for LRF-FSH or LH secretion. Inhibin and follistatin specifically suppress, while activin enhances FSH release by the pituitary. Inhibin increases, but activin and TGFβ (a protein widely distributed in the body) decrease testosterone production by Leydig cells. Modified and redrawn from Ying, 1988.[4]

nate in the GnRH-producing areas of the hypothalamus.[14]

2. In men, inhibin production can apparently be stimulated directly by FSH and also indirectly by LH; this latter is a phenomenon not seen in any animal species.[15]
3. Inhibin levels are elevated in the presence of hydatidiform moles and may prove to be a clinically useful marker of postevacuation persistent trophoblastic disease.[16]
4. Granulosa cell tumors secrete elevated levels of inhibin, and inhibin has been suggested as a serum tumor marker.[17]
5. Activin can elicit oxytocin secretion, and antibodies to inhibin (which probably cross-react with activin) attenuate oxytocin secretion and lactation.[14]
6. Activin has been confirmed to stimulate erythropoiesis in laboratory animals, as well as tissue cultures.[18]
7. Both inhibin and activin may be mediators of the immune response through action on thymocytes.[19]

The rapid sequence of discovery of activin and follistatin obviously raises the possibility of other factors regulating FSH control of the gonads. In addition, the role of inhibin itself in normal human physiology is essentially unknown. The hopes of rapid development of inhibin as an antifertility drug were obviously premature. The revelations of recent years have, if nothing else, reminded us of our ignorance of the complex workings of biological systems.

Artificial regulation of FSH may eventually prove to be a feasible means of contraception for men, women, or both, but even speculation on how this can be achieved must be postponed for several years.

Brain-Enhanced Estrogen Delivery

The development of combination oral contraceptives was undoubtedly the greatest achievement in reversible contraception since the elucidation of the fertile phase of the menstrual cycle in the 1930s. After more than 25 years of general use, "the pill" remains one of the most popular, effective, and safe forms of birth control in the world.

The primary mechanism of oral contraceptives is the inhibition of LH and FSH release. Together these prevent ovarian follicular development and ovulation. This suppression is due to the effects of the estrogenic and progestogenic components on both the anterior hypophysis and the hypothalamus. Secondary antifertility effects are those exerted by the hormones directly on the female reproductive organs, including changes in endometrial proliferation, uterine tube ciliary motility, and cervical mucus production and consistency.

Despite the general safety and popularity of oral contraceptives, their use is prevented in or avoided or discontinued by many women as a

result of the many other effects of estrogen. Risk is increased for thrombotic events, hypertension, benign liver tumors, and gallbladder disease, and the issue of breast cancer seems never to be resolved. Serum triglycerides can be elevated. Subjective side effects include weight gain, depression, nausea, and headache. Drug interactions have been reported with antibiotics, barbiturates, anticonvulsants, antidepressants, theophylline, and vitamins. All of these adverse effects are due largely to the peripheral actions of the estrogen in oral contraceptives. Oral contraceptive use could presumably increase significantly if these effects were reduced or eliminated by delivering the hormone selectively to the pituitary or hypothalamus and avoiding its distribution to other tissues.

A chemical delivery system (CDS) to accomplish this goal is being developed by a group at the University of Florida and two proprietary interests, Gynex (Deerfield, IL) and Pharmatec (Alachua, FL).

The brain-enhanced delivery of estrogen is a specific application of general pharmacologic agents developed to overcome the difficulty of drug penetration of the blood-brain barrier.[20] The principal component of the blood-brain barrier is a network of tight junctions between capillary endothelial cells, which force blood-borne substances to migrate through, rather than between, these cells, either to enter or to exit the brain parenchyma. This passage is enhanced by lipophilia (to traverse the luminal and abluminal membranes) and by the ability to use any of a number of specific carrier systems present in the endothelial cells. Many drugs do not have these properties and are excluded from central nervous system entry. In the case of the steroid hormones, the problem is slightly different; their lipophilia allows easy passage into the brain, but an equally easy escape from it, thus necessitating constant serum levels to maintain constant brain levels.

