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# Puberty in monkeys is triggered by chemical stimulation of the hypothalamus

(*N*-methyl-D-aspartate receptor/gonadotropin-releasing hormone/pulsatile gonadotropin/primates/spermatogenesis)

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**ABSTRACT** **Gonadal quiescence prior to puberty in primates results from a diminished secretion of the pituitary gonadotropic hormones, follicle-stimulating hormone and luteinizing hormone, which, in turn, is occasioned by an interruption of pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus during this phase of development. A discharge of GnRH may be provoked from the hypothalamus of prepubertal monkeys, however, by an i.v. injection of *N*-methyl-D-aspartate (NMDA), an analog of the putative excitatory neurotransmitter, aspartate. Since this action of NMDA is blocked by the specific NMDA receptor antagonist, DL-2-amino-5-phosphonopentanoic acid, the release of GnRH is likely mediated by NMDA receptors located either on the GnRH neurons themselves or on afferents to the GnRH cells. We report here that prolonged intermittent NMDA stimulation of GnRH neurons within the hypothalamus of the juvenile monkey for 16–30 wk results, with surprising ease, in the onset of precocious puberty with full activation of the hypothalamic–pituitary–Leydig cell axis and initiation of spermatogenesis. These findings demonstrate that, in primates, the network of hypothalamic GnRH neurons, which in adulthood provides the drive to the gonadotropin-secreting cells of the anterior pituitary gland, must now be viewed together with the pituitary and gonads as a nonlimiting component of the control system that governs the onset of puberty in these species.**

In the rhesus monkey and other primates including man, the hypothalamic–pituitary component of the control system that governs gonadal function attains a high degree of organization during fetal development (1). Thus, with loss of the inhibitory action of placental steroids at birth, the behavior of this neuroendocrine axis during early neonatal development is reminiscent of that associated with adulthood. Infancy in primates, however, is not followed by an immediate transition into a state of sexual maturity; instead, gonadotropin secretion declines, thus guaranteeing the protracted phase of gonadal quiescence that characterizes prepubertal development in these species. Puberty occurs several years later when the hypothalamic–pituitary axis is reawakened from its prepubertal dormancy (1).

An interruption in the pulsatile release of gonadotropin-releasing hormone (GnRH), the hypothalamic-releasing factor that provides the major drive to the gonadotropin-secreting cells of the anterior pituitary gland (gonadotrophs), appears to underlie the prepubertal hiatus in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion (refs. 2–5, 7; ¶). The seemingly dormant GnRH neurons of the hypothalamus of the prepubertal monkey, however, may be excited by peripheral injections of *N*-methyl-D-aspartate (NMDA) into producing an intermittent discharge of their releasing factor into the hypophysial portal circulation that results in a sustained hypophysiotropic drive to the gonadotrophs (8, 9). This and other central neural actions of NMDA, which is an analog of the putative excitatory neurotransmitter, aspartate, are considered to be mediated by the specific and widely distributed NMDA receptor (10). Administration of the specific NMDA receptor antagonist, DL-2-amino-5-phosphonopentanoic acid, prevents NMDA from eliciting LH release in the monkey (8). NMDA-induced release of GnRH is probably a reflection of a general excitation of many neurons in the vicinity of the median eminence (8), a circumventricular area of the brain (11) presumably accessible to systemically administered acidic amino acids and their analogs.

The foregoing findings suggest that the network of GnRH neurons in the hypothalamus of the prepubertal macaque possesses the neurosecretory capacity to generate an "adult" hypophysiotropic drive to the pituitary gonadotrophs. Therefore, it appears that the hypothalamic GnRH neuron may have to be viewed, along with the pituitary and the gonad (2–5), as a nonlimiting component of the control system that triggers puberty in primates. The purpose of the present study was to test directly this hypothesis.

