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Evidence for Activation of the Central Nervous System-Pituitary Mechanism for Gonadotropin Secretion at the Time of Puberty in the Male Rat*

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ABSTRACT. During sexual development in the male rat, serum testosterone (T) levels increase markedly at 45–60 days of age. At the time of the pubertal rise in T levels, activation of the hypothalamic-pituitary axis is difficult to demonstrate, since there is little change in serum LH levels and a decrease in serum FSH levels. We determined whether experimental maintenance of stable pubertal T levels in these animals as they passed through the normal age of puberty would allow demonstration of a major increase in serum gonadotropin levels. At 14–15 days of age, male rats were castrated and outfitted with either T-containing or empty Silastic capsules. Another group of rats was left intact and outfitted with empty capsules. At various times between 29 and 58 days of age, blood was drawn for measurement of serum LH, FSH, and T levels. In the T-implanted castrated rats, serum T levels were comparable to those in midpubertal intact rats, without significant differences among age groups. In this setting of stable T levels, serum LH and FSH were suppressed to levels at or below those in pubertal intact rats until 51 days of age, when they increased significantly into the untreated castrate range. In contrast, untreated castrate animals demonstrated markedly reduced serum T and elevated LH and FSH levels that did not change significantly throughout the entire study. In intact rats, serum T levels were stable until 58 days of age, when they increased over 2-fold; serum LH levels did not change significantly with age, and serum FSH levels decreased significantly by 54 days of age. A separate group of rats was castrated and outfitted with T-containing Silastic capsules at 21 days of age. In these animals, there were significant increases in hypothalamic LHRH, norepinephrine (NE), and dopamine levels and NE turnover rate at 56 compared to 36 days of age.

We conclude that stable pubertal levels of T are able to suppress gonadotropin levels in castrated rats until the normal age of puberty, at which time LH and FSH levels increase markedly. This decrease in sensitivity of the hypothalamic-pituitary axis to T negative feedback at puberty is accompanied by increases in hypothalamic LHRH, NE, and dopamine levels and NE turnover rate. These results provide direct evidence for activation of the central nervous system-pituitary mechanism regulating gonadotropin secretion at puberty in the male rat. The T-implanted castrate rat is a good model for studying changes in the hypothalamic-pituitary axis underlying normal puberty in the presence of controlled stable T levels and the absence of other testicular factors. (*Endocrinology* **119:** 362–369, 1986)

PUBERTY is a period of development in which a complex interaction of physiological processes results in full sexual maturation and the capacity to reproduce. Important changes in all components of the hypothalamic-pituitary-gonadal axis occur at the time of puberty. In primates, there is a pronounced rise in gonadotropin levels during sexual maturation (1–3). The pubertal increase in gonadotropin secretion also occurs in the absence of functioning testicular tissue (4–7), providing direct evidence for a change in the central nervous system (CNS)-pituitary mechanism regulating gonadotropin secretion at the time of puberty.

Activation of the hypothalamic-pituitary axis during sexual maturation in rats is not as easily demonstrated as it is in primates. In the male rat, pubertal development occurs from 35–60 days of age (8). During this period, serum testosterone (T) levels increase, and sperm maturation is completed (8). Unlike the situation in primates, serum LH levels change very little, and serum FSH levels decline during sexual maturation in the male rat (8–10). Furthermore, orchidectomy at any time during development in these animals results in marked increases in gonadotropin secretion, without further in-

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creases at the time of puberty (9, 11, 12).

In the present study, we determined whether experimental maintenance of stable pubertal T levels in male rats as they passed through the normal age of puberty would allow the demonstration of a major increase in serum gonadotropin levels. Prepubertal male rats were orchidectomized and, at the same time, outfitted with capsules containing T to maintain serum T at intact pubertal levels. The effect on serum LH and FSH levels and on hypothalamic catecholamine and LHRH levels of maintaining stable pubertal T levels in developing castrated immature rats was determined. We reasoned that if serum gonadotropin levels increased and hypothalamic factors known to regulate gonadotropin secretion changed during development, in a setting of stable T levels, this would provide direct evidence for activation of the CNS-pituitary mechanism regulating gonadotropin secretion at the time of puberty in the male rat.

