0013-7227/91/1284-1881$03.00/0
Endocrinology
Copyright © 1991 by The Endocrine Society
Vol. 128, No. 4
Printed in U.S.A.

Testosterone Regulates Pro-Opiomelanocortin Gene Expression in the Primate Brain*

LIZBETH A. ADAMS†, LINDA VICIAN‡, DONALD K. CLIFTON, AND ROBERT A. STEINER

Departments of Obstetrics and Gynecology (L.V., D.K.C., R.A.S.), Physiology and Biophysics (L.A.A. and R.A.S.), Zoology (R.A.S.), the Population Center for Research in Reproduction and the Regional Primate Research Center at the University of Washington, Seattle, Washington 98195

ABSTRACT. Endogenous opioid peptides such as β-endorphin, derived from proopiomelanocortin (POMC), have been widely implicated as serving an important role in the neuroendocrine regulation of the primate reproductive axis. In both human and nonhuman primates, POMC neurons are thought to mediate, at least in part, the negative feedback action of sex steroids on GnRH. Sex steroids, such as testosterone, are thought to inhibit GnRH secretion by enhancing the inhibitory activity of β-endorphin; however, the cellular mechanisms by which steroid hormones regulate the activity of POMC neurons in the primate brain are unknown. In this study, we tested the hypothesis that testosterone stimulates POMC gene expression within the primate brain and that this regulation occurs within a specific subset of POMC neurons residing in the arcuate nucleus of the hypothalamus. We used *in situ* hybridization to compare cellular levels of POMC messenger RNA in intact ($n = 4$), castrated ($n = 4$), and castrated/testosterone-treated ($n = 4$) monkeys. We report that after castration of the male macaque (*Macaca fascicularis*), cellular POMC messenger RNA levels decline significantly ($P < 0.05$) in neurons within the arcuate nucleus and that this decline is prevented by replacement with physiological doses of testosterone. Moreover, we found that this testosterone-dependent modulation of POMC gene expression is restricted to a small fraction of the numerous POMC neurons located within the most anterior region of the arcuate nucleus in the brain of this primate species. These observations provide evidence that sex steroids regulate expression of the POMC gene in the primate brain. (*Endocrinology* **128:** 1881–1886, 1991)

ENDOGENOUS opioid peptides in the brain are important neurotransmitters implicated in the regulation of numerous physiological functions, including feeding behavior, learning, analgesia, and immune responses (1). This, coupled with the observation made more than 50 years ago that female morphine addicts are often infertile or fail to have regular menstrual cycles (see Ref. 2), has generated considerable interest in understanding how endogenous opioids may be involved in the physiological regulation of reproductive function in humans. Moreover, the possibility that abnormalities in opioid peptide function may underlie the development of disorders of reproduction, such as chronic exercise- and stress-induced amenorrhea, has intensified studies of the regulation of opioidergic activity in the brain.

The mammalian reproductive axis is coordinated by the hypothalamic secretion and trophic effects of GnRH, which itself is controlled by the negative feedback action of gonadal steroids operating through a mechanism that involves the opioid peptides (3–8). In the presence of gonadal steroids, β-endorphin acts through μ-receptors to inhibit GnRH secretion; however, this does not occur in an animal whose gonads have been removed. Although it is clear that gonadal steroids, such as testosterone, are required for the interaction between β-endorphin and GnRH neurons, the mechanisms by which testosterone influences the activity of β-endorphin neurons are largely unknown (9, 10). The effects of endogenous opioid peptides on reproduction have been described in a number of mammalian species; however, the neuroendocrine reproductive axis of the primate is unique in several important respects, including the anatomical organization of the hypothalamic neurons involved in the neuroendocrine regulation of GnRH secretion (11–13). The primary objective of this study was to test, in the primate brain, whether testosterone stimulates expression of the POMC gene, which encodes the precursor of β-endorphin. A secondary objective was to determine whether an effect of testosterone might be restricted to a subpop-

Received October 29, 1990.
Address all correspondence and requests for reprints to: Dr. Robert A. Steiner, Department of Obstetrics and Gynecology, RH-20, University of Washington, Seattle, Washington 98195.
* This work was supported by NIH Research Grants HD-12625–11, HD-12629–11, and RR-00166.
† Current address: GRECC 182-B, VA Medical Center, 1660 S. Columbian Way, Seattle, Washington 98108.
‡ Current address: c/o Dr. Harvey Herschman, Warren Hall, University of California at Los Angeles, 900 Veteran Avenue, Los Angeles, California 90024.

ulation of POMC neurons from among the large number of POMC neurons within the arcuate nucleus of the brain.