## MATERIALS AND METHODS

**Animals.** Five male rhesus monkeys (*Macaca mulatta*) were studied during the prepubertal phase of development, which, in this species, is characterized by an apparent hiatus in intermittent hypothalamic GnRH secretion between 6 and 30 mo of age (12). All animals were catheterized between 15 and 16 mo of age. Body weight (BW) at the time of catheterization ranged from 2.2 to 2.9 kg, and all experiments were completed prior to 2 yr of age. The general maintenance of the animals under a controlled photoperiod (lights on from 0600 to 1800 hr) has been described in detail (13).

**Drugs.** *N*-Methyl-DL-aspartic acid (Sigma) was dissolved in 0.9% NaCl (10–30 mg/ml) and used at room temperature within 3 days of preparation. Prior to infusion the solution of the aspartate analog was passed through a 0.22-$\mu$m filter unit (Millen-GS, Millipore) installed in the infusion line.

Two different GnRH receptor antagonists were used. Short-term antagonism was achieved with [*N*-Ac-D-Nal(2)$^1$, D-pCl-Phe$^2$,D-Trp$^3$,D-hArg(Et$_2$)$^6$,D-Ala$^{10}$]GnRH (RS68439, Syntex Research, Palo Alto, CA). RS68439 was dissolved in 0.9% NaCl (0.25 mg/ml) and injected as an i.v. bolus at a dose of 500 $\mu$g/kg of BW. This dose has been shown to inhibit LH secretion for 24 hr in intact adult male macaques (14). For

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Abbreviations: GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone; GH, growth hormone; NMDA, *N*-methyl-D-aspartate; BW, body weight.

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chronic inhibition of GnRH action, [Ac-D-2Nal[1],4Cl-Phe[2], D-Trp[3],D-Arg[6],D-Ala[10]]GnRH-HOAc (Contraceptive Development Branch, National Institute of Child Health and Human Development), an antagonist previously established to inhibit GnRH action in the monkey (15), was employed. The latter GnRH antagonist was suspended in peanut oil (6 mg/ml) and injected (2.3 mg/kg of BW i.m.) every 3½ days.

**Surgical Procedures.** To permit withdrawal of sequential blood samples and the simultaneous i.v. administration of NMDA, monkeys were implanted via an internal jugular vein with two chronic indwelling cardiac catheters that were exteriorized through a cranial platform. Animals were anesthetized with sodium pentobarbital (Nembutal, Abbott; 30 mg/kg of BW, plus supplemental doses of 5 mg/kg of BW as required). Following surgery, analgesia was achieved with meperidine hydrochloride (Demerol, Winthrop-Breon, New York; 1 mg/kg of BW injected i.v. as required) for a minimum of 4 days. Animals were housed in remote sampling cages, which permitted continuous access to the venous circulation without restraint or tranquilization. Full details of these procedures have been provided elsewhere (16). In one animal it was necessary to implant a new catheter after 10 wk of NMDA treatment. Subsequently, this animal was maintained with a tether system that utilizes a nylon jacket, instead of a cranial platform, to protect the catheters (17).

Hemiorchidectomy was performed through a scrotal incision under sodium pentobarbital anesthesia as described above.

**Collection of Blood Samples.** To describe moment-to-moment changes in circulating concentrations of LH, FSH, testosterone (T), and growth hormone (GH), sequential blood samples (1.4–2.0 ml) were withdrawn at 15- to 60-min intervals, as described (18).

**Hormone Assays.** Plasma LH concentrations were estimated by a cynomolgus LH:anti-human chorionic gonadotropin (rabbit 13, pool D) RIA system that employs rhesus pituitary preparation WP-XV-20 (National Institute of Child Health and Human Development rh LH) for standard. FSH was determined by a human FSH (National Institutes of Health FSH HS-1):anti-human FSH (batch 5, National Institute of Diabetes and Digestive and Kidney Diseases, National Pituitary Agency) RIA system that employs a rhesus pituitary preparation (WP-XIII-21-42) as standard. These assays, and the RIAs used to measure plasma T and GH concentrations, have been described (8, 19).