Materials and Methods

Animals

Male Sprague-Dawley rats were obtained from Tyler Laboratories (Bellevue, WA). Male rat pups from different litters were obtained at 9–11 days of age with mothers and raised in litters of 8–10 until the time of weaning at 21 days of age. After weaning, five animals were housed together per cage. All rats were housed in polypropylene cages in an air-conditioned temperature-controlled room (22 C) under controlled artificial lighting conditions (12 h of light and 12 h of dark). All animals had rat chow and water available *ad libitum*.

Surgery

Bilateral orchidectomies were performed under light ether anesthesia through a midline scrotal incision. At the time of castration, either an empty or a T-containing Silastic capsule was implanted in the sc space along the posterior-dorsal aspect of the animals.

Silastic capsules were prepared as described previously (13) using medical grade Silastic tubing (id, 0.062 in.; od, 0.125 in.) sealed with Silastic adhesive, both obtained from Dow-Corning (Midland, MI). T was obtained from Sigma Chemical Co. (St Louis, MO). Capsules containing T were washed and equilibrated with three changes of PBS 0.5% gelatin over a period of 48 h before implantation.

Experimental protocol

Exp 1. At 14–15 days of age, 160 male rat pups were randomly divided into 3 groups.

Intact group: Forty rats were left intact and outfitted with an empty 5-mm long Silastic capsule.

Untreated castrate group: Forty rats were castrated and outfitted with an empty 5-mm long Silastic capsule at the time of orchidectomy.

T-implanted castrate group: Eighty rats were castrated and outfitted with a 5-mm (n = 40) or 10-mm (n = 40) long T-containing Silastic capsule at the time of orchidectomy.

At 29, 36, 40, 43, 47, 51, 54, and 58 days of age, blood was drawn by cardiac puncture under light ether anesthesia from 5 animals in the intact group, 5 animals in the untreated castrate group, and 10 animals from the T-implanted castrate group (5 animals each from the 5- and 10-mm T-implanted group). Blood samples were allowed to clot at room temperature and centrifuged at 4 C. Serum was stored at −20 C until assayed for LH, FSH, and T levels by RIA. Because there was considerable overlap and no statistically significant difference in the serum T levels in castrate rats bearing 5- and 10-mm T-containing capsules, these groups were combined for analysis.

Exp 2. At 22 days of age, 48 male rats were castrated and outfitted with 10-mm long T-containing Silastic capsules at the time of orchidectomy. At 36 and 56 days of age, 12 animals at each time point were killed by decapitation. To determine norepinephrine (NE) turnover rate at 36 and 56 days, another 12 animals at each time point received a dopamine (DA) β-hydroxylase inhibitor diethyldithiocarbamic acid, sodium salt (DDC; Sigma Chemical Co.; 500 mg/kg, ip), to inhibit norepinephrine synthesis. These rats were decapitated 3 h after injection of DDC (14, 15). The NE turnover rate was estimated using a nonsteady state method, described by Brodie *et al.* (16). In this method, the rate constant (k) of first order decline in NE levels after blockade of synthesis was determined using the formula: log [NE] = log [NE]i −0.434 kt, where [NE]i is the NE level before blockade of NE synthesis by DDC, and [NE] is the NE level 3 h after inhibition of NE synthesis by DDC. Then NE turnover rate (K) was calculated by the formula: K = k [NE]i.

To control for any effects of differing food intake and metabolic status on brain catecholamine levels, all animals in this experiment were fasted for 12 h before decapitation.

After decapitation, brains were rapidly removed from the skull, and whole hypothalamic sections were quickly dissected and frozen onto glass microscope slides on dry ice. Hypothalamic sections were stored at −70 C until extracted. Boundaries of the hypothalamic dissection were the optic chiasm rostrally, the mammillary bodies caudally, the optic tracts laterally, and the level of the fornix dorsally.

Sample preparation

Individual whole hypothalamic sections were placed in 500 μl boiling 1 N acetic acid containing 50 ng/ml 3,4-dihydroxybenzylamine (DHBA; Sigma Chemical Co.) for 20 min and sonicated using a Branson sonifier (Danbury, CT) at 20 kHz for 20 sec. DHBA was added as an internal standard to correct for procedural losses as a result of extraction techniques. Validation of DHBA as an internal standard was accomplished by demonstration of similar (within 5%) recoveries of NE, epinephrine (E), DHBA, and DA in spiked rat cerebellar tissue (n = 7) subjected to acid, then alumina, extraction (outlined below). A 10-μl aliquot of the hypothalamic homogenate was removed for protein determination using the micromethod of Bradford *et al.* (17), with reagents obtained from Bio-Rad (Richmond, CA) and for determination of LHRH levels by RIA. The remaining homogenate was then centrifuged at 15,000

× *g* at 4 C for 10 min. The supernatant was removed, aliquoted, and stored at −70 C.