Materials and Methods

Animal care and surgical procedures

Adult male macaques, *Macaca fascicularis,* were used for these studies. Animals were cared for at the Regional Primate Research Center at the University of Washington in accordance with institutional guidelines. They were housed in individual cages under controlled conditions of heat (25.5 ± 1 C), humidity (65%), and light (on 0600 h; off 1800 h). Animals were fed Purina monkey chow and given water *ad libitum* and received fresh fruit twice, and chewable vitamin and iron tablets once weekly. Adult animals weighed 4.5–9.0 kg and were sexually mature as assessed by plasma testosterone levels.

Castrations were performed under aseptic conditions on animals anesthetized with fluothane. At the time of castration, animals received either blank Silastic (od 0.465 cm, Dow-Corning Co., Midland, MI) capsules or capsules containing powdered testosterone (Sigma Chemical Co., St. Louis, MO) packed to a final length of 4 cm, and implanted in a sc pocket in the midscapular region, where they remained for 3 weeks, until the time of death. Blood samples were taken by saphenous venipuncture at the time of castration and again at the time of death, and used for the evaluation of testosterone levels.

Tissue preparation

Before removal of the brain, mature male macaques were preanesthetized with ketamine hydrochloride (Parke-Davis, Morris Plains, NJ) at a dose of 10 mg/kg body wt, killed with sodium pentobarbital (25 mg/kg body wt; Abbot Laboratories, North Chicago, IL), and perfused intracardially with the following series of solutions: 500 ml warm lactated Ringers with 5% dextrose (Travenol, Deerfield, IL) and 0.2% sodium heparin, then 2 liters of ice-cold, fresh 4% paraformaldehyde (Poly Sciences, Warrington, PA)/0.1% EM grade glutaraldehyde (Electron Microscopic Services, Fort Washington, PA) in 0.1 M phosphate buffer, pH 7.4 (PB). After a 15-min waiting period, 2 liters of ice-cold, fresh 4% paraformaldehyde in PB was infused, and this was followed, after a 45-min wait, by 500 ml lactated Ringers with 5% dextrose. A final 15-min wait was followed by 1 liter 20% sucrose in PB.

When the perfusion was complete, the head was removed and placed in a Kopf (Tujunga, CA) stereotaxic instrument. Using a stainless steel cannula mounted on the stereotaxic instrument, we made bilateral tracks in the brain at 28 mm and 30 mm anterior to ear-bar-zero, 7 mm off midline on each side. This provided a means of aligning the hypothalamic block during subsequent sectioning so that sections in a frontal plane could be obtained. The hypothalamus and basal forebrain was blocked by making cuts at the inflection point of the olfactory nerves (anterior), corpus callosum (superior), posterior to the mammillary bodies (posterior), and 9 mm from the midline bilaterally. The block was frozen on dry ice and then stored at −80 C until slicing. Frontal sections (20 μm), cut at −18 C were mounted onto cold poly-L-lysine (50 μg/ml; Sigma, St. Louis, MO)-coated glass slides. For analyses of POMC messenger RNA (mRNA) containing cells, tissue sections (200 μm apart) throughout the arcuate nucleus were processed for *in situ* hybridization.

POMC complementary RNA probe synthesis

A 993-basepair ribonucleotide probe, derived from a *Macaca nemestrina* pituitary complementary DNA (cDNA) library, was transcribed in the presence of ^{35}S-α-thio-uridine triphosphate (New England Nuclear, Boston, MA) to a final specific activity of 1–2 × 10^8 dpm μg RNA. After purification, the RNA was hydrolyzed in 100 mM bicarbonate buffer (pH 10.2) to yield fragments approximately 150 bases in length. On the day of the assay, the riboprobe (final concentration, 1.2 μg/ml·kilobase) was mixed in hybridization buffer (final concentrations in this mixture were: 500 μg/ml total yeast RNA, 50% deionized formamide, 0.3 M NaCl, 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 10 mM dithiothreitol (DTT), 10% dextran sulfate, 1× Denhardt's).