**Testicular and Epididymal Morphology.** The width ($w$) and length ($l$) of the testis *in situ* were measured with calipers, and testicular volume ($v$) was calculated as described by $v = \pi w^2 l/6$. When both gonads were present, the volumes of the left and right testes were summed. In hemiorchidectomized animals, the volume of the remaining testis was doubled to obtain an estimate of total testicular volume.

Following hemiorchidectomy, testis and epididymis were separated and each was promptly weighed. A small fragment (<100 mg) of testis and epididymis was separately minced at 37°C in 200 μl of 0.9% NaCl. The resulting suspensions were viewed under a light microscope to establish the presence or absence of sperm. The testis and epididymis were then cut into several pieces and immersed in Bouins' fixative for 18–24 hr. After fixation, sections were cut (4 μm) and stained with periodic acid/Schiff's reagent and counterstained with hematoxylin.

## EXPERIMENTAL DESIGN

**Experiment 1.** In four of the five monkeys, chemical stimulation of the hypothalamus was imposed for 16–30 wk with a chronic intermittent i.v. infusion of *N*-methyl-DL-aspartate: a brief infusion of 1-min duration was administered every 3 hr. The interval between the NMDA injections was selected to impose a pattern of hypothalamic stimulation that would elicit a discharge of GnRH at a frequency comparable to that produced spontaneously by the hypothalamus of an adult male (18). The initial dose of *N*-methyl-DL-aspartate (≈5 mg/kg of BW) was based on the earlier finding that this dose, administered as a single i.v. injection, elicited a 50% maximal response (8). Over the course of the experiment the dose of *N*-methyl-DL-aspartate was progressively increased to as much as 14 mg/kg of BW to maintain a robust response to hypothalamic stimulation.[‖] The precise dose of NMDA, the active isomer, was not known, since a racemic mixture was administered.

The response of the pituitary–testicular axis to sustained intermittent hypothalamic stimulation was assessed by measuring moment-to-moment changes in circulating concentrations of LH, FSH, and T for 6 hr during the first two infusions of NMDA and thereafter at weekly or biweekly intervals during two representative and consecutive infusions.

Testicular volume and BW were determined at weekly intervals. For this purpose animals were briefly sedated with an i.v. injection of ketamine hydrochloride (Ketarlar, Parke–Davis; ≈50 mg per animal) administered via the catheter used for the infusion of NMDA. Since a rapid induction of sedation following injection of ketamine indicated continued access to the venous circulation, the weekly measurement of somatic indices provided an opportunity to confirm the patency of the catheter used to infuse NMDA. As a consequence of this procedure, the pattern of intermittent NMDA administration was transitorily perturbed.

To evaluate the effect of sustained hypothalamic stimulation on testicular and epididymal morphology, the four monkeys were hemiorchidectomized during the 10th, 18th (two animals), or 26th wk of NMDA treatment, respectively. For this purpose the intermittent i.v. infusion of NMDA was briefly disrupted while animals underwent surgery. The remaining testis was removed at the termination of the experiment after 16–30 wk of NMDA treatment (see below).

In three of the four animals used in this experiment, two additional observations were made following hemiorchidectomy. The purpose of the first of these was to confirm that the effects of NMDA upon the pituitary–testicular axis were secondary to hypothalamic stimulation. Three to 11 days after removal of the first testis, the profiles of circulating gonadotropin and T concentrations were characterized during a 6-hr period of sequential blood collection from 0900 to 1500 hr. Approximately 9–34 hr later the GnRH antagonist, RS68439, was administered as an i.v. bolus without disrupting the pulsatile administration of NMDA. The following day, plasma gonadotropin and T concentrations were monitored while maintaining the intermittent infusion of NMDA. Five to 6 days later sequential samples were again collected to establish that the effects of the GnRH antagonist had dissipated.

