IGF-I administration advances the decrease in hypersensitivity to oestradiol negative feedback inhibition of serum LH in adolescent female rhesus monkeys

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Abstract

Developmental increases in serum LH were assessed in female rhesus monkeys to test the hypotheses that (1) the final stages of puberty are characterized by a decrease in hypersensitivity to oestradiol negative feedback of LH and (2) that increases in IGF-I secretion accelerate this decrease in hypersensitivity. In order to test the first hypothesis, serum LH in the absence of oestradiol and in response to three doses of oestradiol were compared between ovariectomized adult (n=6) and adolescent female monkeys (control group; n=6). The control females were not treated with oestradiol until serum LH had risen to within the 95% confidence interval of serum LH observed in ovariectomized adults. Doses of oestradiol achieved serum levels of approximately 80 (‘low’), 160 (‘intermediate’), and 250 (‘high’) pmol/l. For control group females, treatment with the next higher dose of oestradiol was not initiated until serum LH was no longer suppressed by the lower dose. Treatment with oestradiol produced a dose-dependent suppression in serum LH in adults. In contrast, low-dose oestradiol maximally suppressed serum LH throughout the initial treatment period in the control group compared with the adult females. The low oestradiol dose effectively suppressed serum LH throughout the study period in 4/6 of the control group and became ineffective at suppressing LH after 8 months of treatment in 2/6 control group females. Initiation of the intermediate dose of oestradiol to these females again maximally suppressed LH compared with adult females.

In order to determine whether IGF-I regulates this change in hypersensitivity to oestradiol negative feedback, a second group of ovariectomized, adolescent monkeys (n=6) were treated chronically with IGF-I to elevate serum IGF-I levels above those of control group females. Using the same protocol described for the control females, developmental changes in serum LH in the absence of oestradiol and in response to oestradiol negative feedback were evaluated. Treatment with IGF-I had no effect on the initial increases in serum LH occurring in the absence of oestradiol. In contrast, the decrease in hypersensitivity to the negative feedback effects of the low oestradiol dose was significantly accelerated in IGF-I-treated females, as the interval from the initiation of treatment to the point at which serum LH was no longer suppressed was shorter in IGF-I-treated (4.4 ± 0.7 months; mean ± s.e.m.) compared with control group females (8.4 ± 1.9 months). Although none of the control group females escaped from the negative feedback effects of the intermediate dose of oestradiol during the course of the study, 2/7 of the IGF-I-treated females did so within 5.5 ± 1.4 months of the initiation of the treatment.

The present data indicate that the later stages of puberty in female monkeys are characterized by a decreasing in sensitivity to oestradiol negative feedback inhibition of serum LH and that the timing of this decrease is regulated by circulating concentrations of IGF-I. These data confirm earlier reports that the developmental increases in the GH axis accelerate the tempo of puberty without affecting its onset.


Introduction

The onset and progression of primate puberty is characterized by an increase in pulsatile luteinizing hormone-releasing hormone (LHRH) and luteinizing hormone (LH) secretion (Watanabe & Terasawa 1989). However, different control mechanisms regulating this release may be operative during various stages of puberty. Following neonatal activation of the hypothalamic-pituitary-ovarian axis (Plant 1986), a prolonged hypogonadotrophic state ensues in which LHRH and LH secretion is present but at a reduced frequency and amplitude (Watanabe & Terasawa 1989). This hypogonadotrophic state may be due to an active restraint (Plant & Zorub 1982) or an absence of stimulatory input (Plant et al. 1989, Bourguignon et al. 1990, Urbanski & Ojeda 1990), neither of which involve the negative feedback action of gonadal steroids. This is confirmed by the observation that
ovariectomy at this age does not result in an immediate, open-loop release of LH (Terasawa et al. 1984, Wilson et al. 1986) or LHRH (Chongthammakun et al. 1993). Rather, LH secretion is low and only increases gradually, reaching patterns similar to gonadal adults at the expected age of menarche (Terasawa et al. 1984, Wilson 1989).