In situ hybridization

The tissue received the following treatment immediately before application of the probe: postfixation (3 min) in 4% paraformaldehyde in 0.1 M PB, two 5-min washes in PB, proteinase K (0.5 mg/ml PK in 0.1 M Tris-HCl pH 8.0, 50 mM EDTA for 30 min at 37 C), acetic anhydride (0.25% in 0.1 M triethanolamine pH 8.0 for 10 min at 25 C), 2× SSC (3 min at 25 C; 1× SSC = 150 mM NaCl, 15 mM sodium citrate), dehydration through graded ethanols, delipidation in chloroform (5 min), and rehydration through 95% ethanol. Tissue was air dried from ethanol, and 45 μl/hypothalamic slice of the probe mixture was applied to dry tissue. Parafilm was used as a coverslip, and the edges of the parafilm were sealed with rubber cement. Slides were incubated in a moist chamber overnight at 60 C. The next day, parafilm coverslips were pulled off with forceps, and the slides were rinsed briefly in 4× SSC/10 mM DTT. The slides were then rinsed in fresh 4× SSC/10 mM DTT for 15 min at ambient temperature, treated with RNase A (30 μg/ml in 10 mM Tris pH 8.0, 1 mM EDTA, 0.5 M NaCl for 30 min at 37 C), stirred 1 h in 2× SSC/2 mM DTT/20% deionized formamide at 60 C for 30 min. The slides were then dehydrated through graded ethanols (50%, 80%, and 100%; the first two alcohols contained 300 mM ammonium acetate), then air dried. The slides were dipped in photographic emulsion (Kodak NTB-2, Eastman Kodak, Rochester, NY, diluted 1:1 with 600 mM ammonium acetate), air dried for 30 min, placed in a moist chamber for 3 h, and dried again on the bench for 45 min. Slides were exposed in light-tight boxes at 4 C for 6.5 days, developed (Kodak D-19, diluted 1:1 with distilled water) for 4 min and fixed (Kodak fixer) for 10 min at 15 C, rinsed 30 min in running tap water, and counterstained with cresyl violet.

Image analysis

Tissue sections 200 μm apart throughout the rostro-caudal extent of the arcuate nucleus were processed for *in situ* hybridization. Tissue was visualized under darkfield illumination to identify clusters of photographic grains overlying cells, and lightfield illumination was used to verify that a cresyl-stained

nucleus was associated with each cluster. Twenty pro-opiomelanocortin (POMC) mRNA-containing cells were analyzed on each tissue slice. Sixteen slices from each animal were analyzed, so that a total of 320 cells per animal were subjected to an automated grain detection and quantification process by use of a computerized image processing system (14). Before analysis, the slides were anatomically matched among animals, then were randomized and assigned a 3-letter code so that the operator was blinded to the slide's treatment group assignment. The total number of photographic grains overlying a cell, corrected for background, is referred to as the number of grains per cell. An arithmetic mean number of grains per cell was calculated for each animal, and this mean was used in a one-way analysis of variance to test for differences between treatment groups. All variance measures are SEM, calculated with $n = 4$, representing the number of animals in each treatment group.

Probe specificity

Incubation of tissue sections with a mixture of unlabeled and labeled riboprobe in a ratio of 100:1 (unlabeled:labeled) was performed to control for nonspecific binding; grain clusters were not apparent on these sections.

Testosterone assay

Testosterone levels in ether-extracted serum samples were measured by RIA with an antiserum raised against testosterone linked at the 3-position by carboxymethoxime to BSA. The antiserum exhibited a cross-reactivity of 14% with dihydrotestosterone, 6% with α-androstanediol, and 2% or less with all other steroids tested. The minimum sensitivity of the assay was less than 0.1 ng/ml, and the intra- and interassay coefficients of variation were 3.0% and 8.5%, respectively.

