

Pubertal Changes in Gonadotropin-Releasing Hormone and Proopiomelanocortin Gene Expression in the Brain of the Male Rat*

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ABSTRACT. Pubertal development in mammals is in part attributable to a brain-dependent process, whereby increased pulsatile GnRH secretion leads to the awakening of the entire reproductive system. However, the brain mechanisms controlling this event are unknown. The apparent increase in GnRH secretion at puberty could reflect an autonomous change in the activity of GnRH neurons themselves or in the afferent networks leading to GnRH neurons. If there were a significant increase in the secretion of GnRH with puberty onset, we hypothesized that there would be a commensurate increase in the biosynthetic capacity of GnRH neurons to meet the increasing demand. We tested this hypothesis by comparing the level of cellular prepro-GnRH mRNA (GnRH mRNA) observed between prepubertal (25-day-old; n = 5) and adult (75-day-old; n = 4) male rats by *in situ* hybridization. We detected no significant change with puberty in GnRH mRNA signal levels in any of the anatomical areas examined, which included the vertical limb of the diagonal band of Broca, medial septum, lateral preoptic area, and medial preoptic area. Given the variance of our analytical technique, we determined that there was a greater than 90% probability that we would have detected a 20% increase in GnRH mRNA

had there been one. Endogenous opioid peptides have been implicated in timing the onset of puberty in the rat, with the argument being that a loss in opioid tone could effect a disinhibition of GnRH secretion. One opioid peptide, β -endorphin, is among several peptides cleaved from the precursor POMC. We hypothesized that with puberty, POMC neurons in the arcuate nucleus would have an attenuated capacity to produce β -endorphin. We tested this hypothesis by comparing cellular pre-POMC mRNA (POMC mRNA) levels in the arcuate nuclei of prepubertal (n = 6) and adult (n = 7) male rats with *in situ* hybridization. We observed an increase in POMC mRNA levels with puberty; prepubertal rats had relative POMC mRNA signal levels of 119 ± 10 grains/cell, while adult rats contained 167 ± 12 grains/cell ($P < 0.02$). This increase in cellular POMC mRNA was confined to the rostral portion of the arcuate nucleus. We conclude that the GnRH gene is fully expressed well before the time of normal puberty onset and that the increase in POMC mRNA that occurs with the onset of puberty may be important for the development of pulsatile GnRH secretion. (*Endocrinology* 124: 1760-1767, 1989)

PUBERTY in the rat is heralded by increasing plasma levels of pituitary gonadotropins, which are believed to be attributable to the augmentation of pulsatile GnRH secretion. The factors responsible for the development of pulsatile GnRH secretion remain enigmatic, indeed virtually unknown (for review, see Ref. 1). It is conceivable that increased activity of the GnRH pulse generator at the time of puberty reflects a slow maturational process of the GnRH neuron itself, which before puberty may be incapable of synthesizing and secreting GnRH. In support of this argument is the observation that over the course of puberty in the rat, the hypothalamic GnRH content gradually increases (2,

3), suggesting that GnRH synthesis, and hence secretion, is a rate-limiting event for the timing of puberty onset. This pubertal accretion in hypothalamic GnRH content could be due to either an autonomous change in the activity of GnRH neurons or an alteration in the activity of afferent pathways leading to GnRH neurons, which could, in turn, stimulate GnRH synthesis and secretion. While these possibilities are neither mutually exclusive nor exhaustive, we sought evidence to support the concepts that first, GnRH synthetic capacity may be limiting for the onset of puberty, and second, a change in neuronal afferent pathways controlling GnRH secretion could account for the change in GnRH secretory activity at the time of puberty.

Endogenous opioid peptide (EOP)-containing neurons form an afferent pathway to GnRH neurons that has been implicated in controlling the onset of puberty in the rat (4). Opiates are potent inhibitors of GnRH secretion (5, 6), and EOPs are thought to mediate, at least in

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part, the negative feedback effects of testosterone on GnRH release in the male rat (7-9). An important member of the EOP family, β -endorphin is one of several peptides cleaved from the precursor molecule POMC (10-12), and β -endorphin secreted from neurons in the arcuate nucleus figures prominently in the control of GnRH secretion (13, 14). One theory for the onset of puberty contends that the inhibitory tone on GnRH secretion exerted by EOP activity diminishes over the course of pubertal development, thereby disinhibiting GnRH secretion and awakening the cascade of sexual maturation (4).

