ever. Many mechanisms for learning and pattern recognition have been proposed (*17*) and may be incorporated into this model. Central analyzers in our model are no longer the last neuronal processing stage. Instead, their responses are directed peripherally, where they select, modify, or generate stimulus patterns.

REFERENCES AND NOTES

1. D. H. Hubel and T. N. Wiesel, *J. Physiol. (London)* **160**, 106 (1962); *J. Neurophysiol.* **28**, 229 (1965); *Proc. R. Soc. London Ser. B* **189**, 1 (1977).
2. J. S. Lund, R. D. Lund, A. E. Hendrickson, A. H. Hunt, A. F. Fuchs, *J. Comp. Neurol.* **164**, 287 (1975); W. Singer, *Physiol. Rev.* **57**, 386 (1977); R. T. Marocco, J. W. McClurkin, R. A. Young, *J. Neurosci.* **2**, 256 (1982); S. M. Sherman and C. Koch, *Exp. Brain Res.* **63**, 1 (1986).
3. M. Steriade, N. Ropert, A. Kitsikis, G. Oakson, in *The Reticular Formation Revisited,* J. A. Hobson and M. A. B. Brazier, Eds. (Raven, New York, 1980), pp. 125–167; G. Ahlsén and F. S. Lo, *Brain Res.* **238**, 433 (1982); H. C. Hughes and W. H. Mullikin, *Exp. Brain Res.* **54**, 253 (1984); G. Ahlsén, S. Lindström, F. S. Lo, *J. Physiol. (London)* **347**, 593 (1984); *Exp. Brain Res.* **58**, 134 (1985); M. Steriade, L. Domich, G. Oakson, *J. Neurosci.* **6**, 68 (1986).
4. L. S. Ide, *J. Comp. Neurol.* **210**, 317 (1982).
5. L. F. Kromer and R. Y. Moore, *Neuroscience* **5**, 255 (1980).
6. S. M. Hersh and E. L. White, *Neurosci. Lett.* **24**, 207 (1981); K. S. Rockland and D. N. Pandya, *Brain Res.* **212**, 249 (1981); D. C. Van Essen, in *Cerebral Cortex,* A. Peters and E. G. Jones, Eds. (Plenum, New York, 1985), vol. 3, pp. 259–329.
7. W. Singer, F. Tretter, M. Cynader, *Brain Res.* **102**, 71 (1976).
8. E. Harth, *Biol. Cybern.* **22**, 169 (1976); ——— and K. P. Unnikrishnan, *Int. J. Psychophysiol.* **3**, 101 (1985).
9. Dynamics of the system can be extended by making these coefficients variable through introduction of learning and association algorithms.
10. N. Metropolis, A. W. Rosenbluth, M. N. Rosenbluth, A. H. Teller, E. Teller, *J. Chem. Phys.* **21**, 1087 (1953).
11. S. Kirkpatrick, C. D. Gelatt, Jr., M. P. Vecchi, *Science* **220**, 671 (1983); G. E. Hinton and T. J. Sejnowski, *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition* (1983), p. 448; D. H. Ackley, G. E. Hinton, T. J. Sejnowski, *Cognit. Sci.* **9**, 147 (1985); J. J. Hopfield and D. W. Tank, *Biol. Cybern.* **52**, 152 (1985); *Science* **233**, 625 (1986); L. T. Wille, *Nature (London)* **324**, 46 (1986).
12. Alopex is an acronym for ALgorithm Of Pattern Enhancing CROSScorrelations.
13. E. Harth and E. Tzanakou, *Vision Res.* **14**, 1475 (1974); E. Tzanakou, R. Michalak, E. Harth, *Biol. Cybern.* **35**, 161 (1979).
14. E. Harth, A. S. Pandya, K. P. Unnikrishnan, *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition* (1986), p. 662; E. Harth, K. P. Unnikrishnan, A. S. Pandya, in *Computer Simulation in Brain Science,* R. Cotterill, Ed. (Cambridge Univ. Press, Cambridge, in press).
15. A. A. Verveen and H. E. Derksen, *Kybernetik* **2**, 152 (1965).
16. F. H. C. Crick, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4586 (1984).
17. E. Harth and S. Edgar, *Biophys. J.* **7**, 689 (1967); G. M. Edelman and G. N. Reeke, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2091 (1982); S. Grossberg, in *Competition and Cooperation in Neural Nets,* S. Amari and M. A. Arbib, Eds. (Springer-Verlag, New York, 1982), pp. 295–341; J. J. Hopfield, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2554 (1982); G. L. Shaw, D. J. Silverman, J. C. Pearson, *ibid.* **82**, 2364 (1985).