Once puberty has begun and LH secretion has been re-established, oestradiol negative feedback becomes important, regulating further increments in LH release (Wilson et al. 1986, Winter et al. 1987, Wilson 1989). Serum LH in agonal females is elevated at an earlier age compared with intact or agonal females treated with low-dose oestradiol. However, serum LH eventually rises in the face of these low oestradiol concentrations. Furthermore, oestradiol levels which suppress LH secretion prior to first ovulation fail to do so once ovulation has occurred (Rapisarda et al. 1983). Although these data suggest that the later stages of puberty may be characterized by a changing sensitivity to oestradiol inhibition of LH, dose-response studies of oestradiol negative feedback during primate development have not been evaluated. This period of apparent changing sensitivity to oestradiol negative feedback during adolescence, experimentally demonstrated in agonal females, characterizes the interval between menarche and first ovulation in intact females (Terasawa et al. 1984, Wilson 1989) and is not of a fixed length, but is quite variable in both girls (Apter & Vilko 1985) and monkeys (Wilson 1989). An understanding of the mechanisms which regulate this final stage of puberty will provide insight into the factor(s) which set the tempo of reproductive maturation.

Since developmental increases in LH secretion occur concurrently with accelerated growth in children (Tanner 1978) and monkeys (Tanner et al. 1990), attention has focused on whether the growth hormone (GH) axis may regulate LHRH and LH release during puberty. During adolescent growth, serum GH and insulin-like growth factor-I (IGF-I) increase in response to some non-gonadal influence (Wilson 1989, Lui et al. 1991) yet are further elevated during the perimenarcheal period as oestradiol levels increase (Mauras et al. 1987, Wilson 1989, Lui et al. 1991). Consequently, it has been proposed that factors which regulate growth may affect reproductive development (Bourguignon 1991). Clinical studies report that puberty is delayed by GH deficiency (Tanner & Whitehouse 1975, Ogilvy-Stuart & Shalet 1992). However, GH treatment to GH-deficient children does not affect the onset of puberty but accelerates the time to its completion (Darendeliler et al. 1990, Stanhope et al. 1992). Similarly, long-term administration of GH to normal monkeys does not advance the age at menarche but shortens the interval between menarche and first ovulation (Wilson et al. 1989) while treatment with the long-acting analogue of somatostatin has no effect on the age at menarche but delays the timing of first ovulation (Wilson & Tanner 1994). Consequently, the GH axis may not affect the onset but may regulate the tempo of puberty. Since the tempo, or rate at which an individual attains the capacity to reproduce, may be due to how quickly hypersensitivity to oestradiol negative feedback of LH release diminishes, the GH axis may be important in mediating this change in gonadal inhibition of LH secretion during this late stage of primate puberty.

The goals of the present study were to determine whether the final stages of primate puberty are characterized by a decreasing sensitivity to oestradiol negative feedback and whether IGF-I regulates this developmental change in LH secretion. Specifically, the hypothesis tested was that, as puberty progresses, increasing doses of oestradiol are required to suppress LH. Dose-response characteristics of oestradiol negative feedback were evaluated in ovariectomized adolescent monkeys throughout development and were compared with patterns observed in agonal adults. The second hypothesis tested was that the administration of IGF-I would not advance the onset of puberty, defined by the initiation of LH secretion in ovariectomized females, but would accelerate the decrease in hypersensitivity to oestradiol negative feedback thereby advancing the progression of puberty.