Before catecholamine assay, an aliquot of the boiling acetic acid extract was extracted further using a modification of the alumina extraction technique described by Anton and Sayre (18). One hundred and fifty microliters of the acetic acid extract were added to a 1.9-ml conical polypropylene tube containing 10 mg acid-washed aluminum oxide (type WN-2 chromatographic alumina, grade I, Sigma Chemical Co.) and 1.0 ml 0.5 M Tris buffer (pH 8.6). The contents were shaken well for 5 min using a Roto-torque mixer (Cole-Palmer Instrument Co., Chicago, IL) and then centrifuged in a Beckman Microfuge II (Beckman Instruments, Berkley, CA) at 11,600 × *g* for 30 sec. The supernatant was aspirated and discarded, and the alumina was washed twice with 1.0 ml deionized distilled water. Catecholamines were desorbed from the alumina by adding 150 μl 0.1 N perchloric acid-0.825 mM cysteine, shaking well for 5 min, centrifuging at 11,600 × *g* for 5 min, and aspirating the supernatant. Alumina extracts were stored at −70 C until assayed.

Assays

LH and FSH RIAs. Serum LH and FSH levels were determined by RIA using reagents provided by the NIAMDD. Details of the methodology have been described previously (13). The antisera used were rabbit antirat LH S-5 and FSH S-10; the tracers used were rat LH I-5 and FSH I-5, radioiodinated with ^{125}I using chloramine-T (19). The reference standards used were rat LH and FSH RP-1. The intraassay coefficients of variation for the LH and FSH RIAs were 2.9% and 3.5%, respectively, and the interassay variabilities were 11% and 14%, respectively. The assay sensitivities for the LH and FSH RIAs were 0.6 and 10 ng/tube, respectively.

T RIA. The RIA for serum T used reagents provided by the WHO Matched Reagent Programme. Details of this assay have been described previously (20). The antiserum was raised in rabbits against a T-BSA and exhibited cross-reactivity of 14% with 5α-dihydrotestosterone, 6% with 5α-androstanediol, and less than 2% with the other steroids tested. The T assay was preceded by ether extraction, and separation of bound from free hormone was accomplished using dextran-coated charcoal. The intra- and interassay variables were 5.1% and 9.8%, respectively, and the assay sensitivity was 0.1 ng/ml.

LHRH RIA. LHRH levels in hypothalamic extracts were measured by RIA, as described previously (21). The antiserum (N540) was raised in rabbits against a LHRH-keyhole-limpet hemocyanin conjugate and was kindly provided by Dr. Robert A. Steiner (Departments of Obstetrics/Gynecology, Physiology/Biophysics, and Zoology, University of Washington School of Medicine, Seattle, WA). The tracer was [^{125}I]iodo-LHRH, purchased from New England Nuclear (Boston, MA), and synthetic LHRH, obtained from Peninsula Laboratories (San Carlos, CA), was used as the reference standard. The sensitivity of this assay was 0.5 pg/tube. LHRH values were expressed in picograms per mg protein.

Catecholamine assay. NE and DA levels in hypothalamic extracts were determined using reverse phase ion-pairing HPLC with amperometric electrochemical detection (HPLC/ECD).

Citric acid monohydrate, dibasic sodium phosphate, and HPLC grade methanol obtained from J. T. Baker Chemical Co (Phillipsburg, NJ), EDTA obtained from Fisher Scientific Co. (Fairlaw, NJ) and 1-octanesulfonic acid, sodium salt, obtained from Aldrich Chemical Co. (Milwaukee, WI) were used in preparation of the mobile phase. NE hydrochloride, DA hydrochloride, and DHBA hydrobromide, obtained from Sigma Chemical Co., were used to prepare catecholamine standard solutions. L-Cysteine, obtained from Sigma Chemical Co., was used as an antioxidant in samples and standards.