18. This research was supported by research contracts DAAG 29-83-K-0167 and DAAL 03-87-K-0034 of the Army Research Office and BRSG grant S07 RR077068-21 of the Biomedical Research Support Grant Program, NIH.

2 January 1987; accepted 1 May 1987

Localization, Secretion, and Action of Inhibin in Human Placenta

FELICE PETRAGLIA, PAUL SAWCHENKO, ALAN T. W. LIM, JEAN RIVIER, WYLIE VALE

Inhibin is a gonadal glycoprotein hormone that regulates the production of follicle-stimulating hormone (FSH) by the anterior pituitary gland and exhibits intragonadal actions as well. The present study shows that inhibin-like immunoreactivity (inhibin-LI) is present in cells of the cytotrophoblast layer of human placenta at term and in primary cultures of human trophoblasts. Human chorionic gonadotropin (hCG) stimulated secretion of inhibin-LI from these cultured placental cells. This effect was mimicked by 8-bromo–cyclic adenosine monophosphate (8-bromo–cAMP), forskolin, and cholera toxin, suggesting that the mechanism of hCG induction of placental inhibin-LI secretion is cAMP-dependent. Incubation with an antiserum that binds the α-subunit of human inhibin increased the secretion of hCG and gonadotropin-releasing hormone–like immunoreactivity (GnRH-LI) from trophoblast cells in culture, suggesting a local tonic inhibitory action of endogenous inhibin on hCG and GnRH-LI release. The action of inhibin on hCG secretion may partially require the presence of placental GnRH, as suggested by evidence that a synthetic GnRH antagonist partially reverses the hCG increase induced by inhibin immunoneutralization. Results suggest paracrine roles for both inhibin and GnRH in the regulation of placental hCG production.

THE PLACENTA SYNTHESIZES AND SECRETES protein and peptide hormones that are active both in the maternal-fetal compartment and within the placenta. Human chorionic gonadotropin (hCG) is the polypeptide hormone that regulates progesterone production from corpus luteum and placenta (*1*); a placental gonadotropin-releasing hormone (GnRH) has been proposed as a local modulator of hCG secretion (*2*). Because peptides participate in the mechanism of control of hormonogenesis in the chorionic system (*3*), the presence of inhibin-like bioactivity and immunoreactivity in rabbit and human placenta at term (*4, 5*) suggests a possible role for inhibin in the endocrinology of pregnancy. Inhibin, a heterodimeric protein with α and β subunits, has been isolated from porcine and bovine follicular and ram rete testis fluids; it selectivity inhibits the release of follicle-stimulating hormone (FSH) from the pituitary (*6*). The amino acid structure of porcine, bovine, and human inhibin has been determined from complementary DNA (cDNA) sequences (*7*). We have also identified the human inhibin α-chain messenger RNA (mRNA) in a term placental cDNA library (*8*). The aim of the present study was to localize placental inhibin and explore its local action and regulation.

To determine where inhibin-like immunoreactivity (inhibin-LI) is present in placental cells, we conducted immunohistochemical localization studies on immersion-fixed samples of three fresh human placentas collected at term. Indirect immunofluorescence staining (*9*) with antiserum to porcine inhibin-α-(1–25)-Gly-Tyr revealed numerous immunoreactive cells in the placental villi (Fig. 1). These appeared to be localized in the central (cytotrophoblast) layer of the villi. When the antiserum was adsorbed with rat synthetic corticotropin-releasing factor (CRF) or GnRH, other peptides present in cytotrophoblasts (*2, 3, 10*) did not interfere with staining for inhibin-LI. The antisera used to detect inhibin-LI in human placenta were used previously to stain porcine and rat ovarian follicular granulosa cells, which are widely acknowledged to be the principal site of inhibin production in the female. Trophoblasts produce placental hormones (*1*), and cytotrophoblast cells show intense positive staining for GnRH, CRF, and somatostatin (*2, 3, 10*).