In this report we explored two possible mechanisms to account for the pubertal increase in hypothalamic GnRH content and, presumably, the capacity to secrete and elaborate GnRH pulses in the male rat. First, we tested the hypothesis that the biosynthetic capacity of GnRH neurons increases as a function of puberty. Using mRNA levels as an index of biosynthetic capacity, we measured levels of prepro-GnRH mRNA (GnRH mRNA) in individual cells of the hypothalamus and forebrain by *in situ* hybridization and compared levels observed between prepubertal and adult male rats. Second, we tested the hypothesis that the activity of POMC neurons declines as a function of puberty. Again using *in situ* hybridization, we measured levels of pre-POMC mRNA (POMC mRNA) in neurons of the arcuate nucleus and compared levels between prepubertal and adult male rats. We report that POMC mRNA levels increase with the onset of puberty and that this increase is localized to a population of neurons in the anterior aspect of the arcuate nucleus; in contrast, we found no evidence that the amount of GnRH mRNA changes as a function of sexual development.

Materials and Methods

Animals

Male Sprague-Dawley rats were purchased (Tyler Laboratories, Bellevue, WA) and housed under light-controlled conditions (14 h of light, 10 h of darkness), with lights on at 0700 h. Animals were provided rat chow and tap water *ad libitum*. Twenty-five-day-old (prepubertal; $n = 6$) and 75-day-old (adult; $n = 7$) animals were anesthetized with ether and decapitated. The animals were brought into the laboratory, and individual rats from the two groups were alternately killed in order to control for the stress of being in the laboratory. The brains were quickly removed, frozen on dry ice, and stored at -80 C. Trunk blood was collected, and the serum fractions were stored at -20 C before assay for testosterone.

GnRH oligoprobe synthesis

We used a 48-base oligodeoxynucleotide probe that was complementary to the entire GnRH coding region and the first 18 bases of the GnRH-associated-peptide region of GnRH

mRNA (15). The probe was synthesized by the Solid Phase Synthesis Center of the Howard Hughes Medical Institute of the University of Washington (Seattle, WA). The full-length oligomer was purified by electrophoresis through a 20% polyacrylamide-50% urea gel. The main band was cut out, and the oligomer was eluted into a solution of 100 mM Tris and 1 mM EDTA (pH 7.8). Final purification of the oligomer was performed on a New England Nuclear (NEN; Wilmington, DE) Nensorb-20 column according to the manufacturer's directions. The final concentration of oligomer was determined by absorbance at 260 nm.

The purified oligomer was 3' end labeled with [35 S]dATP in a reaction containing GnRH oligomer (0.6 μ M), 3 μ M [35 S]dATP (NEN), 0.8 U/ μ l terminal deoxynucleotidyl transferase [Bethesda Research Laboratories (BRL), Gaithersburg, MD], and tailing reaction buffer supplied by BRL. This reaction was incubated at 37 C for 90 min and then stopped by transferring it to a 65 C bath for 5 min. The labeled oligomer was purified on a Nensorb-20 column and reconstituted in TED [10 mM Tris HCl, 1.0 mM EDTA, and 10 mM dithiothreitol (DTT)] to a probe concentration of 0.22 μ M. The specific activity was approximately 8.4×10^8 dpm/ μ g. This stock solution of GnRH probe was then diluted into hybridization buffer consisting of 4 \times SSC (1 \times SSC = 150 mM NaCl and 15 mM Na citrate), 1 \times Denhardt's solution (1% each of Ficoll, polyvinylpyrrolidone, and BSA), 2.5 μ g/ml tRNA, 0.5 mg/ml single stranded salmon sperm DNA, 50% deionized formamide, 10% dextran sulfate, and 10 mM DTT.

POMC riboprobe synthesis

A cDNA clone, pMKSU16, containing the entire coding sequence for the mouse pituitary POMC precursor, was kindly given to us by Michael Uhler. The 925-basepair (bp) *Hind*III-*Eco*RI cDNA fragment from pMKSU16 was subcloned into pSp64 and pSp65 (Promega Biotec, Madison, WI), and the orientation of the fragment in each clone was confirmed by restriction mapping. This fragment consisted of 98 bp of 5' noncoding sequence, 105 bp of 3' noncoding sequence, the entire 705 bp of coding sequence, and 8 bp of synthetic linker at each end (16). The subclone pLV09 contained the fragment cloned into pSp64, and pLV16 contained the insert cloned into pSp65. This was used to produce antisense RNA (cRNA complementary to POMC mRNA), which was the probe used in this experiment. Radioactive cRNA was synthesized *in vitro* from pLV09 and pLV16 as previously described (17). The transcription reaction contained a total of 75 μ mol α -thio-UTP (NEN), of which 5.3 μ mol were 35 S labeled and 69.7 μ mol were unlabeled α -thio-UTP. The final specific activity was 6.58×10^8 dpm/ μ g. The probe was hydrolyzed to an average length of 150 bases and diluted in riboprobe hybridization buffer.