Bodor, et al.[21] reported successful testing of a brain-selective estrogen delivery system. Estradiol is first condensed with trigonelline (1-methylnicotinic acid, a natural metabolite of nicotinic acid [niacin]), then quaternized and reduced to remove the ionic charge. The product is a highly lipophilic chemical delivery system, an estradiol esterified with a carrier. This hybrid molecule has low inherent estrogenic activity be-

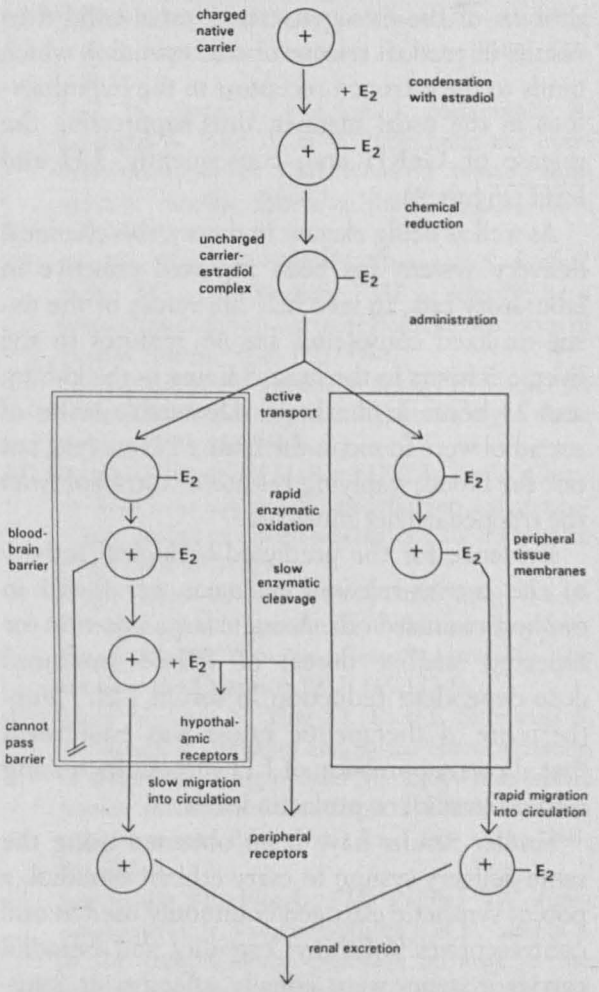


Figure 4. Simplified schematic representation of the synthesis and distribution of a brain-enhanced chemical delivery system for estrogen. Modified from Brewster ME, Estes KS, Bodor N.[23]

cause the 17-esterification prevents binding with estrogen receptors. Pharmatec's developmental designation for the complex is PR-63.

Intravenous administration of this compound in animals results in rapid tissue distribution because of ease of membrane passage. In peripheral tissues, the enzymatically labile dihydropyridine carrier is rapidly oxidized back to its more stable quaternary salt form, rendering it hydrophilic and subject to renal elimination. (It is believed that oxidation is due to the ubiquitous NADH-dehydrogenases.)[20] In the brain, similar transmembrane passage and oxidation of the carrier moiety to its polar form occur, but the blood-brain barrier then prevents outward migration of the now-ionic compound. The carrier-estrogen complex is, in essence, trapped in the central nervous system by its conversion to a polar form. Slow hy-

drolysis of the estrogen-carrier ester bond then results in gradual release of free estradiol, which binds to the estrogen receptors in the hypothalamus in the usual manner, thus suppressing the release of GnRH and, consequently, LH and FSH (Figure 4).

As well as being elegant in theory, this chemical delivery system has been reported effective in laboratory rats. In vivo half-life values of the tissue-oxidized compound are 46 minutes in the liver, 5.5 hours in the lung, 8 hours in the kidney, and 23 hours in the brain. Detectable levels of estradiol were found in the brain of these rats, but not the blood, implying release of estradiol from the trapped carrier molecule.[21]

Evidence for the predicted biological activity of the carrier-released estrogen was found in oophorectomized rats. A single large injection (or repeated smaller doses) of PR-63 produced dose-dependent reduction in serum LH.[22] Furthermore, a therapeutic range was established that allows suppression of LH without increasing serum estradiol or prolactin levels.

Similar results have been obtained using the same delivery system to carry ethinyl estradiol, a potent synthetic estrogen commonly used in oral contraceptives.[23] Ethinyl estradiol and estradiol carrier systems were equally effective at long-term (18 days) suppression of LH. The ethinyl estradiol system was shown to have the same desirable properties as the estradiol system, namely, short peripheral elimination half-life and long brain half-life. (Because the ethinyl estradiol system showed no clear advantage over that using the natural estradiol, future research is expected to focus on the latter.)

Inhibition of ovulation by the estradiol chemical delivery system was reported by a group at the University of California at San Diego.[24] The chemical delivery system was three times as effective as an equimolar dose of free estradiol at lengthening the estrous cycle of rats; to double the length of the cycle required only 1/20th the molar dose of estradiol when coupled to the delivery system. Treated rats had significant delays in LH surge and ovulation. Brain level elevations of estradiol persisted four to five times longer when the hormone was delivered by carrier. These researchers also found elevated levels of GnRH in the hypothalamus and depressed levels in the hypothalamic-hypophyseal portal vein of rats 16 days after estradiol-carrier treatment, suggesting that gradual release of the active estradiol from its carrier suppresses release of GnRH from the parvicellular neurons of the hypothalamus. They also note that, because they did not see a late LH surge, the site of action of the estradiol delivery system is more likely to be the hypothalamus than the pituitary; continual estrogen stimulation of the pituitary changes its negative-feedback loop to a positive-feedback loop (as in the late follicular phase of the menstrual cycle) and should have produced such a surge if the pituitary were the primary repository of the carrier estradiol. (The anterior pituitary is outside the blood-brain barrier and would be expected to demonstrate rapid elimination of administered estrogen carrier, just as do peripheral tissues.)