To study the regulation of inhibin secretion from the placenta, we developed a monolayer primary culture of human trophoblasts. Placenta collected from pregnancy at term was minced, rinsed, and dissected

F. Petraglia, A. T. W. Lim, J. Rivier, W. Vale, The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.
P. Sawchenko, Developmental Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

from the connective tissue. Cells were dispersed enzymatically [0.4% collagenase II, 3000 IU of KUnitz (Cooper Biomedical) deoxyribonuclease type IV, and 0.4% trypsin (Sigma)] for 1 hour in a water-jacketed spinner suspension flask. Dissociated cells were washed, suspended in culture medium (*11*), and plated in 35-mm six-well multidishes. The experiments were carried out 1 week after cells were plated. The inhibin-LI concentration in the culture medium was measured by a specific radioimmunoassay with the same antiserum to the α-subunit of porcine inhibin.

To evaluate the mechanisms regulating inhibin-LI secretion from placental culture, we added either the adenylate cyclase activators forskolin or cholera toxin, or we added 8-bromo–cyclic adenosine monophosphate (8-bromo–cAMP). The addition of any of these substances increased the secretion of inhibin-LI in a dose-dependent manner (Fig. 2A), suggesting that cAMP may be a second messenger that induces the release of inhibin-LI from human placental cells. Because hCG actively increases placental adenylate cyclase and cAMP formation (*12*), we tested the effect of hCG on inhibin-LI secretion from placental cells. Inhibin-LI concentrations were increased by hCG in a dose-related manner (Fig. 2B), and neither the α-subunit of hCG nor human placental lactogen (hPL) significantly modified the inhibin-LI secretion from the culture preparation (Fig. 2B). The presence of estradiol, estriol, or progesterone (from 10^{-9} to $10^{-5}M$) did not influence the basal and the hCG-induced secretion of placental inhibin-LI. These results suggest that hCG may locally stimulate the secretion of inhibin-LI, probably acting through cAMP formation. This result is in agreement with the observation that in rat granulosa cell cultures, FSH is a major stimulus for inhibin-LI secretion, acting through cAMP formation (*13*); it is interesting that hCG in the gonads has FSH-like (*14*) and luteinizing hormone (LH)-like (*15*) activities. The lack of any change in inhibin-LI secretion when steroids are incubated in our placental cell culture supports the previous demonstration that steroids do not play an important role in the secretion of other glycoprotein hormones (hCG or hPL) from placental tissue preparations (*16*).

To determine the possible role of endogenous placental inhibin on the secretion of hCG, we incubated placental cells in culture with antiserum to inhibin. In the presence of this antiserum, the concentration of hCG-LI in cell culture medium was significantly higher than that in cells treated with serum from unimmunized rabbits (NRS) (Fig. 3). Antisera to somatostatin or prolactin did not induce significant changes in hCG-LI concentration. The increase in the concentration of hCG-LI was time-dependent and dose-related. This result suggests that placental inhibin modulates local gonadotropin secretion in an inhibitory fashion, just as gonadal inhibin decreases FSH secretion from the pituitary (Fig. 3).

Because previous reports suggested a stimulatory action of placental GnRH on hCG secretion (*2*), we evaluated the possibility that the effect of inhibin on hCG involves an inhibition of GnRH secretion. The observation that GnRH-LI concentrations in cell cultures treated with antiserum to inhibin was significantly higher than in cells treated with NRS (Fig. 4) suggested a possible tonic inhibitory action of inhibin on placental GnRH secretion. In addition, a synthetic GnRH antagonist [Ac-DNal(2)[1],