Materials and Methods

Subjects were colony-born adult and adolescent female rhesus monkeys (Macaca mulatta). Adult animals were housed indoors in single cages while adolescent animals were housed socially indoors in multi-animal cages (Wilson et al. 1989). Indoor housing provided a constant temperature range (21–24 °C) and lighting conditions (12 h light: 12 h darkness). Females were fed commercial monkey chow (Wayne Products, Chicago, IL, USA) ad libitum twice daily and fresh fruit once daily. Water was available at all times. The experimental protocol was approved by the Emory University Institutional Animal Care and Use Committee in accordance with NIH and USDA standards. All surgical procedures (ovariectomies and s.c. placement of osmotic mini-pumps and oestradiol pellets) were done while the animals were under general anaesthesia (ketamine hydrochloride, 30 mg/kg i.m.; Parke-Davis Pharmaceuticals, Morris Plains, NJ, USA).

Dose-response patterns of oestradiol negative feedback on LH secretion in adults

Adult animals (n=6) had been ovariectomized at 1 year of age and were approximately 12 years of age at the time of the study. Although these animals had received oestradiol in previous protocols (Wilson et al. 1986, 1987), none had been treated during the preceding year. The effect of three doses of oestradiol (Innovative Research of America, Toledo, OH, USA) on serum LH was evaluated. The pellets, designed to release oestradiol for 3 weeks, were
implanted s.c. between the scapulae and contained either 0-30, 0-60, or 0-90 mg, producing serum levels similar to that observed during the early, mid, and late follicular phase respectively. These pellets did not maintain serum oestradiol at a constant level but produced decreasing values over the 3 week treatment period. Females were exposed to alternating periods of 3 weeks of no oestradiol followed by 3 weeks receiving oestradiol, with the order of treatment with a particular dose counterbalanced among the animals. Blood samples were obtained twice weekly for the determination of oestradiol and LH. The design provided dose–response data on the effects of oestradiol negative feedback on serum LH in adults. The 95% confidence intervals of serum LH in the absence of oestradiol were calculated to define the range of serum LH expected for agonadal adults. The dose–response effects of oestradiol on the suppression of serum LH were compared with patterns observed in adolescent females, as described below.

Developmental changes in serum LH

Adolescent females were ovariectomized at ~13 months of age and were randomly assigned to either a control group (n=6) or a group receiving recombinant human IGF-I (n=7). IGF-I (a gift from Genentech Inc., South San Francisco, CA, USA) was administered by a constant s.c. infusion using osmotic mini-pumps (Alza Corporation, Palo Alto, CA, USA). Initially, subjects received IGF-I at a dose of 60 μg/day from 16 to 18 months of age. Since serum IGF-I was not elevated to the desired range of 600 to 800 μg/l (i.e., late pubertal levels (Wilson 1989)) by this treatment, the dosage was increased to 300 μg/day for the remainder of the study. Pumps were implanted s.c. between the scapulae every 4 weeks. Body weights were measured monthly following an overnight fast. Twice weekly blood samples were collected from the control and IGF-I-treated females for the assay of oestra¬diol, LH and IGF-I. Oestradiol treatment was not initiated in these adolescents until 2 months after LH concentrations had risen to within the 95% confidence intervals of values observed in non oestra¬diol-treated adult females. This delay in the initiation of treatment was imposed to ensure that LH secretion had reached adult patterns so that any changes could be attributed to oestradiol negative feedback and not to the continued maturation of the non-gonadal regulation of LH release. The oestradiol treatment protocol was similar to that described for adults in which a female received alternating blocks of 3 weeks of ‘no oestradiol’ followed by 3 weeks of oestradiol. The initial dose of oestradiol (0-25 mg pellet implanted s.c. between the scapulae) achieved serum concentrations similar to those of the low-dose oestradiol treatment used for adults and corresponded to pubertal-early follicular phase values (Wilson et al. 1986). The females continued to receive this dose of oestradiol until it no longer suppressed serum LH, i.e. serum LH under this dose of oestradiol was similar to that observed during the intervening ‘no oestradiol’ treatment periods. Once this occurred, the dose of oestradiol was increased (0-60 mg) to achieve intermediate serum levels similar to those attained in adults. Once this dose no longer suppressed LH, the dose of oestradiol was further incremented (0-75 mg) to achieve serum concentrations corresponding to late follicular phase values. The design permitted an assessment of (a) the timing of the initial rise in LH concentrations following ovariectomy, (b) the interval from this rise to the point ‘adult-like’ levels were attained, (c) the response to oestradiol negative feedback, and (d) the age at which each oestradiol treatment failed to suppress serum LH. The dose–response patterns of oestradiol negative feedback were compared between adults and adolescent controls to determine whether there were developmental differences in the sensitivity to oestradiol negative feedback. The control and IGF-I-treated adolescent groups were compared to determine whether IGF-I treatment affected these developmental parameters of LH secretion. Data collection for the adolescent animals continued until either the highest dose of oestradiol was no longer capable of suppressing serum LH or when the animals reached 37 months of age (the expected age at first ovulation in gonadally intact similarly housed monkeys (Wilson et al. 1989)).