A movement from laboratory to clinical studies is underway on several fronts. First, unpublished animal studies on the feasibility of oral administration have shown no gastrointestinal oxidation of the dihydropyridine carrier, which would prevent its access to the central nervous system and defeat the purpose of the CDS, but the studies have shown some degree of cleavage of the ester bond by which it holds the estradiol. For this reason, sublingual tablets will probably be the form of administration ultimately developed (Kerry S. Estes, Ph.D., personal communication, 13 February 1990). Second, the first human trials of the CDS have already begun. Intravenous administration of PR-63 to 10 postmenopausal women demonstrated sustained, dose-dependent suppression of LH release with no subjective side effects or adverse effects on pulse, blood pressure, or serum chemistry determination.[25] Third, the addition of a cyclodextrin complex to the formulation has been found to extend its shelf life and increase its water and tissue solubility.[26]

Uses for this system other than contraception have been proposed. The goal of estrogen treatment of prostate cancer is the suppression of gonadal testosterone production by artificial inhibition of LH release. This action could presumably be accomplished by hypothalamic suppression of GnRH release by PR-63, greatly reducing the peripheral side effects of traditional estrogen therapy, such as hypertension and edema.[23] The vasomotor symptoms of menopause could probably be suppressed by PR-63,[23] and relief thus provided for the many symptomatic women who

have a contraindication to systemic estrogens. The many beneficial effects of systemic estrogen in menopausal women, however, would be forfeited. Finally, this system might be useful in treating obesity. In laboratory animals, the direct estrogenic stimulation of the hypothalamus produces long-term weight loss, and these effects have been duplicated with PR-63.[27,28]

This system is still in the early testing stages. Full toxicology studies need to be performed, although the carrier itself appears not to be neurotoxic to primates.[29] Much remains to be learned about the pharmacologic fate of the carrier in different tissues, the multiple endocrinologic effects of the gradual central nervous system release of estrogen (e.g., on FSH and progesterone), and the need for concomitant progestin use. Conceptually, there is no obvious barrier to eventual success.

Such success should mean the ability to inhibit LH release and ovulation with much lower doses of estrogen than are used in current oral contraceptives, resulting in fewer problems created by circulating and tissue-bound estrogens and presumably with less frequent dosing. Not only could this achievement supplant all current oral contraceptives, it could greatly enlarge the number of women able to use them.

My thanks to Anne Robin, M.D., and David K. Webb, M.D., for helpful hints and encouragement; to Linda Coates, Karen Brudvig, and the entire medical library staff at the University of Illinois, Urbana, for expert bibliographic assistance; and to my wife, Betsy, for respecting my hours in the library.