The HPLC/ECD system used was composed of the following components. The solvent delivery system consisted of a Beckman model 420 controller and model 112 pump. A Beckman model 210 sample injection valve equipped with a 20-μl sample loop was used to inject samples. A Brownlee NewGuard guard column (Brownlee Labs, Santa Clara, CA; id, 3.2 mm; length, 15 mm) with a 7-μm RP-18 cartridge was used to protect the analytical column from sample contaminants. The analytical column used for chromatographic separation of catecholamines was an Altex Ultrasphere-ODS column (id, 4.6 mm; length 250 mm; particle size, 5 μm). The amperometric electrochemical detector was a Bioanalytical Systems model LC-4B (BAS, West Lafayette, IN), with a TL-5A glassy carbon working electrode and RE-I Ag-AgCl reference electrode. A Hewlett-Packard 3390A integrator (Hewlett-Packard, Avonadale, PA) was used to create calibration tables based on injections of catecholamine standards and to calculate peak areas and quantitate catecholamine levels in samples.

The mobile phase was composed of 0.1 M citrate-phosphate buffer, containing 0.1 mM EDTA and 0.012% (wt/vol) octanesulfonic acid, as an ion-pairing agent. After adjusting the mobile phase to pH 3.50 with concentrated hydrochloric acid, methanol (9%, vol/vol) was added an organic modifier. Before use, the mobile phase was filtered and degassed by vacuum twice through a 0.22-μm Millipore filter (Millipore Corp., Bedford, MA). The working potential of the electrochemical detector was set at +0.65 V relative to the Ag-AgCl reference electrode, and the detector sensitivity was set at 2 nA full scale. Mobile phase was pumped at a flow rate of 1 ml/min (2600–2900 psi) at ambient temperature. Before the injection of any samples, the entire HPLC/ECD system was allowed to equilibrate overnight with the mobile phase.

A standard solution of catecholamines (containing 50 ng/ml each of NE, E, DHBA, and DA in 0.1 N perchloric acid-0.825 mM cysteine) was freshly prepared each day from an aliquot of a more concentrated stock solution (1 mg/ml) which was stored at −70 C. Each day, an internal standard calibration table was created in the integrator with three successive injections of a standard solution. In addition, two whole rat brain pools (prepared at the same time stock standard solution was prepared) were included in each assay run. The injection volume for standards, pools, and samples was 20 μl. Quantitation of catecholamines in samples was performed by the integrator by comparison of peak areas of the unknowns to those of known quantities of catecholamine standards and the internal standard DHBA. Catecholamine values were expressed in nanograms per mg protein.

A typical elution profile of catecholamine standards and a rat hypothalamic extract is shown in Fig. 1. Using the same mobile phase preparation, there was less than 1% variability in the retention times with successive injections of same standard solution (n = 15) or hypothalamic extract (n = 10). The linear response range of the detector with the sensitivity set at 2 nA full-scale for standards and hypothalamic extracts was 60 pg to 8 ng. The intraassay coefficient of variation, determined by repeated injections of a standard solution (n = 24) or a pooled rat hypothalamic extract (n = 11), was 2.1% for NE and 3.1% for DA. The interassay coefficient of variation determined on two rat whole brain pools run in 30 consecutive assays was 6% for NE and 9% for DA.

To compare values of catecholamines measured with the assay described above with those reported by other investigators, hypothalami from 15 adult male Sprague-Dawley rats were isolated, weighed, extracted, and assayed for NE and DA in our HPLC/ECD assay. Hypothalamic NE levels averaged 1.92 ± 0.16 ng/mg wet weight, and DA levels averaged 0.46 ± 0.04 ng/mg wet weight. These values were comparable to those reported by several other laboratories (22–26). To further validate our assay, rat hypothalamic and whole brain extracts (n = 11) were assayed simultaneously in the laboratory of Dr. Richard C. Veith (GRECC, V.A. Medical Center, Seattle, WA) using a different HPLC/ECD system with different mobile phase and detector conditions. Comparison of catecholamine values in these two HPLC/ECD systems revealed an excellent correlation (r = 1.00 and slope = 0.92 for NE; r = 0.98 and slope = 0.95 for DA). Finally, catecholamine standards (n = 14), ranging from 60–8000 pg, and rat hypothalamic extracts (n = 11), ranging from 265–1935 pg, were assayed blindly for catecholamines in the laboratory of Dr. Richard C. Veith (GRECC, V.A. Medical Center, Seattle, WA) using a radioenzymatic assay that has been described previously (27). There was excellent correlation between catecholamine values measured in the HPLC/ECD and radioenzymatic assays for both standards (r = 0.995 and slope = 1.08 for NE; r = 0.999 and slope = 0.97 for E; r = 0.998 and slope = 1.06 for DA) and hypothalamic extracts (r = 0.996 and slopes = 0.89 for NE; r = 0.994 and slope = 1.06 for DA).