Analyses

Serum oestradiol was measured by a direct RIA as described previously (Wilson et al. 1989). The assay had a sensitivity of 26 to 37 pmol/l. The interassay coefficient of variation (CV) and the intra-assay CV were 11-1 and 4-3% respectively. Serum IGF-I was measured by RIA following removal of IGF-binding proteins by acid treatment as described previously (Osterud et al. 1984). The assay had a sensitivity of 75 μg/l and inter- and intra-assay CV values of 9-6 and 5-2% respectively. The reference and material for iodination was recombinant human IGF-I (Amgen Biologicals, Thousand Oaks, CA, USA) and the anti-IGF-I serum was obtained as a gift from the National Hormone Pituitary Program (NHPP), NIDDK, NICHD, and USDA. Serum LH was measured by RIA as previously described (Walker et al. 1984) using reagents also supplied as a gift by NHPP. The assay had a sensitivity of 5 to 10 μg/l with inter- and intra-assay CV values of 12-6 and 4-5% respectively.

Data were expressed as means±s.e.m. Differences between either adults and the control group or the control and IGF-I-treated groups were evaluated with t-tests or Mann–Whitney U tests for single time points, or ANOVA for repeated measures for data collected sequentially. In the latter case, differences between groups at specific time points were evaluated with Fisher's least square difference post hoc tests (Keppel 1980). Statistical comparisons having P<0.05 were considered significant.
Results

Developmental differences in oestradiol negative feedback on serum LH

The 95% confidence interval of serum LH for adult, non-oestradiol-treated, ovariectomized animals was $284 \leq 323 \geq 361 \mu g/l$. Following ovariectomy at $\sim 13$ months of age, serum LH for the adolescent control group females was low (5 to 10 $\mu g/l$) and unvarying. The rise in serum LH from ovariectomy to values within the 95% confidence interval of adult females was gradual, taking $10.0 \pm 1.7$ months. Once these levels were attained, the adolescent female began the oestradiol treatment protocol 2 months later.

Oestradiol produced a dose–response suppression of serum LH in adult females (Fig. 1). The intermediate and high doses of oestradiol actually induced acute surges in LH secretion, as evidenced by abrupt increases +0.4 weeks after treatment. Serum LH was maximally suppressed at +1.8 weeks following each treatment at which point concentrations increased in a dose-dependent manner. Serum oestradiol gradually declined throughout the ensuing 6 week period. At +5.4 weeks following treatment, serum LH had increased to pre-treatment levels following the low and intermediate oestradiol dose but were still suppressed by the high dose.

A comparison of developmental dose–response differences in oestradiol negative feedback on serum LH indicated that adolescent females were more sensitive to oestradiol inhibition than were adults and that this hypersensitivity decreased as puberty progressed. Despite similar profiles in circulating oestradiol (Fig. 2), serum LH was completely suppressed during the initial 3 week low oestradiol treatment period in adolescent controls compared with adult females. From the second to the third weeks of treatment, serum LH levels were significantly lower in the adolescent control group females ($10.0 \pm 0.5 \mu g/l$) compared with adults.