Tissue preparation

Coronal slices (20 μ m) were cut in a -15 C cryostat with consecutive slices thaw mounted onto three sets of poly-L-lysine-coated slides, thereby placing every third slice into a given set. Sections were collected from the point where the corpus callosum joins at the midline, through the preoptic area

and the hypothalamus. Slides were stored in airtight boxes at -80°C until hybridization histochemistry was conducted. One set of slides from each animal was then processed for GnRH mRNA and another for POMC mRNA *in situ* hybridization.

GnRH cDNA *in situ* protocol

Tissue section slides were taken out of the -80°C freezer, dried, and postfixed in 4% paraformaldehyde (pH 7.4) for 5 min. The slides were then washed in 0.1 M phosphate buffer (20 mM $\text{NaH}_2\text{PO}_4\text{-H}_2\text{O}$ and 80 mM $\text{Na}_2\text{HPO}_4\text{-7H}_2\text{O}$) and placed into 0.1 M triethanolamine-0.14 M NaCl, pH 8.0, with 0.25% acetic anhydride for 10 min. Next, the tissue was rinsed in $2 \times$ SSC and delipidated by passing the slides through a graded series of alcohols, ending in chloroform. The slides were then dried, 60 μl GnRH oligoprobe were applied to each slide, and a Parafilm coverslip was placed over the tissue sections. The slides were incubated in moist chambers at 37°C for approximately 20 h. After incubation, the coverslips were floated off in $1 \times$ SSC, and the slides were washed in a second dish of $1 \times$ SSC for 15 min. This was followed by four separate 15-min washes in $1 \times$ SSC at 48°C and two 1-h washes in $1 \times$ SSC (room temperature). The slides were then rinsed in distilled H_2O and dried.

POMC cRNA *in situ* protocol

Tissue sections were dried, fixed in 4% paraformaldehyde, and washed in 0.1 M phosphate buffer. Next, the slides were treated with 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min. The slides were then rinsed in $2 \times$ SSC and blotted, and 60 μl POMC riboprobe were placed on a slide, followed by a Parafilm coverslip. Tissue sections were incubated with riboprobe for approximately 20 h in moist chambers at 45°C . After incubation the coverslips were floated off in $4 \times$ SSC containing 4.2 mM DTT. After a series of washes and RNase treatment, the slides were passed through a series of alcohols and air dried (for more detail, see Ref. 17).

Autoradiography and histological staining

After *in situ* hybridization histochemistry, the slides were dipped into NTB-2 photographic emulsion (Eastman Kodak, Rochester, NY) that was diluted 1:1 with 600 mM ammonium acetate and heated to 45°C . The slides were air dried for 30 min and placed in a moist chamber for 45 min. Next, the slides were placed in light-tight boxes with desiccant and stored at 4°C until processed for autoradiographic exposure of silver grains and counterstained with cresyl violet.

Anatomical matching and image analysis

GnRH mRNA. Ten coronal slices from each animal were anatomically matched across animals to measure relative cellular GnRH mRNA content. These slices were taken from the anterior septal region, through the diagonal band of Broca, and the preoptic area, with the most caudal slice at the rostral portion of the supraoptic nuclei and lateral hypothalamus. The slides were analyzed by an operator unaware of the animal's experimental group. All cells that labeled positively for GnRH

mRNA were analyzed, and approximately 145 cells were measured in each animal. The specific anatomical area in which each labeled cell was located was entered into the image-processing computer.

POMC mRNA. We divided the arcuate nucleus into four sections of approximately equal length in a rostral to caudal orientation using a rat brain atlas (18) as an anatomical guide. These subdivisions corresponded to the following: 1) arc A began rostrally at the retrochiasmatic area (plate 18) where POMC-positive cells first appear, and continued caudally to the elongation of the third ventricle (plate 19; see Ref. 18 for plate identification); 2) arc B was continuous with arc A, beginning at plate 19 and continuing caudally to the appearance of the dorsal medial nucleus (DM; plate 20); 3) arc C was defined as the area containing the DM, beginning rostrally at plate 20 and continuing caudally through plate 21; and 4) arc D began with the disappearance of the DM and continued caudally to the end of the arcuate nucleus and the disappearance of POMC-positive neurons (plate 22). Three tissue sections/arcuate subdivision/animal were anatomically matched across animals and analyzed in a blind fashion. The signal levels in at least 10 individual cells of the arcuate nucleus/section (usually 5 cells/side) were measured by an automated computerized image-processing system (19). This same system was used to analyze the GnRH mRNA data. Clusters of silver grains were identified as being over a cell nucleus with bright-field optics, and then individual grains were counted with a $\times 40$ epiillumination darkfield objective. For the POMC mRNA data, the signal from 65–100 cells was measured in each animal.