The purpose of the second control observation, which was conducted between the 20th and 29th wk of hypothalamic stimulation and after recovery from the effects of RS68439, was to monitor the consequences of acutely withdrawing intermittent hypothalamic stimulation. For this purpose, the NMDA infusion was terminated in the evening between 1900 and 2300 hr. At this time, the NMDA that was contained in the dead space of the infusion catheter (≈60 mg of *N*-methyl-DL-aspartate) was flushed into the animal with 5–10 ml of saline to eliminate further delivery of the aspartate analog. The following morning a final series of sequential blood samples were collected between 0900 and 1500 hr. After a 16- to 23-hr

[‖]The NMDA doses for each animal were as follows. Animal 1: wk 1–8, 4 mg/kg; wk 9–19, 7 mg/kg; wk 20–30, 9 mg/kg. Animal 2: wk 1–3, 6 mg/kg; wk 4–13, 10 mg/kg; wk 14–20, 13 mg/kg. Animal 3: wk 1–7, 5 mg/kg; wk 8–16, 8 mg/kg. Animal 4: wk 1 and 2, 5 mg/kg; wk 3 and 4, 10 mg/kg; wk 5–22, 14 mg/kg.

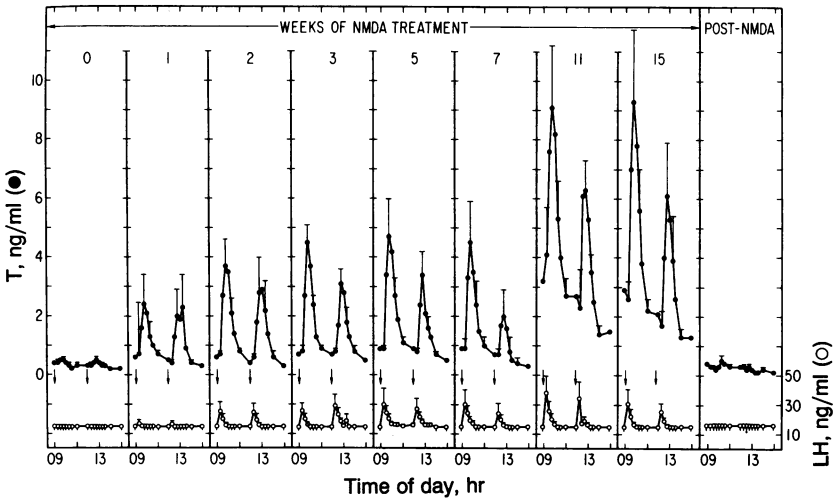


FIG. 1. Premature activation of the hypothalamic–pituitary–testicular axis in prepubertal rhesus monkeys ($n$ = 4) induced by repetitive stimulation of the hypothalamus with brief i.v. infusions of NMDA administered once every 3 hr for 15 wk. When NMDA treatment was initiated at week 0, the animals were between 15 to 16 mo of age: 1½–2 yr before the age puberty is normally initiated in this species. Although the intermittent infusions of NMDA were administered without interruption, circulating LH and T concentrations (means ± SEMs) were only monitored during a 6-hr window at weekly or biweekly intervals. Here data for weeks 0, 1, 2, 3, 5, 7, 11, and 15 of treatment are shown, together with those obtained 4–14 wk later following the temporary interruption (16–23 hr) in the intermittent i.v. infusion of NMDA.

hiatus in hypothalamic stimulation, the intermittent infusion of NMDA was reestablished. Two to 20 days after reinitiating the NMDA infusion, the second testis was removed.

In the remaining animal studied in the first experiment, the second testis was removed 6 wk after the initial hemiorchidectomy. In this monkey uninterrupted hypothalamic stimulation was maintained throughout the interval between unilateral castrations.

**Experiment 2.** To further determine whether the effects of NMDA upon the pituitary–testicular axis were mediated exclusively by the release of hypothalamic GnRH, the fifth animal was treated, during the initial 6 wk of hypothalamic stimulation, with a long-acting preparation of the GnRH antagonist, [Ac-D-2Nal[1],4Cl-Phe[2],D-Trp[3],D-Arg[6],D-Ala[10]]-GnRH-HOAc. This drug was injected every 3½ days while the animal was briefly sedated with sodium thiamyal (Biotal, Boehringer Mannheim; 20 mg per animal) infused i.v. For this experiment, *N*-methyl-DL-aspartate (≈14 mg/kg of BW per pulse infusion) was administered at the standard frequency of one pulse per 3 hr throughout the entire experiment. Following withdrawal of the GnRH antagonist after 6 wk of hypothalamic stimulation, the intermittent infusion of NMDA was continued for an additional 6 wk, during which time control injections of the vehicle used to inject the antagonist were administered every 3½ days. The response of the pituitary–testicular axis during concomitant hypothalamic stimulation and treatment with the GnRH receptor antagonist, and during the selective withdrawal of the GnRH antagonist, was assessed by using procedures identical to those described above for animals receiving NMDA alone.