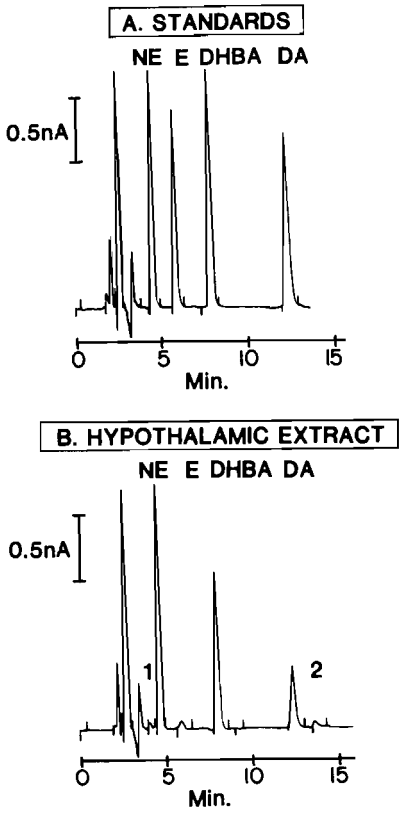

FIG. 1. A, Chromatogram of a standard solution containing 1 ng each of NE, E, DHBA (internal standard), and DA. B, Chromatogram of a whole hypothalamic extract from an intact 56-day-old male rat. Both standard solution and hypothalamic extract were diluted in 0.1 N perchloric acid-0.825 mM cysteine and injected at a volume of 20 μl. The analytical column was a 4.6 mm (id) × 250 mm (length) 5-μm particle size Ultrasphere-ODS column. The mobile phase was composed of 0.1 M citrate-phosphate buffer, 0.1 mM EDTA, and 0.012% (wt/vol) octanesulfonic acid at pH 3.50 and 9% (vol/vol) methanol and was pumped at a flow rate of 1 ml/min. The BAS LC-4B electrochemical detector used a thin layer glassy carbon electrode set at +0.650 V relative to a Ag/AgCl reference electrode. The retention times for NE, E, DHBA, and DA were 4.4, 5.7, 7.9, and 12.1 min, respectively. In the rat hypothalamic extract (B), the small peak (1) eluting before NE was 3,4-dihydroxyphenylglycol and the peak (2) eluting after DA was 3,4-dihydroxyphenylacetic acid.

Statistical analysis

In Exp I, mean serum LH, FSH, and T levels were determined at each age for the intact, untreated castrate, and T-implanted castrate groups. One-way analysis of variance and Duncan's multiple range test were used to assess the effect of age on hormone levels in each of the three groups of animals. In Exp 2, mean hypothalamic LHRH, NE, and DA levels were determined at the two ages studied and compared using Student's unpaired *t* test. The rate constants for NE turnover were compared using analysis of variance of regression coefficients.

Results

Exp 1

In intact rats (Fig. 2), mean serum T levels rose gradually from 29 to 54 days of age, but did not exceed 1.20 ng/ml. At 58 days of age, there was a significant increase in T levels to 3.25 ± 0.91 ng/ml (mean ± SEM). Serum LH levels in intact rats also increased gradually from 29 to 54 days of age. Mean LH levels throughout the study were 89 ± 7 ng/ml, and the slight increase in LH levels was not statistically significantly. Serum FSH levels in these animals decreased significantly from 682 ± 18 ng/ml at 29 days of age to 360 ± 28 and 448 ± 41 ng/ml by 54 and 58 days of age, respectively.