Control experiments

GnRH mRNA. A saturation curve was constructed to determine the concentration of GnRH cDNA probe needed to saturate the endogenous GnRH mRNA molecules. Figure 1 illustrates that saturation was achieved at a probe concentration of approximately $0.21 \mu\text{g}/\text{ml}\cdot\text{kb}$. We performed our experiment with a GnRH probe concentration of $0.42 \mu\text{g}/\text{ml}\cdot\text{kb}$.

To demonstrate the binding specificity of the GnRH probe,

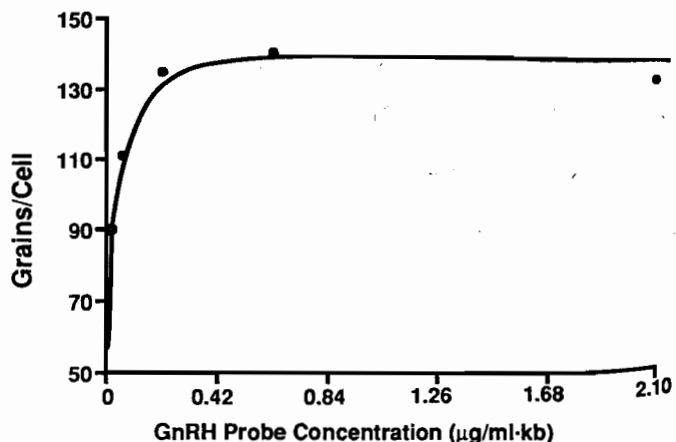


FIG. 1. Saturation curve for the prepro-GnRH oligonucleotide probe. The tissue is saturated at a concentration of approximately $0.21 \mu\text{g}/\text{ml}\cdot\text{kb}$. Experiments were performed with a probe concentration of $0.42 \mu\text{g}/\text{ml}\cdot\text{kb}$.

brain slices taken through the medial preoptic area of intact adult male rats were incubated with a saturating concentration of ^{35}S -labeled probe and a 100-fold excess of unlabeled GnRH oligomer. An absence of grain clusters over cells on the slides treated with excess cold probe indicated that binding of the GnRH cDNA probe was specific.

POMC mRNA. The POMC cRNA probe concentration required to saturate the cells in the arcuate nucleus of male rats was also determined by producing a saturation curve (not shown). The arcuate cells were saturated at a probe concentration of approximately $0.3 \mu\text{g}/\text{ml}\cdot\text{kb}$. Our experiment was carried out with a probe concentration of $0.6 \mu\text{g}/\text{ml}\cdot\text{kb}$.

To demonstrate the binding specificity of the POMC cRNA probe, three control experiments were performed. First, ^{35}S -labeled sense POMC cRNA probe, which had the same, not the complementary, sequence as POMC mRNA, was applied to tissue sections containing the arcuate nucleus. The fact that there were no grain clusters in these sections indicated that the silver grain clusters achieved with the use of antisense POMC cRNA probe were due to the probe binding to POMC mRNA and not the result of nonspecific binding. The second control experiment consisted of treating tissue with 20 mg/ml RNase for 30 min at 37 C before *in situ* hybridization with our antisense POMC cRNA probe. No silver grain clusters were observed in these sections, which demonstrated that the probe bound specifically to RNA in this experiment. In the third control experiment a 100-fold excess of unlabeled antisense POMC cRNA was added to the saturating concentration of ^{35}S -labeled probe and applied to tissue sections. No grain clusters were discerned in these sections, which indicated that the excess unlabeled probe competed with the radiolabeled probe for specific binding sites.

Testosterone RIA

Blood samples were analyzed for testosterone by RIA with a kit purchased from Leeco Diagnostics, Inc. (Southfield, MI). Samples were run in duplicate. The assay had a sensitivity of $5 \text{ pg}/100 \mu\text{l}$ and an intraassay coefficient of variation of 3.6%; all samples were measured in a single assay.