References

1. de Jong FH. Inhibin—its nature, site of production, and function. Oxf Rev Reprod Biol 1987; 9:1-53.
2. Sheth AR, Moodbidri SB. Potential application of inhibin in male and female contraception. Adv Contracept 1986; 2:131-9.
3. Li CH, Ramasharma K. Inhibin. Annu Rev Pharmacol Toxicol 1987; 27:1-21.
4. Ying SY. Inhibins, activins, and follistatins: gonadal proteins modulating the secretion of follicle-stimulating hormone. Endocr Rev 1988; 9:267-93.
5. Findlay J. The nature of inhibin and its use in the regulation of fertility and diagnosis of infertility. Fertil Steril 1986; 46:770-83.
6. Meunier H, Rivier C, Evans RM, Vale W. Gonadal and extragonadal expression of inhibin α, β_A, and β_B subunits in various tissues predicts diverse functions. Proc Natl Acad Sci USA 1988; 85:247-51.
7. Tsonis CG, Sharpe RM. Dual gonadal control of follicle-stimulating hormone. Nature 1986; 321: 724-5.
8. Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, et al. Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature 1986; 321:776-9.
9. Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, et al. Pituitary FSH is released by a heterodimer of the β-subunits from the two forms of inhibin. Nature 1986; 321:779-82.
10. Lee W, Mason AJ, Schwall R, Szonyi E, Mather JP. Secretion of activin by interstitial cells in the testis. Science 1989; 243:396-8.
11. Burger HG, Igarashi M, Baird DT, Bardin W, Chappel S, de Jong F, et al. Inhibin: definition and nomenclature, including related substances. Clin Endocrinol (Oxf) 1988; 28:448-9.
12. Ying SY, Becker A, Swanson G, Tan P, Ling N, Esch F, et al. Follistatin specifically inhibits pituitary follicle stimulating hormone release in vitro. Biochem Biophys Res Commun 1987; 149:133-9.
13. Ueno N, Ling N, Ying SY, Esch F, Shimasaki S, Guillemin R. Isolation and partial characterization of follistatin: a single-chain M_r 35,000 monomeric protein that inhibits the release of follicle-stimulating hormone. Proc Natl Acad Sci USA 1987; 84:8282-6.
14. Sawchenko PE, Plotsky PM, Pfeiffer SW, Cunningham ET Jr, Vaughn J, Rivier J, et al. Inhibin β in the central neural pathways involved in the control of oxytocin secretion. Nature 1988; 334: 615-7.
15. McLachlan RI, Matsumoto AM, Burger HG, de Kretser DM, Bremner WJ. Relative roles of follicle-stimulating hormone and luteinizing hormone in the control of inhibin secretion in normal men. J Clin Invest 1988; 82:880-4.
16. Yohkaichiya T, Fukaya T, Hoshiai H, Yajima A, de Kretser DM. Inhibin: a new circulating marker of hydatidiform mole? Br Med J 1989; 298: 1684-6.
17. Lappohn RE, Burger HG, Bouma J, Bangah M, Krans M, de Bruijn HW. Inhibin as a marker for granulosa-cell tumors. N Engl J Med 1989; 321: 790-3.
18. Schwall R, Schmelzer CH, Matsuyama E, Mason AJ. Multiple actions of recombinant activin-A in vivo. Endocrinology 1989; 125:1420-3.
19. Bremner WJ. Inhibin: from hypothesis to clinical application. N Engl J Med 1989; 321:826-7.
20. Bodor N, Brewster ME. Problems of delivery of drugs to the brain. Pharmacol Ther 1982; 19: 337-86.
21. Bodor N, McCornack J, Brewster ME. Improved delivery through biological membranes. XXII. Synthesis and distribution of brain-selective estrogen delivery systems. Int J Pharm 1987; 35:47-59.

22. Anderson WR, Simpkins JW, Brewster ME, Bodor N. Evidence for suppression of serum LH without elevation in serum estradiol or prolactin with a brain-enhanced redox delivery system for estradiol. Life Sci 1988; 42:1493-502.
23. Brewster ME, Estes KS, Bodor N. Improved delivery through biological membranes. 32. Synthesis and biological activity of brain-targeted delivery systems for various estradiol derivatives. J Med Chem 1988; 31:244-9.
24. Sarkar DK, Friedman SJ, Yen SS, Frautschy SA. Chronic inhibition of hypothalamic-pituitary-ovarian axis and body weight gain by brain-directed delivery of estradiol-17β in female rats. Neuroendocrinology 1989; 50:204-10.
25. Howes J, Bodor N, Brewster M, Estes K, Eve M. A pilot study with PR-63 in postmenopausal volunteers [abstract]. J Clin Pharmacol 1988; 28:951.
26. Brewster ME, Estes KS, Loftsson T, Perchalski R, Derendorf H, Mullersman G, et al. Improved delivery through biological membranes. XXXL: solubilization and stabilization of an estradiol chemical delivery system by modified β-cyclodextrins. J Pharm Sci 1988; 77:981-5.
27. Simpkins JW, Anderson WR, Dawson R Jr, Seth A, Brewster M, Estes K, et al. Chronic weight loss in lean and obese rats with a brain-enhanced chemical delivery system for estradiol. Physiol Behav 1988; 44:573-80.
28. Estes KS, Brewster ME, Bodor NS. A redox system for brain targeted estrogen delivery causes chronic body weight decrease in rats. Life Sci 1988; 42:1077-84.
29. Brewster ME, Estes KS, Perchalski R, Bodor N. A dihydropyridine conjugate which generates high and sustained levels of the corresponding pyridinium salt in the brain does not exhibit neurotoxicity in cynomolgus monkeys. Neurosci Lett 1988; 87:277-82.

NOTICE

Certificate of Added Qualifications in Geriatric Medicine Examination April 10, 1992

The next examination for the American Board of Family Practice Certificate of Added Qualifications in Geriatric Medicine will be administered on April 10, 1992. Application materials for this exam will be available July 1, 1991. ABFP Diplomates interested in participating in the exam should request application materials by writing to:

Geriatric Medicine Examination
American Board of Family Practice
2228 Young Drive
Lexington, Kentucky 40505-4294