In the untreated castrate rats (Fig. 3), serum T levels (0.10 ± 0.01 ng/ml) were reduced, and serum LH (882 ± 37 ng/ml) and FSH (1491 ± 50 ng/ml) levels were elevated to castrate levels at all time points. There were no significant age-related changes in serum T, LH, and FSH

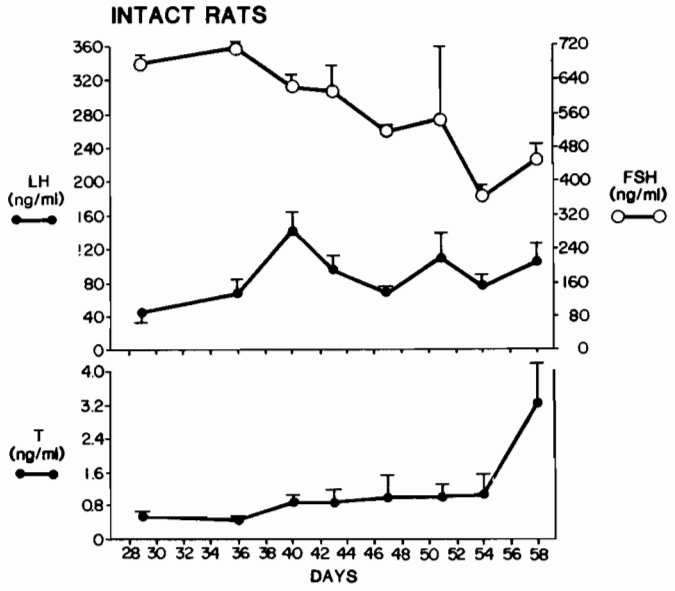

FIG. 2. Mean serum LH and FSH levels (*upper panel*) and T levels (*lower panel*) in male rats outfitted with empty Silastic capsules at 14–15 days of age (intact group) and bled at various ages. Serum T levels increased gradually from 29 to 54 days of age and markedly at 58 days of age. Serum LH levels changed very little, and FSH levels decreased from 29 to 58 days. Each *point* represents the mean ± SEM for five animals.

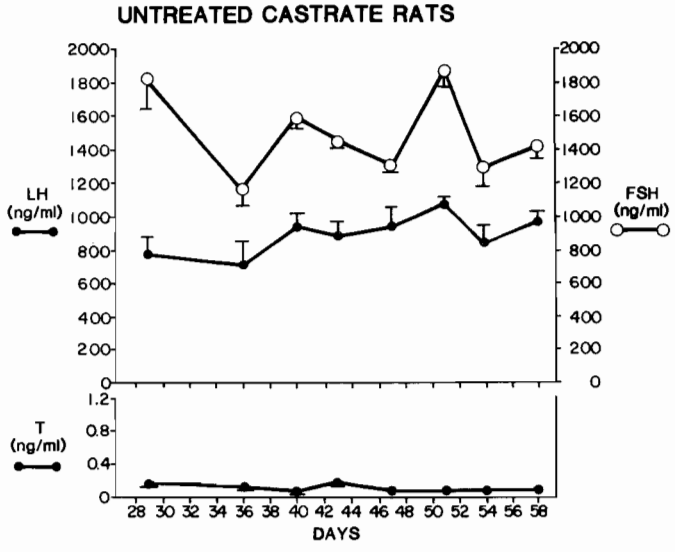

FIG. 3. Mean serum LH, FSH, and T levels in male rats castrated and outfitted with empty Silastic capsules at 14–15 days of age (untreated castrate group) and bled at various ages. Serum T levels were suppressed, and LH and FSH levels were elevated to castrate levels without a consistent trend in values from 29–58 days of age. Each *point* represents the mean ± SEM for five animals.

in untreated castrate rats throughout the entire study.

Silastic T implants maintained stable serum T levels across all age groups in the T-implanted castrate rats (Fig. 4). Serum T levels averaged 1.20 ± 0.08 ng/ml in this group of animals and were similar to those in mid-pubertal intact rats. There were no statistically signifi-