## RESULTS

**Experiment 1.** The intermittent administration of NMDA at 3-hr intervals to prepubertal male monkeys resulted in a progressive activation of the pituitary–Leydig cell axis, as reflected by the time courses of circulating LH and T concentrations during the first 15 wk of treatment with this excitatory amino acid analog (Fig. 1). Circulating FSH concentrations, determined in samples collected approximately every 4th wk, were low prior to initiation of treatment (5.3 ± 2.1 ng/ml, mean ± SEM), but by the 4th wk of NMDA administration they had reached a mean level of 12.2 ± 2.0 ng/ml, where they appeared to be maintained for the duration of the experiment. An interruption of the NMDA infusion between the 20th and 29th wk of treatment resulted, the following day, in a return of the hypothalamic–pituitary–Leydig cell axis to the unstimulated state (Fig. 1). NMDA-induced episodes of LH secretion and the associated discharges of testicular T were abolished by an i.v. injection of a short-acting preparation of the GnRH receptor antagonist, RS68439, that was administered during the final weeks of NMDA treatment (not shown).

The premature activation of the pituitary–Leydig cell axis was associated with dramatic growth and differentiation of the epididymis (Fig. 2) and with a progressive increase in mean testicular volume from <1.0 ml before initiation of treatment to 5.0 ml by the 16th wk of NMDA administration (Fig. 3). The enlargement of the testis was associated with a striking maturation of the seminiferous epithelium. In two animals, motile sperm were recovered from the epididymides at the time of orchidectomy, 16 and 22 wk after initiation of NMDA treatment, and in these and one other animal, which had been treated for 26 wk, testicular spermatozoa were observed when histological sections of the gonad were later examined (Fig. 4). In the remaining animal, although considerable stimulation of the seminiferous tubule was evident after 20 wk of NMDA treatment, testicular spermatozoa were not observed (Fig. 4).

BW increased at a rate of 48 g/wk throughout the period of NMDA treatment.

**Experiment 2.** In one additional animal, activation of the pituitary–testicular axis was prevented for the initial 6 wk of intermittent NMDA treatment by the concomitant administration of a long-acting preparation of a GnRH receptor antagonist (Fig. 5). When treatment with the GnRH antagonist was withdrawn, while maintaining the NMDA infusion,

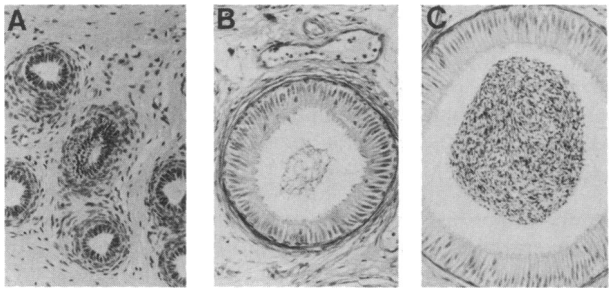


FIG. 2. Photomicrographs (×115) of sections of epididymides, stained with periodic acid/Schiff's reagent and hematoxylin, from a 21-mo-old untreated juvenile (*A*), a 19-mo-old NMDA-treated juvenile (*B*), and an untreated adult monkey (*C*). The NMDA-treated juvenile had been receiving an intermittent infusion of the aspartate analog for 16 wk. The epithelium of the ductus epididymis of the untreated juvenile consisted of low cuboidal cells lacking stereocilia, whereas that of the treated juvenile comprised tall columnar cells possessing stereocilia similar to those found in the epididymis of the adult. The epididymides from untreated monkeys were obtained during the course of other studies.