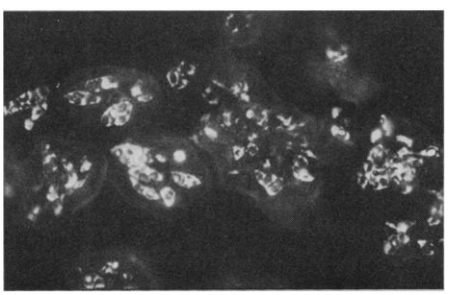

Fig. 1. Fluorescence photomicrograph of inhibin-immunoreactive cells in human placenta (×460). Stained cells are localized in the centrally situated cytotrophoblast layer of the placental villi. The more peripherally situated syncytiotrophoblasts are unstained. Placentas were fixed in 4% paraformaldehyde in 0.1*M* phosphate buffer for 4 to 6 hours at room temperature and postfixed overnight at 4°C in the same fixative, which also contained 10% sucrose. Frozen sections (20 μm) were incubated for 48 hours at 4°C in a 1:1000 dilution of rabbit antiserum to porcine inhibin-α-(1–25)-Gly-Tyr, which was conjugated via bisdiazotized benzidine to human α-globulin. Primary antisera were preadsorbed with an excess of human α-globulin, diluted in 0.02*M* phosphate-buffered saline containing 0.3% Triton X-100 and 5% bovine serum albumin (BSA), and localized by use of a biotin-streptavidin detection system (Amersham) with a Texas red fluorophore. Nonfat dry milk (5%) was added to the biotinylated secondary antiserum to minimize nonspecific binding (*21*). Specific staining was abolished by preincubation of the primary antisera with 100 μg/ml of synthetic porcine inhibin-α-(1–25)-Gly-Tyr and was not affected by addition of similar concentrations of rat CRF or GnRH.

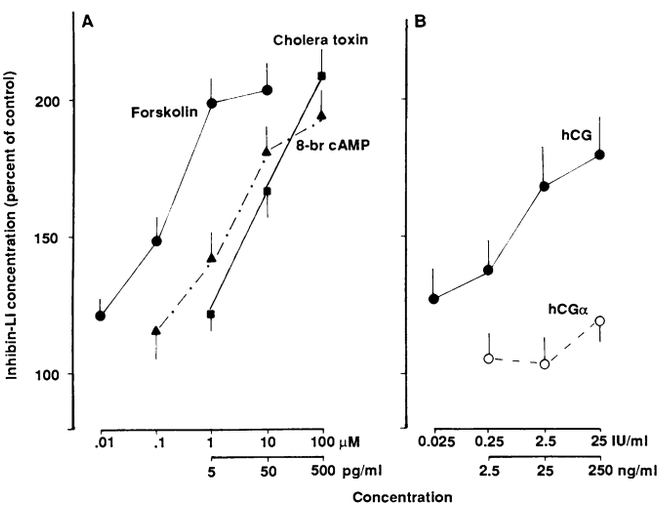

Fig. 2. (**A**) Forskolin (●) (μ*M*), 8-bromo-cAMP (▲) (μ*M*), and cholera toxin (■) (pg/ml) increase inhibin-LI concentrations in cultured human placental cells. The cultures were maintained at 37°C, in a water-saturated atmosphere containing 5% CO_2; the medium was changed every 2 days. Seven to ten days after cells were plated, triplicate incubation mixtures were prepared. Forskolin (Boehringer Mannheim), 8-bromo–cAMP, and cholera toxin (Sigma) dissolved in Hepes-dissociated buffer (HDB) or 100% ethanol, were made up as 100-fold concentrates. Small volumes of substances were added into wells coated with 1 ml of serum-free medium containing 0.1% BSA. After 48 hours of incubation at the above-described conditions, the conditioned medium was collected and assayed for inhibin-LI. Vehicle-treated wells were present in each experiment. Inhibin-LI was measured by double-antibody radioimmunoassay. We used rabbit antiserum to a synthetic fragment of the α-subunit of porcine inhibin, porcine inhibin-α-(1–25)-Gly-Tyr. The ^{125}I-labeled human inhibin-α-(1–25)-Gly-Tyr was purified by high-performance liquid chromatography (HPLC). Antibody-bound radioactivity was separated from free radioactivity with the use of sheep antiserum to rabbit γ-globulin. Increasing volumes of pooled and extracted medium produced a displacement curve parallel to that of the peptide standard. Luteinizing hormone, FSH, GnRH, hCG, and transforming growth factor–β did not displace antibody-bound radioactivity in the inhibin assay. Intra- and intercoefficients of variance were 3.5% (n = 6) and 4.5% (n = 6), respectively. The concentrations of inhibin are reported here and in the text as percent change from control values, because no pure human inhibin is available to use as reference standard. In this and in subsequent figures, each point represents the mean ± standard error of the mean of triplicate wells to prepare the standard curve. Each experiment was repeated at least three times and a representative experiment is shown. In this and in the other studies, the statistical analysis of the results was performed by using analysis of variance and the multiple range test of Duncan when needed. (**B**) hCG (●) (IU/ml), but not hCG α-subunit (○) (ng/ml), increases inhibin-LI concentrations in cultured human placental cells; the hCG was purchased from Sigma.