Results

We compared the relative cellular levels of POMC mRNA signal, expressed as grains per cell, in the brains of male crab-eating macaques (*Macaca fascicularis*) that were either intact ($n = 4$), castrated ($n = 4$), or castrated and implanted with Silastic capsules designed to maintain physiological levels of testosterone in the peripheral circulation (castrate + T) ($n = 4$). The majority of POMC neurons in the primate brain were observed to be located in two large clusters, residing on both sides of the tip of the third ventricle within the arcuate nucleus (Fig. 1). The overall POMC mRNA signal levels in the arcuate were: intact, 152.3 ± 4.9 grains per cell; castrate, 111.7 ± 11.9 grains per cell; castrate + T, 157.3 ± 14.0 grains per cell. Thus, when POMC cells throughout the arcuate nucleus were analyzed, we observed that castration resulted in a 27% decrease in the mean number of grains per cell over POMC mRNA-containing cells compared with cells from intact controls ($P < 0.05$). We also observed that testosterone replacement prevented this decrease in POMC message, as there was no statistical difference in grains per cell between intact and castrate + T animals (Figs. 2 and 3).

Fig. 1. Distribution of cells containing POMC mRNA in the arcuate nucleus of adult male macaques, *Macaca fascicularis*, shown on schematic drawings of frontal sections of the macaque brain with the indicated anterior-posterior coordinates, based on the atlas of Shantha *et al.* (35).

To determine whether the effects of castration and testosterone treatment occur only in particular subsets of POMC neurons, we divided the arcuate nucleus into four divisions of equal length (about 0.8 mm each) along the anterior-posterior axis. The anterior-posterior coordinates of these regions correspond roughly to those of the plates shown in Fig. 1, defining the anterior boundary of the nucleus as the point at which the optic chiasm first indents at the midline, and the posterior boundary as the point of appearance of the fourth ventricle. The castration-induced decrease in cellular POMC mRNA signal levels was most pronounced in the most rostral