Statistical analysis

For both GnRH mRNA and POMC mRNA determinations, the mean grains per cell from a single animal and mean grains per cell from discrete anatomical areas in individuals were used to calculate the mean \pm SEM for each group. The difference between prepubertal and adult animals in grains per cell for all of the cells counted in an animal was assessed by Student's *t* test. To examine differences in GnRH message in specific anatomical regions, any area in which there were at least 10 cells/animal measured in all animals was evaluated individually. Variations across anatomical areas were evaluated by two-way analysis of variance with repeated measures (ANOVA). Power calculations were performed on the GnRH mRNA data to determine the resolution capacity of the protocol.

Results

GnRH mRNA analysis

GnRH mRNA-containing cells were identified in the anterior hypothalamic area, bed nucleus of stria terminalis, horizontal and vertical limbs of the diagonal band of Broca, lateral hypothalamus, lateral preoptic area, lateral septum, lateroanterior hypothalamic nucleus, median nucleus of the preoptic area, medial preoptic area, medial septum, nucleus accumbens, olfactory tubercle, organum vasculosum of the lamina terminalis, retrochiasmatic area, subfornical organ, septohypothalamic nucleus, and ventral pallidum. When all of the cells for the two age groups were analyzed and compared, no difference in message signal levels was detected (Fig. 2). The four anatomical areas that warranted a statistical evaluation (diagonal band of Broca, lateral preoptic area, medial preoptic area, and medial septum) were compared by ANOVA, and no changes were found between age groups or across anatomical areas, and there was no interaction between age and area (Fig. 3). Power calculations indicated that the quantitative analysis had sufficient sensitivity to detect a 20% increase in grains per cell between groups 90% of the time at the $P < 0.05$ level.

POMC mRNA analysis

Analysis of POMC mRNA levels in individual cells of the entire arcuate nucleus (Figs. 4 and 5) revealed that prepubertal animals had 119 ± 10 grains/cell, while adult cells contained an average of 167 ± 12 grains/cell ($P < 0.02$, by Student's *t* test), representing a signal increase of 40%. When the data from the four anatomical subdivisions of the arcuate nucleus were evaluated, it was

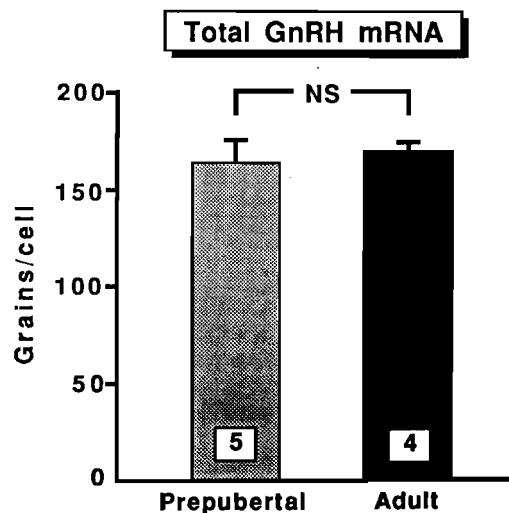


FIG. 2. A comparison between prepubertal and adult male rats in GnRH mRNA signal levels (grains per cell \pm SEM) in all cells counted throughout the septal, preoptic, and hypothalamic regions. The number of rats is given at the base of each column.

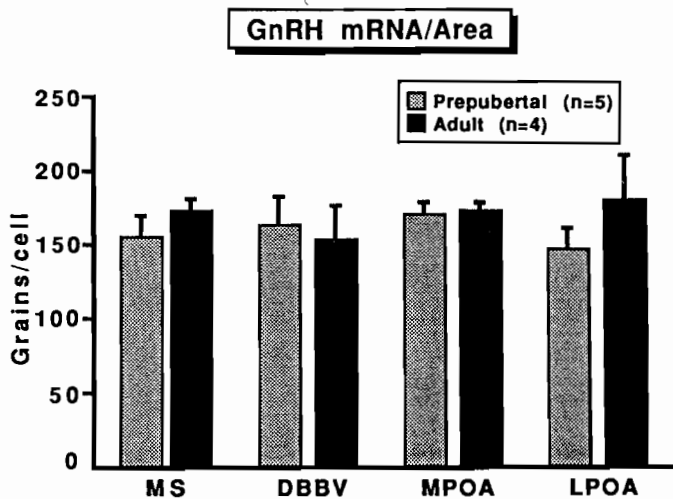


FIG. 3. A comparison between prepubertal and adult male rats in GnRH mRNA signal levels (grains per cell \pm SEM) in cells from four anatomical regions, including the medial septum (MS), the vertical limb of the diagonal band of Broca (DBBV), the medial preoptic area (MPOA), and the lateral preoptic area (LPOA).