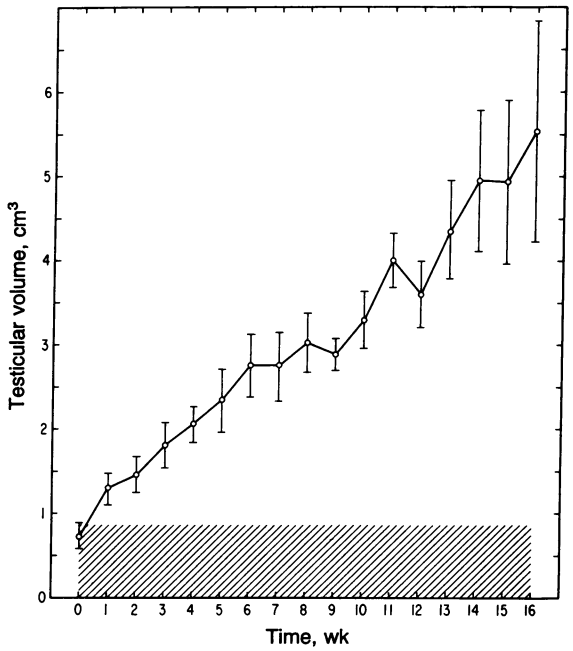


FIG. 3. Changes in total testicular volume (mean ± SEM) in prepubertal rhesus monkey ($n$ = 4) during the first 16 wk of hypothalamic stimulation imposed by a pulsatile i.v. infusion of NMDA. In one of the four animals, hemiorchidectomy was performed after 10 wk of NMDA treatment (see text for calculations of testicular volume). The hatched area indicates the mean testicular volume of untreated animals aged between 12 to 18 mo (20).

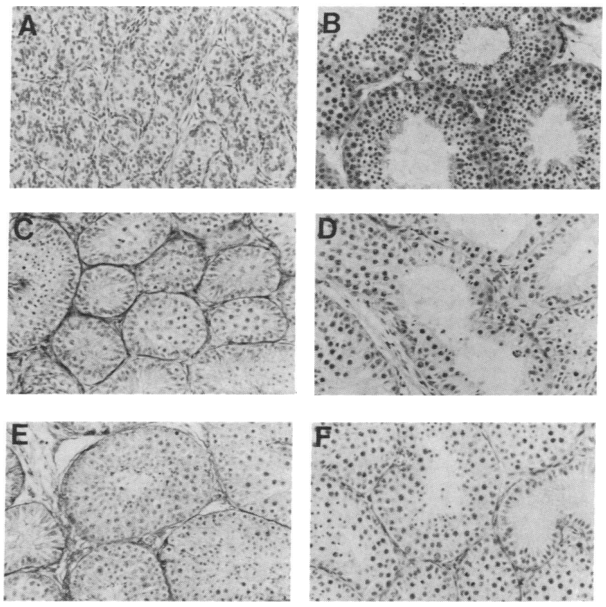


FIG. 4. Photomicrographs (×115) of sections of testis stained with periodic acid/Schiff's reagent and hematoxylin from an untreated 21-mo-old juvenile (*A*), an untreated adult (*B*), and four juvenile monkeys treated with NMDA (*C–F*). The sections from NMDA-treated animals were taken from the testis removed at the second hemicastration, 16–30 wk after initiation of the intermittent i.v. infusion of the aspartate analog, when the ages of the monkeys ranged from 19 to 23 mo of age. The testis from the untreated juvenile monkey was comprised of seminiferous cords surrounded by undistinguished interstitial cells. The seminiferous cords contained only the dark staining nuclei of immature Sertoli cells and stem spermatogonia. In contrast, the seminiferous tubules of testes of NMDA-treated monkeys contained epithelia comprised of mature Sertoli cells and differentiated germ cells, including spermatogonia, spermatocytes, and spermatids. In testicular sections from three NMDA-treated animals (*D–F*), all stages of germ cell development, including testicular spermatozoa, were identified.

a progressive increase in the pulsatile activity of the pituitary–Leydig cell axis (Fig. 5) and in testicular volume was observed. As had been the case in all animals studied in experiment 1, discharges of GH were tightly coupled to NMDA infusions in the monkey treated chronically with the GnRH antagonist (Fig. 5).