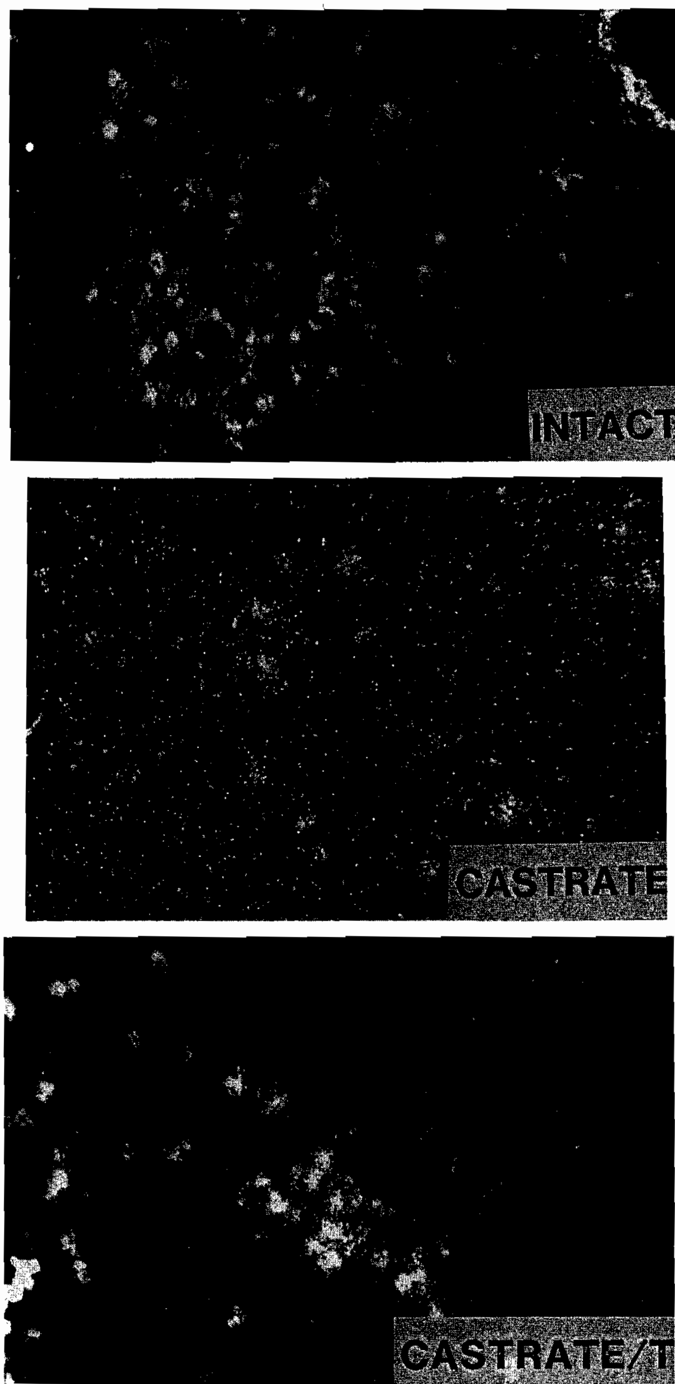

FIG. 2. Darkfield photomicrographic images taken from frontal sections through the rostral arcuate nucleus of representative intact (*top*), castrate (*middle*), and castrate + T macaques (*bottom*). The grain clusters identify the presence of POMC mRNA-containing cells.

portion of the nucleus, where we observed a 38% decrease in average grain counts ($P < 0.05$), and here again, testosterone replacement completely prevented this decrease. The POMC mRNA levels in the most anterior part of the arcuate were: intact, 168.3 ± 19.0 grains per cell; castrate, 108.3 ± 8.9 grains per cell; castrate + T,

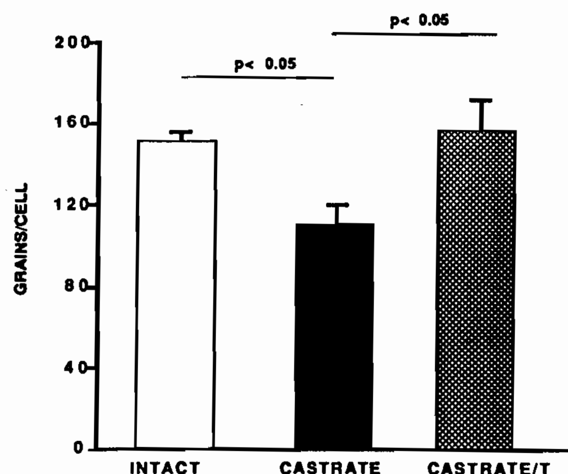

FIG. 3. The effect of castration and steroid replacement on the mean cellular POMC mRNA levels throughout the entire arcuate nucleus of intact (n = 4), castrated (n = 4), and castrated + T (n = 4) adult male macaques.

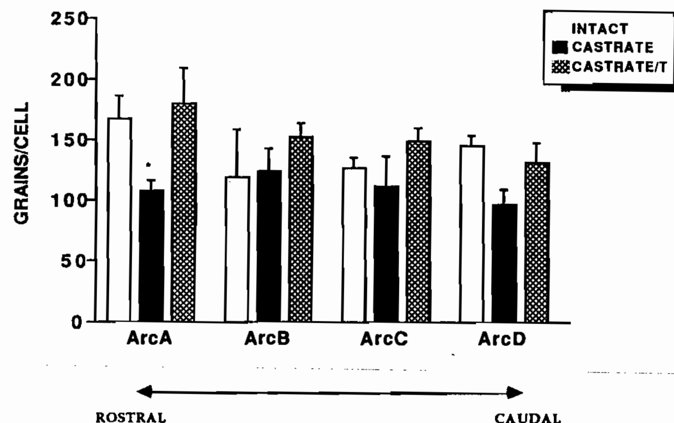

FIG. 4. The effect of castration and testosterone replacement on cellular POMC mRNA levels in four regions of the arcuate nucleus of male macaques ($P < 0.05$). As described in the text, the nucleus is divided into four divisions of equal length along the anterior-posterior axis and designated ArcA, ArcB, ArcC, and ArcD such that ArcA is the most anterior and Arc D is the most posterior portion of the nucleus.

180.0 ± 29.8 grains per cell. Changes in POMC mRNA signal levels associated with castration were not statistically significant in the other three divisions of the arcuate nucleus (Fig. 4).

The effectiveness of the castration and testosterone replacement regimens was demonstrated by the mean serum testosterone levels in each group, which were: intact, 4.8 ± 0.8 ng/ml; castrated, 0.25 ± 0.07 ng/ml; and castrated + T, 8.6 ± 0.4 ng/ml. The testosterone values in castrated + T animals is within the range of normal testosterone values for intact adult males (15).