FIGURE 1. Time course of serum concentrations (mean ± S.E.M.) of (a) LH and (b) oestradiol in ovariectomized adult females during the low-dose (open squares), intermediate-dose (hatched squares), and high-dose (solid squares) oestradiol treatments. Error bars are absent where their value is smaller than the symbol.

FIGURE 2. Time course of serum concentrations (mean ± S.E.M.) of (a) LH and (b) oestradiol for ovariectomized adult females (solid circles) and control adolescent females during their initial low-dose oestradiol treatment (open squares) and the low-dose oestradiol treatment in which serum LH was no longer suppressed (solid squares). Error bars are absent where their value is smaller than the symbol.
(73·1±8·9 µg/l; $t_{10}=7·89$). During these initial low-dose oestradiol treatment regimens, serum LH did not return to ‘pre-treatment’ concentrations (further illustrated in Fig. 5).

This low-dose oestradiol treatment suppressed serum LH in 4/6 of the control females throughout the study period, representing an average of 10·0±2·0 months of treatment. However, this dose became ineffective at suppressing LH in the remaining two control females after 8·3±4·0 months of treatment. Unlike the initial series of low-dose oestradiol treatments (Fig. 2), LH concentrations during the second and third week of this last low-dose treatment period were similar between adult (73·1±8·9 µg/l) and control group females (51·6±19·5 µg/l; $t_{6}=0·43$). Once this low dose of oestradiol became ineffective at suppressing serum LH in these two control females, treatment with the intermediate dose was initiated. Serum LH was again suppressed to levels significantly lower than that observed for adults females (data not shown). During the second and third week of this oestradiol treatment, serum LH was significantly lower in control (11·4±0·7 µg/l) compared with adult females (36·1±7·0 µg/l; $t_{6}=3·95$). This intermediate dose maximally suppressed serum LH in these two control females until 37 months of age, the preset age for the cessation of data collection.

**Effects of IGF-I on developmental changes in serum LH**
Administration of IGF-I significantly elevated serum IGF-I concentrations (Fig. 3). Although the initial dose of IGF-I used (60 µg/day) elevated serum concentrations in IGF-I-treated animals above that of control group females, the dosage was increased (300 µg/day) to elevate serum IGF-I levels 50 to 75% above those in control females. Average serum IGF-I concentrations were significantly higher in IGF-I-treated compared with control females prior to the initiation of oestradiol treatment and during the 3 week oestradiol treatments (Fig. 3 inset; $F_{1,11}=14·40$). Oestradiol significantly elevated serum IGF-I in both groups of females ($F_{1,11}=40·86$). IGF-I administration did not affect body weight gain (kg/month).
from ovariectomy to the initiation of oestradiol treatment (controls, 0·08±0·01 vs IGF-I-treated, 0·07±0·01; $t_{11}$=0·36) or from the initiation of oestradiol treatment to the release from low-dose negative feedback (controls, 0·14±0·01 vs IGF-I-treated, 0·12±0·01; $t_{11}$=1·40).

Treatment with IGF-I had no effect on development changes in serum LH prior to the initiation of oestradiol treatment. The interval from ovariectomy to the initial rise in serum LH was similar in IGF-I-treated (4·6±0·9 months) and control group females (4·1±0·7 months; $t_{11}$=0·37), as was the interval from this rise to the age at which adult-like concentrations had been attained (Fig. 4; 5·0±0·8 vs 5·9±1·1 months; $t_{11}$=0·69). Serum LH reached levels within the 95% confidence interval for adults of similar ages for IGF-I-treated (22·5±1·3 months) and control females (23·8±1·9 months; $t_{11}$=0·14). Once it had been confirmed that serum LH had reached adult-like levels, low-dose oestradiol treatment began and this occurred at similar ages for IGF-I-treated (25·5±1·5 months) and control females (26·5±1·7 months; $t_{11}$=0·46).