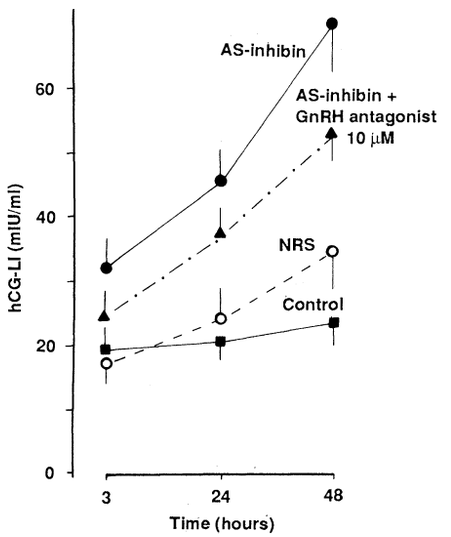

Fig. 3. Time-dependent effect of antiserum to inhibin (●) (1:100) or serum from unimmunized rabbits (○) (NRS) on hCG-LI concentration in human placental cell cultures. Antiserum to inhibin increased hCG-LI concentration, and this effect was partially reversed by the GnRH antagonist (▲) [Ac-DNal(2)[1], 4FDPhe[2], DTrp[3], DArg[6]]GnRH (10 μ*M*). (■) Control. In the dose-relation study, antiserum to inhibin was also used at final dilutions of 1:500 and 1:1000. The antiserum to the α-subunit of inhibin was treated as described in Fig. 1, and for this experiment we used the procedures described in Fig. 2. Concentrations of hCG were measured by double-antibody radioimmunoassay (Diagnostic Products). The assay was standardized against the World Health Organization's First International Reference Preparation No. 75/537. The tracer was ^{125}I-labeled hCG. Antibody-bound radioactivity was separated from free radioactivity by precipitation of the first antibody with goat antiserum to rabbit γ-globulin and polyethylene glycol. No cross-reactions were found with GnRH, CRF, or somatostatin. Intra- and intercoefficients of variations were 3% (n = 6) and 4% (n = 6), respectively.

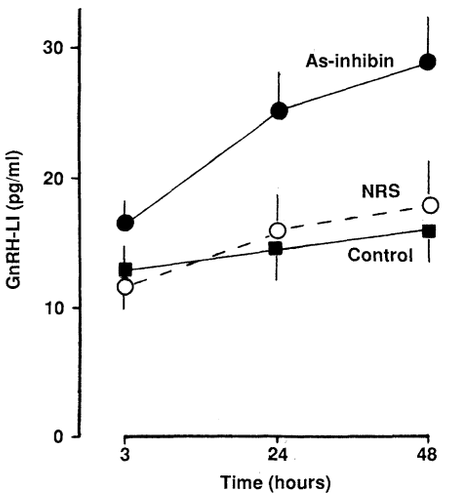

Fig. 4. Time-related effect of antiserum to inhibin (●) (1:100) or serum from an unimmunized rabbit (○) (NRS) on GnRH-LI concentration in human placental cell cultures. The antiserum to inhibin increased GnRH-LI concentrations. The secretion of another placental hormone [adrenocorticotropin (ACTH)] or peptide (CRF) from cultured cells was not modified by the antiserum to inhibin. To measure GnRH-LI we used a double-antibody radioimmunoassay with HPLC-purified ^{125}I-labeled GnRH as tracer and rabbit [Lys[8]]GnRH conjugated by glutaraldehyde to human α-globulins as antigen to generate the first antibody. The antiserum is directed to the NH_2-terminal, and shows less than 1% cross-reactivity with [Ac-His[2]]GnRH(2–10) or [desHis[2]] GnRH. The assay sensitivity to standard GnRH was 0.5 pg per tube, with a median effective dose (ED_{50}) of 20 ± 0.5 pg per tube. The intra- and intercoefficients of variation were 3.5% (n = 6) and 7.0% (n = 6), respectively.