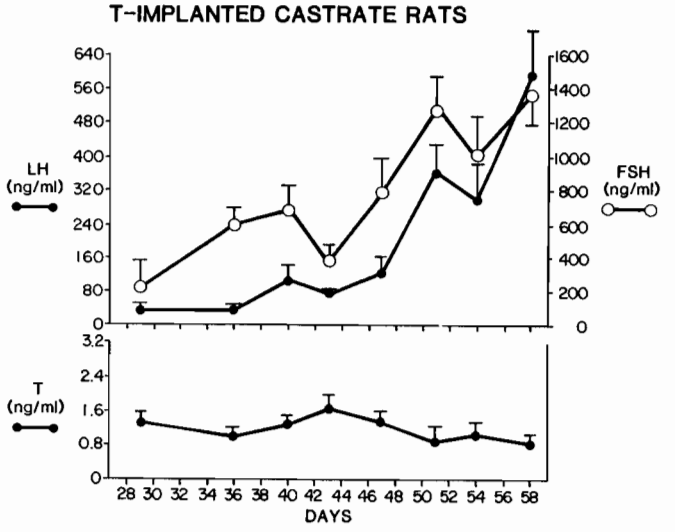

FIG. 4. Mean serum LH and FSH levels (*upper panel*) and T levels (*lower panel*) in male rats castrated and outfitted with a Silastic capsule containing T (T-implanted castrate group) and bled at various ages. Serum T levels were stable from 29–58 days of age. Serum LH and FSH levels were within the intact pubertal range from 29–47 days and then increased markedly to untreated castrate levels at 51–58 days of age. Each *point* represents the mean ± SEM for 10 animals.

cant changes in serum T levels with age by analysis of variance. In this setting of stable T levels, there was a significant age-related increase in serum LH levels in T-implanted castrate animals. In this group, LH levels were stable from 29 to 47 days of age and were at or below levels found in intact rats. At 51 days of age, LH levels increased significantly, reaching a mean of 593 ± 104 ng/ml by 59 days, a level in the range of untreated castrate rats. Serum FSH also increased significantly with age in the T-implanted castrate rats. In these animals, FSH levels increased slightly at 36 days of age and then remained stable at levels similar to those in intact rats until 47 days. At 51 days of age, serum FSH increased significantly to untreated castrate levels, reaching a mean of 1365 ± 177 ng/ml by 58 days.

Exp 2

Hypothalamic LHRH levels (Table 1) increased about 1.5-fold from 635 ± 198 pg/mg protein at 36 days of age to 979 ± 391 pg/mg protein at 56 days of age.

Hypothalamic levels of both NE and DA (Table 1) increased approximately 2-fold from 36 to 56 days of age. The rate constant for NE turnover at 56 days of age was significantly greater than that at 36 days, and NE turnover rate increased 2.5-fold from 15.9 ng/mg protein·h at 36 days to 40.0 ng/mg protein·h at 56 days of age.

Discussion

Our results demonstrate a marked pubertal increase in serum gonadotropin levels in castrated immature male

TABLE 1. Hypothalamic LHRH, NE, and DA levels and NE turnover rates

	Day 36	Day 56
LHRH (pg/mg protein)[a]	636 ± 198	979 ± 391[b]
NE (ng/mg protein)[a]	48.4 ± 3.6	107.3 ± 12.2[c]
DA (ng/mg protein)[a]	9.2 ± 0.7	22.6 ± 2.1[c]
NE rate constant, k (liters/h)[d]	0.327 ± 0.007	0.376 ± 0.013[e]
NE turnover rate (ng/mg protein·h)[d]	15.9	40.0

[a] Values are the mean ± SEM for 10–12 animals at each age.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] Calculated by the method of Brodie *et al.* (16).
[e] $P < 0.001$.

rats, whose serum T was maintained at nearly constant levels across the normal time of puberty. Rats that were castrated and outfitted with T-containing Silastic capsules maintained essentially stable T levels from 29 to 58 days of age. In the setting of stable serum T levels, serum LH and FSH levels in T-implanted castrate animals remained comparable to those in midpubertal intact rats from 29 to 47 days of age. By 51 days of age, both serum LH and FSH increased markedly to castrate levels despite unchanging serum T levels. In contrast, rats that were castrated and given empty capsules demonstrated markedly diminished serum T values and elevated serum LH and FSH levels, typical of adult castrate animals, throughout the study. These results demonstrate an age-related decrease in the sensitivity of the hypothalamic-pituitary axis to the negative feedback effect of T on gonadotropin secretion during puberty in the male rat. The change in CNS-pituitary sensitivity to T negative feedback occurred just before (51 days) the age at which intact rats demonstrated a marked pubertal rise in serum T levels (between 54 and 58 days of age). This change in sensitivity to T negative feedback provides an explanation for the finding that serum LH levels change very little in intact rats from 54 to 58 days of age, a time when serum T levels increase over 2-fold in these animals.