In contrast to these developmental changes in serum LH following ovariectomy, IGF-I treatment significantly advanced the maturational decrease in oestradiol negative feedback inhibition (Fig. 5). As noted above, the low-dose oestradiol treatment suppressed serum LH until the cessation of data collection in 4/6 of the control females. In contrast, the low-dose oestradiol became ineffective at suppressing serum LH in 6/7 of the IGF-I-treated females. Since data collection was terminated prior to the release from low-dose oestradiol negative feedback for the four control and one IGF-I-treated females, age calculations for specific developmental parameters related to oestradiol negative feedback were underestimated for these animals as their age at the cessation of data collection was used for analysis (Table 1). Nevertheless, the low oestradiol dose became ineffective at suppressing serum LH at a younger age for IGF-I-treated compared with control females (Table 1; $t_{11}$=2·54). An analysis of the subset of females which did release from low-dose oestradiol negative feedback supported this comparison (IGF-I-treated, 28·8±1·1 months, $n=6$ vs controls, 32·8±0·1 months, $n=2$; Mann–Whitney U test, $P=0·04$). Holding constant the specific age at which low-dose treatment was initiated, a covariance analysis revealed that the interval between the initiation of treatment and the release from low-dose oestradiol negative feedback was significantly shorter in IGF-I-treated (4·4±0·7 months) than in control females (8·4±1·9 months; $F_{1,10}=9·73$). Furthermore, the intermediate dose of oestradiol was initiated at a significantly younger age in IGF-I-treated than in control females (Table 1; $t_{11}$=2·65). This intermediate dose of oestradiol became ineffective at suppressing serum LH in 2/7 of the IGF-I-treated females within 5·5±1·4 months of the start of treatment. The high-dose oestradiol treatment was initiated in these two IGF-I-treated females at 32·6 (±0·2) months, but data collection was terminated prior to any change in LH in response to oestradiol for these animals (Fig. 5).

**Discussion**

These results indicate that puberty in female monkeys is characterized by an initial increase in serum LH which
occurs independently of any gonadal steroid regulation and, once this becomes established, a progressive decrease in hypersensitivity to oestradiol negative feedback on LH release. Although IGF-I administration had no effect on this non-gonadally-mediated increase in serum LH at the onset of puberty, such treatment significantly advanced the timing of the decrease in hypersensitivity to oestradiol negative feedback, thereby accelerating the progression of puberty. The IGF-I treatment prematurely elevated serum levels of this peptide to those observed during late puberty in females (Harris et al. 1985, Cutler et al. 1985, Wilson 1989). Serum IGF-I was increased in both the control and IGF-I-treated groups by oestradiol, confirming the facilitative effects of oestradiol on IGF-I release (Harris et al. 1985) and indicating that exogenous IGF-I does not affect endogenous IGF-I secretion (Wilton et al. 1991).

The present data support previous observations that a change in hypersensitivity to oestradiol negative feedback is functionally important in the regulation of LH secretion during late puberty in female primates (Rapisarda et al. 1983, Wilson et al. 1986, Winter et al. 1987, Wilson 1989). It has been proposed (Ryan 1986) that this decrease in sensitivity to oestradiol negative feedback may be explained by continued maturation of the non-gonadally mediated mechanism which results in the re-initiation of LHRH and LH secretion, such that any inhibitory

![Graph showing developmental changes in serum LH](chart)