Discussion

We have shown that expression of the POMC gene in the hypothalamic arcuate nucleus is reduced after cas-

tration of adult male macaques, and that this decrease can be prevented by administration of physiological levels of testosterone. Our data are consistent with the hypothesis that the β-endorphin system requires the presence of gonadal steroids to exert its physiological effects on the reproductive system. According to this model, when circulating levels of gonadal steroids are low (*e.g.* after castration or during the prepubertal state), the inhibition of GnRH neurons by β-endorphin is so small that its functional removal (by the pharmacological application of a μ receptor antagonist, such as naloxone) has no perceptible effect on GnRH secretory activity (16–20). The present study provides an explanation for such an attenuation of β-endorphin activity: the castration-induced reduction in POMC gene expression we have observed may account for a diminution of β-endorphin secretory activity that persists in states of low circulating sex steroids.

The effects of testosterone on β-endorphin secretion *per se* are not well characterized in any species, and have not been described at all in the primate brain. The hypothalamic content of β-endorphin increases after castration of male rats, but this could be attributed either to an increased synthesis or decreased release of the peptide; an *in vitro* reduction in β-endorphin release from hypothalamic slices of castrated male rats favors the latter explanation (21, 22). Because POMC neurons interact with a variety of physiological systems in addition to the reproductive axis, it cannot be asserted that the POMC neurons we analyzed make functional connections with GnRH neurons. Nevertheless, the data presented here are consistent with the proposed model of POMC interaction with the reproductive system.

The manner in which testosterone exerts its effect on POMC gene expression is also unknown, since testosterone can act on the genome through multiple pathways. Testosterone itself may act through androgen receptors to influence a variety of cellular events, including gene expression, or it can be metabolized to other steroidal compounds (for example, to dihydrotestosterone in the presence of 5-α-reductase or to estradiol by the action of aromatase) which themselves exert physiological effects. In the rat, it appears that testosterone acts on POMC neurons through conversion to estradiol (23), but this has not yet been established in a primate species.

This study in the primate brain, as well other studies from our lab in the rat brain documenting the effects of testosterone on POMC mRNA levels in neurons of the arcuate nucleus, are at variance with other reports in the rat (24, 25). Several important differences between these studies and the present study bear discussion, in addition to the obvious consideration of species differences. Perhaps the most salient point is that we measured mean POMC mRNA levels in individual neurons, whereas in the other studies, changes in POMC mRNA relative to total mRNA were measured by solution hybridization. It is conceivable that after castration the POMC mRNA content of individual neurons in the anterior part of the arcuate decreases, but the total number of neurons expressing POMC mRNA (and thus the amount of hypothalamic POMC mRNA) in the arcuate increases. Additionally, there could be a decrease in the amount of total mRNA in the hypothalamus, or some other change in baseline levels of mRNA which could decrease the standard to which POMC mRNA levels are compared. These discrepancies are not yet resolved, but one important consideration may be the heterogeneity of POMC neurons in the hypothalamus.

Our demonstration that castration induces a greater decrease in POMC mRNA in the anterior part of the arcuate nucleus emphasizes this heterogeneity. Regional specificity of steroid effects on POMC gene expression has also been demonstrated in the rat (26, 27) and could result from regional variations in steroid hormone receptors, steroid metabolism, or in steroid-sensitive afferent inputs to these neurons. Indeed, it has been shown in the female rat hypothalamus that of those neurons which are immunoreactive for β-endorphin, only about 30% have progesterone receptors (28), and something less than 30% have estrogen receptors (29, 30). Thus, there are distinct subpopulations of POMC neurons upon which steroid hormones could act directly. There is also heterogeneity in the projections of POMC neurons from different regions of the arcuate. Although careful mapping studies have not been performed in the macaque brain to indicate where POMC neurons within the arcuate project, studies in the rat have examined the projections of cells containing ACTH, another cleavage product of the POMC precursor molecule. They show that a population of ACTH-containing cells whose somata are in the arcuate nucleus project to the medial preoptic area, and that in the medial preoptic area there are numerous synaptic connections between ACTH neurons and GnRH neurons (31, 32). Functional heterogeneity is not unique to the POMC neurons within the arcuate but is evident in other systems as well, including the dopaminergic cells in the arcuate (33, 34). These observations underscore the fact that the functional organization of the brain defies dissections along classical anatomical boundaries and suggests that multiple criteria must be used to define and study the regulation of neurotransmitter systems, even within individual brain nuclei.