found that the difference in POMC mRNA content between prepubertal and adult animals was confined to the most rostral areas (Fig. 6). In arc A, prepubertal animals had 138 ± 17 grains/cell, while adults had 216 ± 12 grains/cell ($P < 0.005$, by Student's *t* test), representing a 57% increase in signal. Arc B in prepubertal rats contained 113 ± 20 grains/cell, and in adults there were 177 ± 12 grain/cell ($P < 0.05$, by Student's *t* test), again representing a 57% increase in signal intensity. There was no statistically significant difference between prepubertal and adult males in the two caudal areas of the arcuate. A significant difference across areas was detected by two-way ANOVA ($P < 0.003$).

Testosterone analysis

The RIA for testosterone showed that the mean (\pm SEM) plasma level in the prepubertal rats was less than 0.1 ng/ml, confirming their prepubertal status. The mean plasma testosterone level in adult rats was 3.33 ± 0.81 ng/ml.

Discussion

Measuring GnRH mRNA in neurons throughout the hypothalamus and forebrain of the male rat, we detected no change in message content as a function of puberty. Given the variance of our analytical technique, we determined that there was a greater than 90% probability that we would have detected a 20% increase in GnRH mRNA had there been one. There is, however, a possibility that GnRH message levels do change with sexual development, but that this change is restricted to a relatively small population of GnRH neurons dispersed among a

larger heterogeneous population. If this were the case, even a large change in GnRH mRNA in cells within this subset of GnRH neurons could go undetected. Assessing this possibility will require double and triple labeling techniques combined with *in situ* hybridization.

Finding no significant change in GnRH mRNA with pubertal maturation suggests that the biosynthetic capacity of GnRH neurons of prepubertal animals is essentially in place, at least through the point of GnRH gene transcription and delivery of processed message to the cytoplasm. Evidence from primate species suggests that the prepubertal animal is capable of translating this GnRH message and secreting mature peptide, albeit in small amounts (20). Reinforcing this concept is the observation that pulsatile administration of the excitatory amino acid analog *N*-methyl-D,L-aspartic acid to prepubertal rats (21) and macaques (22) can stimulate GnRH secretion and, in rats, initiate full pubertal maturation. Furthermore, after castration in the prepubertal rat, gonadotropin secretion increases, with a time course similar to that in the adult animal (23, 24), again suggesting that the prepubertal animal is capable of producing GnRH well before the onset of puberty. Together, these findings argue that the biosynthetic elements required for synthesizing and secreting GnRH are in place in the prepubertal animal, but held in abeyance by some unknown mechanism.

Whereas our results suggest no change in the biosynthetic capacity of GnRH neurons, others have shown that hypothalamic GnRH content increases with sexual development (2, 3). The mechanism underlying the pubertal accretion of hypothalamic GnRH could involve changes in the patterning and efficiency of GnRH secretion, remodeling the prepubertal secretory pattern from a relatively constant delivery mode to one that is pulsatile and more efficient at stimulating gonadotropin secretion (22, 25). The accumulation of hypothalamic stores of the mature peptide could be explained on the basis of a simple disparity between synthesis and release. This putative remodeling of GnRH secretion could occur as a result of alterations in synaptic input to GnRH neurons or other modifications in the activity of the GnRH neuronal ensemble (26–28). It is also conceivable that pubertal changes in afferent activity to GnRH neurons mediate this possible remodeling event, and here, changes in EOP activity could play an important role. This possibility is reinforced by the fact that β -endorphin neurons from the arcuate nucleus project to the medial preoptic area and make direct, presumably inhibitory, synaptic contact onto GnRH neurons (29, 30).

We have shown that with the onset of puberty in the male rat POMC mRNA levels increase in neurons of the rostral arcuate nucleus. This result would argue against the concept that a reduction in POMC-derived EOP tone