4FDPhe[2], DTrp[3], DArg[6]]GnRH, partially reversed the increase of hCG-LI concentration in cell cultures treated with antiserum to inhibin (Fig. 3). Thus, the local effect of placental inhibin on hCG secretion may depend on the presence and action of an endogenous GnRH-like peptide.

The presence of a feedback loop between inhibin and hCG at the placental level may have a physiological significance. In pregnancy, maternal plasma hCG levels are high during the first trimester, slowly decline until the end of the second trimester, and remain stable until delivery (*1*). The metabolic clearance rate of hCG does not change throughout pregnancy (*17*), thus suggesting a decrease in hCG production rate (*18*). It is possible that an increased expression of placental inhibin may contribute to a decrease in hCG production. In agreement with McLachlan *et al.* (*4*) we find that the mean concentration of plasma inhibin-LI in seven samples of umbilical cord blood collected at the time of delivery was significantly higher than the concentration in control men and nonpregnant women (mean ± standard error, 225 ± 19%) ($P < 0.01$). However, a possible fetal source of inhibin in cord blood cannot be excluded, because in our samples venous and arterial blood were mixed. If we assume that inhibin also circulates in maternal blood, this hormone may contribute to the very low maternal FSH concentration (*19*) and the lack of gonadotropin responses to GnRH administration during pregnancy (*20*).

In conclusion, the present study, shows that placental trophoblast tissue contains and secretes inhibin-LI, which could play a role in the regulation of hCG secretion.