Acknowledgments

We thank Pam Kolb, Emilia Kabigting, and the staff of the Regional Primate Research Center at the University of Washington for excellent technical support. We also thank Dr. Stanley Watson for providing us

with the *Macaca nemestrina* POMC cDNA, and Dr. William Bremner for supervising the testosterone assays.

References

1. Morley JE 1987 Neuropeptide regulation of appetite and weight. Endocr Rev 8:256–287
2. Barraclough CA, Sawyer CH 1955 Inhibition of the release of pituitary ovulatory hormone in the rat by morphine. Endocrinology 57:329–337
3. Plant TM, Hess DL, Hotchkiss J, Knobil E 1978 Testosterone and the control of gonadotropin secretion in the male rhesus monkey (*Macaca mulatta*). Endocrinology 103:535–541
4. Plant TM 1982 Effects of orchidectomy and testosterone replacement treatment on pulsatile luteinizing hormone secretion in the adult rhesus monkey (*Macaca mulatta*). Endocrinology 110:1905–1913
5. Plant TM, Dubey AK 1984 Evidence from the rhesus monkey (*Macaca mulatta*) for the view that negative feedback control of luteinizing hormone secretion by the testis is mediated by a deceleration of hypothalamic gonadotropin-releasing hormone pulse frequency. Endocrinology 115:2145–2153
6. Cicero TJ, Schainker BA, Meyer ER 1979 Endogenous opioids participate in the regulation of the hypothalamic-pituitary-luteinizing hormone axis and testosterone's negative feedback control of luteinizing hormone. Endocrinology 104:1286–1291
7. Cicero TJ, Meyer ER, Gabriel SM, Bell RD, Wilcox CE 1980 Morphine exerts testosterone-like effects in the hypothalamus of the castrated male rat. Brain Res 202:151–164
8. Cicero TJ, Badger TM, Wilcox CE, Bell RD, Meyer ER 1977 Morphine decreases luteinizing hormone by an action on the hypothalamic-pituitary axis. J Pharmacol Exp Ther 203:548–555
9. Simonds WF 1988 The molecular basis of opioid receptor function. Endocr Rev 9:200–212
10. Drouva SV, Epelbaum J, Tapia-Arancibia L, Laplante E, Kordon C 1981 Opiate receptors modulate LHRH and SRIF release from mediobasal hypothalamic neurons. Neuroendocrinology 32:163–167
11. Khachaturian H, Lewis ME, Haber SN, Akil H, Watson SJ 1984 Proopiomelanocortin peptide immunocytochemistry in rhesus monkey brain. Brain Res Bull 13:785–812
12. King JC, Anthony ELP 1984 LHRH neurons and their projections in humans and other mammals: species comparisons. Peptides 5 (Suppl 1):195–207
13. Goldsmith PC, Lamberts R, Brezina LR 1983 Gonadotropin-releasing hormone neurons and pathways in the primate hypothalamus and forebrain. In: Norman RL (ed) Neuroendocrine Aspects of Reproduction. Academic Press, New York, vol 2:7–45
14. Rogers KV, Vician L, Steiner RA, Clifton DK 1988 The effect of hypophysectomy and growth hormone administration on preprosomatostatin messenger ribonucleic acid in the periventricular nucleus of the rat hypothalamus. Endocrinology 122:586–591
15. Adams LA, Bremner WJ, Nestor Jr JJ, Vickery BJ, Steiner RA 1986 Suppression of plasma gonadotropins and testosterone in adult male monkeys (*Macaca fascicularis*) by a potent inhibitory analog of gonadotropin-releasing hormone. J Clin Endocrinol Metab 62:58–63
16. Bhanot R, Wilkinson M 1983 Opiatergic control of LH secretion is eliminated by gonadectomy. Endocrinology 112:399–401
17. Bhanot R, Wilkinson M 1984 The inhibitory effect of opiates on gonadotrophin secretion is dependent upon gonadal steroids. J Endocrinol 102:133–141