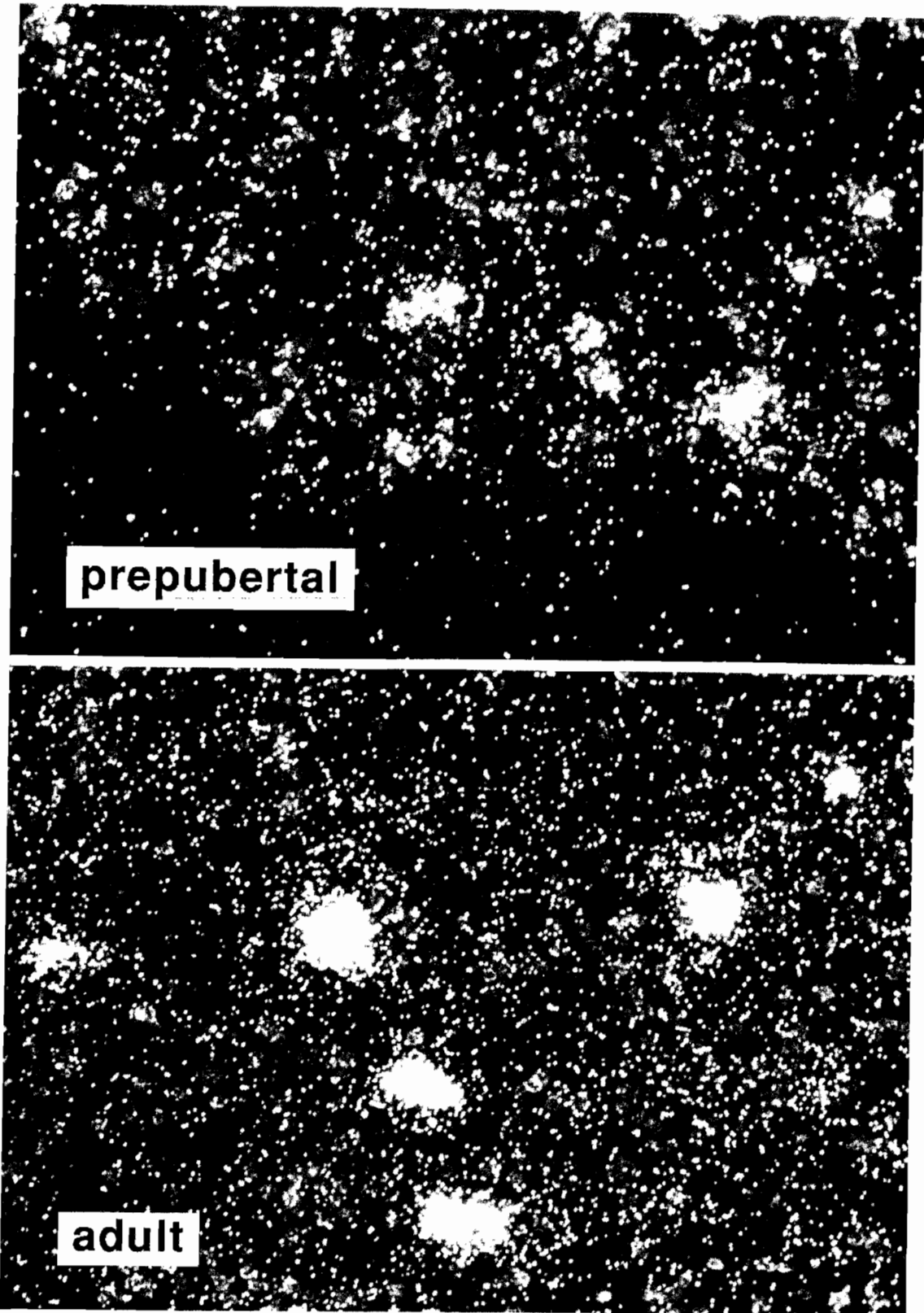


FIG. 4. Dark-field photomicrographs of the rostral arcuate nucleus showing cells labeled with a probe for POMC mRNA in sections obtained from prepubertal (*upper panel*) and adult (*lower panel*) male rats.

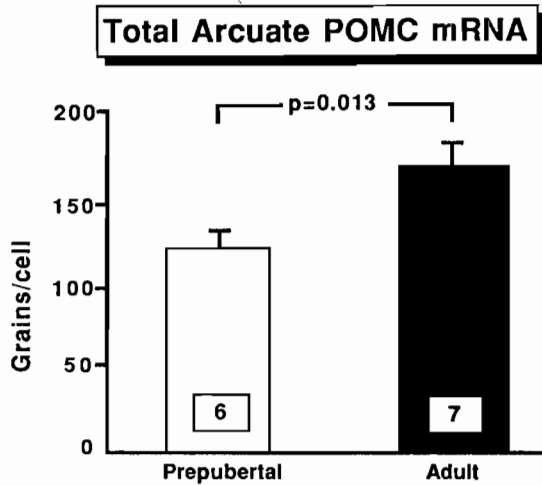


FIG. 5. A comparison between prepubertal and adult male rats in POMC mRNA signal levels (grains per cell \pm SEM) in labeled cells throughout the arcuate nucleus. The number of rats is given at the base of each column.

is directly responsible for mediating the change in GnRH secretory activity during puberty. It is conceivable that the pubertal increase in gonadal steroids is responsible for stimulating POMC gene expression in these neurons, *i.e.* the change in POMC message is a steroid-dependent process. Indeed, POMC neurons in the rostral arcuate of the adult male rat appear to be steroid sensitive, reflected by the fact that with castration, POMC message levels decline, and this decline can be prevented with testosterone replacement (31).