Our findings confirm those of other investigators who have reported previously that more T was required to prevent the acute postcastration rise in serum gonadotropin levels in adult compared to prepubertal male rats (28–30). In those studies, T was administered, beginning at the time of castration, for relatively short periods of time (2–5 days) before blood sampling at a single time point after orchidectomy. In the present study, T was also started at the time of castration, but was administered for a longer period of time (14–44 days) before blood sampling at various times after orchidectomy. Using a paradigm similar to that used in the present study, Sisk and Turek (31) demonstrated a similar pubertal decrease in sensitivity to T negative feedback in castrated male hamsters bearing T implants that maintained chronically stable T levels across the normal time of puberty.

Despite the fact that T-implanted castrate rats increased in body size and their blood volume probably increased during the prolonged period of T treatment, reasonably stable serum T levels were maintained in these animals when a single Silastic capsule was left in place throughout the entire study. Smith *et al.* (30) reported that plasma T levels produced by a Silastic capsule of a given length were greater in adult rats than in prepubertal rats. They suggested that this was due to a reduced metabolic clearance of T in adult compared to prepubertal animals. Decreased clearance of T with aging would have tended to offset the dilutional effects of increasing blood volume on the serum T levels produced by the T-containing Silastic capsules and may have contributed to the maintenance of stable T levels in our study.

Studies performed previously in adult rats that were castrated and outfitted with T-containing capsules to maintain intact serum T levels demonstrated sustained gonadotropin suppression for periods of 21–76 days (13, 32). In contrast, maturing animals in our study demonstrated increased serum gonadotropin levels 36–37 days after castration and implantation of a T-containing capsule. Therefore, the decreased sensitivity to T negative feedback appears to be a phenomenon unique to maturing animals.

Although their exact neuroregulatory role is unclear, both NE and DA are known to be important neurotransmitters regulating LHRH secretion (33, 34). In addition, NE is thought to be an important neuromodulator of T negative feedback in the CNS. NE content and turnover have been demonstrated to increase after orchidectomy and decrease with T replacement of orchidectomized rats (35, 36). LHRH is the major hypothalamic releasing factor regulating both LH and FSH secretion from the anterior pituitary gland (37). LHRH content has been reported to decrease after orchidectomy and increase with T replacement in castrated animals (38). Other investigators have reported increases in hypothalamic LHRH content (39) and catecholamine levels and turnover (40–42) with sexual maturation in intact rats. However, since both LHRH and catecholamine content and turnover are affected by changes in gonadal status, changes in these variables across puberty in intact animals could simply reflect changes in gonadal hormone production.

We found significant increases in hypothalamic LHRH, NE, and DA levels and NE turnover rate at 56 days (an age when there is a demonstrable decrease in the hypothalamic-pituitary sensitivity to T negative feedback) compared to those at 36 days of age. These

changes were found using a paradigm that eliminated the increase in gonadal hormone production that normally occurs at this age. Therefore, these age-related changes in LHRH and catecholamine metabolism presumably reflect a primary CNS event, not an alteration in gonadal hormone production. The increases in these important neuroregulators of gonadotropin secretion suggest that at least part of the decrease in sensitivity of T negative feedback occurring after 51 days of age occurs at the level of the CNS. The increase in hypothalamic neurotransmitter activities in the presence of stable circulating T levels provides direct evidence for activation of the CNS at the time of puberty in the male rat. This increase in central drive (43) can explain, at least in part, the decrease in hypothalamic-pituitary sensitivity to T negative feedback, known as the gonadostat theory of the onset of puberty (11).

Whether an independent change also occurs in the negative feedback effect of T on the anterior pituitary was not addressed in this study. In intact male rats, previous studies have reported no difference in the LH response and a decreased FSH response to LHRH in adult compared to prepubertal animals (44, 45). However, in those studies, it is likely that serum T levels were higher in the adult compared to prepubertal rats, and the effect of sexual maturation on gonadotropin responsiveness to LHRH in a setting of stable T levels is not known.

In conclusion, constant pubertal levels of T are able to suppress gonadotropin levels in castrated immature male rats until the normal age of puberty, at which time LH and FSH levels increase markedly. This decrease in the negative feedback effect of T on the hypothalamic-pituitary axis is accompanied by increases in hypothalamic LHRH, NE, and DA content and NE turnover rate. These results provide direct evidence for activation of the CNS-pituitary mechanism regulating gonadotropin secretion at puberty in the male rat. Finally, the T-implanted castrate rat is a good model for studying changes in the hypothalamic-pituitary axis underlying normal puberty in the presence of controlled stable T levels and the absence of other testicular factors.

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