## DISCUSSION

The first herald of spontaneous puberty in the male rhesus monkey—namely, a nocturnal elevation of testicular T secretion—is first observed at 2½ yr of age (12) and spermatogenesis is usually initiated during the fourth year of life (21). In the present study, the pulsatile patterns of LH and T secretion observed in 18- to 19-mo-old monkeys after 11 wk of NMDA treatment were comparable to those previously observed in fully adult male macaques (18). Moreover, spermatogenesis was initiated in three of the four NMDA-treated animals between 19 and 22 mo of age. Thus, the activation of the hypothalamic–pituitary–testis axis that was achieved in this study by intermittent administration of NMDA was unequivocally precocious.

Somatic growth was also markedly increased during treatment with NMDA. Although BW during prepubertal development in unstimulated animals increases at a rate of ≈20 g/wk (12), NMDA treatment resulted in a growth velocity (48 g/wk) in excess of that observed (12) during spontaneous puberty (37 g/wk). The relative contribution of testicular androgen and pituitary GH to the acceleration in somatic growth was not evaluated.

The mechanisms whereby the chronic administration of a pulsatile infusion of NMDA triggered precocious puberty are probably identical to those previously established to underlie the acute release of LH induced by a single i.v. injection of the excitatory amino acid analog (8)—namely, release of GnRH into the hypophysial portal circulation as a result of activation of NMDA receptors located either on the network of GnRH neurons that project to the median eminence or on an excitatory neuronal pathway afferent to the releasing factor system. This view is underlined by the present findings that the NMDA-induced episodes of pituitary LH and testicular T secretion were interrupted following injection of a short-acting preparation of a GnRH receptor antagonist and that activation of the pituitary–testicular axis was completely prevented when a long-acting preparation of another GnRH receptor antagonist was concomitantly administered with the pulsatile infusion of NMDA. Here, it is to be noted that NMDA-induced GH discharges, which are presumably mediated by the release of GH-releasing factor, were not abolished during treatment with the GnRH antagonist, indicating that hypothalamic excitation had been achieved during blockade of GnRH receptors.

The failure of the hypothalamic–pituitary–Leydig cell axis to respond immediately to NMDA stimulation is probably a reflection of the low gonadotropin content and the impaired responsivity of the pituitary gland in unstimulated prepubertal monkeys (8, 22). In animals in which pituitary response to GnRH is first enhanced by prior exposure to chronic intermittent stimulation with the synthetic decapeptide, a robust LH discharge is observed in response to the first injection of NMDA (8).

The slow and progressive amplification of pulsatile activity in the hypothalamic–pituitary–testicular axis of the prepubertal monkey in response to intermittent NMDA stimulation may be contrasted to the situation in the immature rat, where a single injection of NMDA elicits a LH discharge with a magnitude similar to those observed spontaneously in puber-