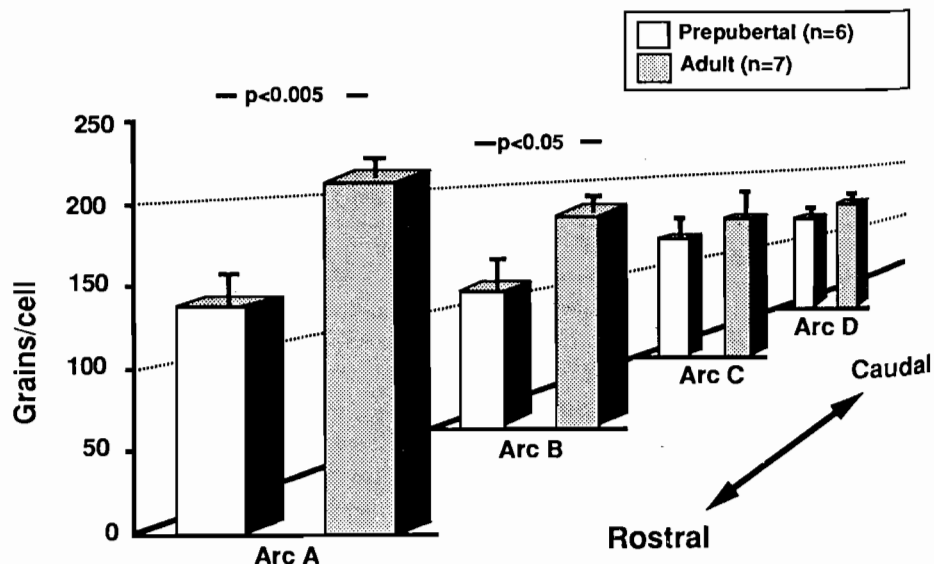
It is also plausible that the increase in POMC mRNA

may reflect the autonomous (steroid-independent) development of an important inhibitory neurotransmitter system which has, as its function, the orchestration of discrete GnRH pulses. In such a scheme, adult-like GnRH pulses become manifest with the periodic disinhibition of GnRH neurons, brought about by a transitory decrease in the activity of β -endorphin neurons. Although unproven, this hypothesis is attractive, as it provides a rational explanation for the fact that GnRH biosynthetic capacity, as reflected by GnRH mRNA, does not appear to change during puberty. In this model system we infer that GnRH secretion is present long before puberty, but that what is missing in prepubertal life is the ability of the brain to generate discrete GnRH pulses (22), and that the developing POMC (β -endorphin) system could provide the necessary regulatory element.

The pubertal increase in POMC mRNA appears to be restricted to a small subset of neurons located in the anterior aspect of the arcuate nucleus. This finding underscores the heterogeneity of POMC neurons, which could be subserved in part by a rostro-caudal gradient in steroid hormone receptors (32) and, moreover, identifies this region of the arcuate nucleus as an area that is potentially important for mediating the events controlling the onset of puberty. A differing neuronal environment between various areas of the arcuate could affect POMC neurons and bring about the observed regional difference in message levels. Björklund, *et al.* (33) have demonstrated that the arcuate nucleus dopaminergic neurons within the tubero-hypophyseal system are het-

POMC mRNA Content in 4 Anatomical Regions of the Arcuate Nucleus

FIG. 6. A comparison between prepubertal and adult male rats in POMC mRNA signal levels (grains per cell \pm SEM) in each of four regions of the arcuate nucleus divided in a rostral to caudal plane with reference to the atlas of Paxinos and Watson (18). Arc A is the most rostral section, and Arc D is the most caudal (see *Materials and Methods* for a full description).



erogeneous, thereby establishing a precedent for differential organization of a single neurotransmitter system within the arcuate nucleus. It appears that the arcuate POMC system, like the dopaminergic system, should also be regarded as a diverse assemblage of functionally distinct subsystems, all using a common neurotransmitter.

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