18. Foresta C, Marra S, Scanelli G, Scandellari C 1983 Gonadal steroids and opioid control of gonadotropin secretion in man. Fertil Steril 40:798–801
19. Petraglia F, Berrasconi S, Iughetti L, Loche S, Romanini F, Facchinetti F, Marcellini C, Genazzani AR 1986 Naloxone-induced luteinizing hormone secretion in normal, precocious, and delayed puberty. J Clin Endocrinol Metab 63:1112–1115
20. Veldhuis JD, Kulin HE, Warner BA, Santner SJ 1982 Responsiveness of gonadotropin secretion to infusion of an opiate-receptor antagonist in hypogonadotropic individuals. J Clin Endocrinol Metab 55:649–653
21. Wardlaw SL 1986 Regulation of β-endorphin, corticotropin-like intermediate lobe peptide, and α-melanotropin-stimulating hormone in the hypothalamus by testosterone. Endocrinology 119:19–24
22. Almeida OFX, Nikolarakis KE, Herz A 1987 Significance of testosterone in regulating hypothalamic content and *in vitro* release of beta-endorphin and dynorphin. J Neurochem 49:742–747
23. Chowen-Breed JA, Argente J, Vician L, Clifton DK, Steiner RA 1990 Proopiomelanocortin messenger RNA in hypothalamic neurons is increased by testosterone through aromatization to estradiol. Neuroendocrinology 52:581–588
24. Blum M, Roberts JL, Wardlaw SL 1989 Androgen regulation of proopiomelanocortin gene expression and peptide content in the basal hypothalamus. Endocrinology 124:2283–2288
25. Wardlaw SL, Blum M 1990 Biphasic effects of orchiectomy on proopiomelanocortin gene expression in the hypothalamus. Neuroendocrinology 52:521–526
26. Chowen-Breed JA, Clifton DK, Steiner RA 1989 Regional specificity of testosterone regulation of proopiomelanocortin gene expression in the arcuate nucleus of the male rat brain. Endocrinology 124:2875–2881
27. Chowen-Breed JA, Fraser HM, Vician L, Damassa DA, Clifton DK, Steiner RA 1989 Testosterone regulation of proopiomelanocortin messenger ribonucleic acid in the arcuate nucleus of the male rat. Endocrinology 124:1697–1702
28. Fox SR, Harlan RE, Shivers BD, Pfaff DW 1990 Chemical characterization of neuroendocrine targets for progesterone in the female rat brain and pituitary. Neuroendocrinology 51:276–283
29. Jirikowski GF, Merchenthaler I, Rieger GE, Stumpf WE 1986 Estradiol target sites immunoreactive for beta-endorphin in the arcuate nucleus of rat and mouse hypothalamus. Neurosci Lett 65:121–126
30. Morrell JI, McGinty JF, Pfaff DW 1985 A subset of beta-endorphin or dynorphin-containing neurons in the medial basal hypothalamus accumulates estradiol. Neuroendocrinology 41:417–426
31. Leranth C, MacLusky N, Naftolin F 1986 Interconnections between neurotransmitter- and neuropeptide-containing neurons involved in gonadotrophin release in the rat. In: Moody TW (ed) Neural and Endocrine Peptides and Receptors. Plenum, New York, p 177–193
32. Zaborszky L, Leranth C 1985 Simultaneous ultrastructural demonstration of retrogradely transported horseradish peroxidase and choline acetyltransferase immunoreactivity. Histochemistry 82:529–537
33. Bjorklund A, Moore RY, Nobin A, Stenevi U 1973 The organization of tubero-hypophyseal and reticulo-infundibular catecholamine neuron systems in the rat brain. Brain Res 51:171–191
34. Marshall PE, Goldsmith PC 1980 Neuroregulatory and neuroendocrine GnRH pathways in the hypothalamus and forebrain of the baboon. Brain Res 193:353–372
35. Shantha TR, Manocha SL, Bourne GH 1968 A Stereotaxic Atlas of the Java Monkey Brain (*Macaca irus*). Williams and Wilkins, Baltimore