REFERENCES AND NOTES

1. R. Osathanondh and D. Tulchinsky, in *Maternal-Fetal Endocrinology*, D. Tulchinsky and K. J. Ryan, Eds. (Saunders, Philadelphia, 1980), p. 17; E. R. Simpson and P. C. MacDonald, *Annu. Rev. Physiol.* **43**, 163 (1981).
2. G. S. Khodr and T. M. Siler-Khodr, *Science* **207**, 315 (1980); P. H. Seeburg and J. P. Adelman, *Nature (London)* **311**, 666 (1984); A. J. Currie, H. M. Fraser, R. M. Sharpe, *Biochem. Biophys. Res. Commun.* **99**, 332 (1981); R. V. Haning, L. Choi, A. J. Kiggens, D. L. Kuzma, J. W. Summerville, *J. Clin. Endocrinol. Metab.* **55**, 213 (1982).
3. T. M. Siler-Khodr, *Clin. Perinatol.* **3**, 553 (1983).
4. R. I. McLachlan *et al.*, *Biochem. Biophys. Res. Commun.* **140**, 485 (1986).
5. Z. Hochberg *et al.*, *Placenta* **2**, 259 (1981).
6. N. B. Schwartz and C. P. Channing, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5721 (1977); F. H. DeJong and R. M. Sharpe, *Nature (London)* **263**, 71 (1976); R. R. Grady, C. Charlesworth, N. B. Schwartz, *Recent Prog. Hormone Res.* **38**, 409 (1982); R. McLachlan *et al.*, *Lancet* **1986-I**, 1233 (1986); C. Rivier, J. Rivier, W. Vale, *Science* **234**, 205 (1986); D. M. Robertson *et al.*, *Biochem. Biophys. Res. Commun.* **126**, 220 (1985); H. Miyamoto *et al.*, *ibid.* **129**, 396 (1985); N. Ling *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7217 (1985); J. Rivier, J. Spiess, R. McClintock, J. Vaughan, W. Vale, *Biochem. Biophys. Res. Commun.* **133**, 120 (1985).
7. A. J. Mason *et al.*, *Nature (London)* **318**, 659 (1985); R. G. Forage *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3091 (1986); A. J. Mason, H. D. Niall, P. H. Seeburg, *Biochem. Biophys. Res. Commun.* **135**, 957 (1986).
8. K. E. Mayo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5849 (1986).
9. P. E. Sawchenko and L. W. Swanson, *Brain Res.* **210**, 31 (1981).
10. G. S. Khodr and T. M. Siler-Khodr, *Fertil. Steril.* **29**, 523 (1978); W. B. Watkins and S. S. C. Yen, *J. Clin. Endocrinol. Metab.* **5**, 969 (1980); F. Petraglia, P. E. Sawchenko, J. Rivier, W. Vale, *Nature (London)*, in press.
11. W. Vale *et al.*, *Methods Enzymol.* **103**, 565 (1983).
12. S. R. Davis *et al.*, *Biochem. Biophys. Res. Commun.* **138**, 1191 (1986); T. A. Bicsak *et al.*, *Endocrinology*, in press.
13. K. M. J. Menon *et al.*, *J. Clin. Endocrinol. Metab.* **36**, 1104 (1973); J. Levilliers, E. Alsat, Ph. Laudat, L. Cedard, *FEBS Lett.* **47**, 146 (1974).
14. J.-P. Louvet *et al.*, *Endocrinology* **99**, 1126 (1976); E. S. Siris *et al.*, *ibid.* **102**, 1356 (1978).
15. F. J. Morgan *et al.*, *ibid.* **94**, 1601 (1974); K. J. Catt, M. L. Dufau, T. Tsuruhara, *J. Clin. Endocrinol. Metab.* **34**, 123 (1972); B. Shome and A. F. Parlow, *ibid.* **36**, 618 (1973).
16. L. Milewich *et al.*, *Placenta* **3**, 165 (1982); P. Zeitler, E. Markoff, S. Handwerger, *J. Clin. Endocrinol. Metab.* **57**, 812 (1983).
17. A. R. Midgley, Jr., and R. B. Jaffe, *J. Clin. Endocrinol. Metab.* **28**, 1712 (1968); T. Rizkallah, E. Gurpide, R. L. Vande Wiele, *ibid.* **29**, 92 (1969); S. S. C. Yen, O. Llerena, B. Little, O. H. Pearson, *ibid.* **28**, 1763 (1968).
18. G. Benagiano, A. Pala, M. Meirinho, M. Ermini, *J. Endocrinol.* **55**, 287 (1972); J. L. Vaitukaitis, *J. Clin. Endocrinol. Metab.* **38**, 755 (1974).
19. F. W. Hanson, J. E. Powell, V. C. Stevens, *Am. J. Obstet. Gynecol.* **36**, 667 (1970); R. B. Jaffe, P. A. Lee, A. R. Midgley, *J. Clin. Endocrinol. Metab.* **29**, 1281 (1969); D. R. Mishell, I. H. Thorneycroft, Y. Nagata, T. Murata, R. M. Nakamura, *Am. J. Obstet. Gynecol.* **117**, 631 (1973); A. F. Parlow, T. A. Daane, W. J. Dignam, *J. Clin. Endocrinol. Metab.* **31**, 218 (1970).
20. L. M. Rubinstein, A. F. Parlow, C. Derzko, J. Hershman, *Obstet. Gynecol.* **52**, 172 (1978); J. R. Sowers, M. Colantino, J. Fayez, H. Jonas, *ibid.*, p. 685.
21. R. C. Duhamel and D. A. Johnson, *J. Histochem. Cytochem.* **33**, 711 (1985).
22. Supported by NIH grants HD13527 and AM26741, and partially by the Consiglio Nazionale delle Ricerche, pos. 350.542, Department of Obstetrics and Gynecology, University of Modena, 41100 Modena, Italy (F.P.), by a C. J. Martin fellowship from National Health and Medical Research Council of Australia (A.T.W.L.), and NIH grant NS21182 and a McKnight Foundation scholar's award (P.E.S.). Research conducted in part by the Clayton Foundation for Research, California Division, where W.V. is a senior investigator. We thank Dr. B. Villanueva and the staff of the labor and delivery room at Scripps Memorial Hospital, La Jolla, CA, for assistance. We also thank S. Sardelli, B. Toth, J. Vaughan, S. Cappel, A. Corrigan, G. Yamamoto, R. Kaiser, J. Dykert, R. Galyean, J. Porter, M. Dowell, C. Arias, and D. Sanger for assistance and B. Connor and S. McCall for manuscript preparation. Moreover, we acknowledge the National Hormone and Pituitary Program (NIDDK) for supplying the hCG α-subunit.

28 November 1986; accepted 17 April 1987