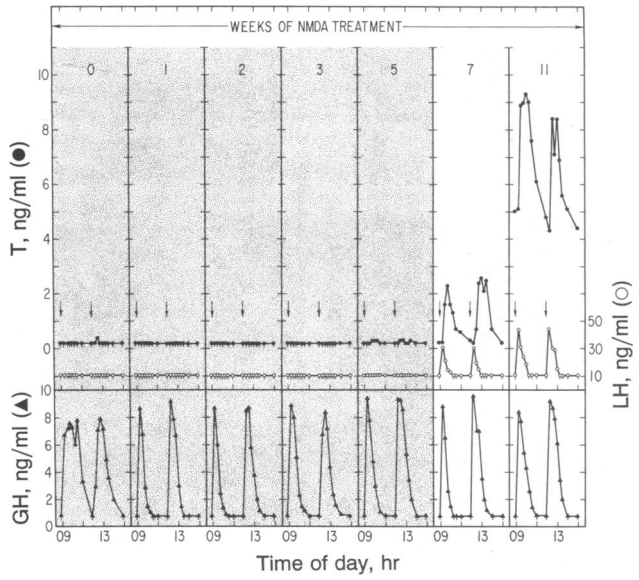


FIG. 5. Treatment with a GnRH antagonist for the first 6 wk of intermittent hypothalamic stimulation with NMDA (stippled areas) in one prepubertal rhesus monkey blocked activation of the pituitary–testicular axis. This animal was 16 mo of age at the start of the experiment. Termination of treatment with the GnRH antagonist on week 6 was followed by a rapid initiation of an adult-like pattern of pulsatile LH and T secretion (*Upper*). Testicular volume remained <1.0 ml during treatment with the GnRH antagonist and increased to 2.7 ml by the 6th wk of treatment with NMDA alone (not shown). Note also the NMDA-induced GH discharges throughout the entire experiment (*Lower*). See Fig. 1 for further details.

tal rats (23). The reason for this may be accounted for by differences in the mechanisms that govern the ontogeny of the hypothalamic–pituitary–gonadal axis in the rat and monkey (1, 24). In the primate, the network of hypothalamic neurons that generate the intermittent GnRH drive to the pituitary gonadotrophs, although functional at birth, is held in a state of "check" throughout the greater part of the prepubertal development. The nature of this restraint upon the GnRH pulse-generating mechanism in the juvenile monkey is such that the prepubertal hiatus in gonadotropin secretion is sustained in the absence of the testis (1). In the rat, on the other hand, the prepubertal phase of development is characterized by relatively unrestrained gonadotropin secretion, as reflected by the capacity of the hypothalamic–pituitary axis to respond to castration with a robust hypersecretion of gonadotropin at any stage of postnatal development (24). Thus, the premature initiation of vaginal opening and first ovulation that follows intermittent NMDA administration in the immature rat (23) should be viewed as an exaggeration of an already extant hypothalamic stimulation of the pituitary–gonadal axis. In the monkey, however, the initiation of precocious puberty following stimulation of the hypothalamus with NMDA represents a premature reawakening of a system that has been held in a state of profound dormancy since late infancy.

The present finding that repetitive chemical excitation of the hypothalamus of the prepubertal male macaque induces a state of sexual precocity graphically reinforces the now established notion that neither pituitary nor gonad is limiting in the onset of puberty in higher primates (2). Additionally, and more significantly, the observation that the network of GnRH neurons within the hypothalamus of the prepubertal monkey may be driven in an adult manner for weeks on end, and with surprising ease, suggests that the prepubertal hiatus in intermittent GnRH release cannot be accounted for by deficits at this stage of development in the mechanisms within the GnRH neuron that govern synthesis and release of the decapeptide.

That hypothalamic NMDA receptors may play a physiological role in the initiation of primate puberty and subsequent transition into adulthood now becomes a pertinent question. In this context, NMDA receptors, located either on the GnRH neurons themselves or on an afferent input excitatory to the GnRH system, might be activated at puberty as a result of either an elevation in the concentrations of the acidic amino acids (aspartate and glutamate) in the systemic circulation or an increased tone across a synapse that utilizes glutamate or some other NMDA receptor agonist as transmitter. Although we have been unable to identify differences in circulating concentrations of aspartate and glutamate, as measured by HPLC, in prepubertal and adult monkeys, a pilot study in the rat has revealed that spontaneous LH release, and presumably therefore that of hypothalamic GnRH, is markedly suppressed following blockade of NMDA receptors with the specific antagonist, DL-2-amino-5-phosphonopentanoic acid (6). The latter observation suggests that excitatory amino acids may be an important component of the neural mechanism that generates the hypophysiotropic drive to the rat gonadotroph. If the release of GnRH in primates involves a similar control, the idea of a role for excitatory neurotransmission in triggering the onset of puberty in these species would become attractive.

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