**Figure 5.** Developmental changes in serum LH (solid circles) in response to oestradiol replacement (solid lines) for two control females (Kz and Zz) and two females treated with IGF-I (Zy and Mz). The initiation of low-, intermediate- and high-dose oestradiol treatments are indicated for each female. The initial oestradiol treatment was given at week 0 and successive treatments were administered every 6 weeks thereafter.
stimulus, including low-dose oestradiol, would effectively suppress the release of these hormones. In order to address this hypothesis, oestradiol treatments in the present study were not initiated until serum LH in untreated, ovariectomized adolescent females had reached concentrations similar to those of untreated, agonalad adults. The assumption was that once these levels had been attained, the non-gonadally mediated mechanism regulating LHRH and LH secretion would have matured and any change in serum LH as the result of subsequent oestradiol replacement could be attributed to steroid negative feedback and not to an immature LHRH-LH secretory system. Although serum LH was similar in adult and adolescent females prior to the initiation of oestradiol treatment, the low oestradiol dose maximally suppressed serum LH in adolescent compared with adult females. Although the adolescent females eventually 'escaped' from the low-dose oestradiol negative feedback, serum LH was again significantly suppressed below that observed for adult females during treatment with the intermediate dose of oestradiol. The importance of a developmental change in oestradiol negative feedback is supported by observations that LHRH release is greater in ovariectomized adolescent monkeys compared with the gonadally intact females (Chongthammakun et al. 1993). Although LHRH release continues to increase during development in gonadally intact females, there is no further increase in LHRH secretion in ovariectomized females between the early and middle stages of puberty, suggesting that LHRH release in the absence of oestradiol is maximal during the early pubertal phase of development. Taken together, these data suggest that this decrease in hypersensitivity to oestradiol negative feedback of LH secretion is a real phenomenon and that the rate of this change establishes the tempo of maturation and the timing of first ovulation (Wilson 1989).

The observation that IGF-I administration accelerated the decrease in hypersensitivity to oestradiol negative feedback during late puberty but did not affect the initiation of LH secretion in non-oestradiol-treated, gonadal juvenile females at the onset of puberty supports previous clinical (Darendeliler et al. 1990, Ogilvy-Stuart & Shalet 1992, Stanhope et al. 1992) and experimental findings (Wilson et al. 1989, Wilson & Tanner 1994) that the GH axis, including IGF-I, regulates the tempo but not the onset of puberty (Wilson 1989). Supporting data indicate that the response to gonadal steroid negative feedback on serum LH is greater in GH-deficient mice which have suppressed IGF-I levels, an effect which is diminished by GH replacement (Chandrashekhar & Bartke 1993). Also, serum LH increases at an earlier age in

<p>| TABLE 1. Individual data for control and IGF-I-treated female monkeys, for the age at the initiation of low and intermediate doses of oestradiol treatment as well as the age at the release from low-dose oestradiol negative feedback. Also shown are the group means ± S.E.M. for each parameter |</p>
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<th>at start of medium-dose treatment</th>
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*Age at the release from low-dose oestradiol negative feedback was underestimated as females had not yet released by the cessation of data collection. Since the intermediate dose of oestradiol was not given to these four control animals, they did not contribute data to the analysis of age at the initiation of the medium-dose treatment.

*Significant difference between controls and IGF-I-treated group for that parameter (P<0.05, t-test).
gonadally intact, adolescent monkeys treated with GH compared with untreated agemates (Wilson et al. 1989). In the present study, the effectiveness of low and intermediate doses of oestradiol negative feedback diminished more quickly in monkeys treated with IGF-I. These data suggest that increments in serum IGF-I result in a decrease in hypersensitivity to oestradiol negative feedback during adolescence.

The results of the present study cannot address the mechanism by which IGF-I may alter the sensitivity to oestradiol negative feedback on LH release during primate puberty. The locus of the negative feedback effects of oestradiol on LH secretion in adult female monkeys is at the level of both the hypothalamus and pituitary (Knobil 1980, Chappel et al. 1981, Weick et al. 1983, Pau et al. 1990). With this in mind, treatment with IGF-I increases the release of LHRH from the median eminence in vitro (Hiney et al. 1991) and the secretion of LH from pituitary cell cultures in response to LHRH (Kanematsu et al. 1991). IGF-I receptors are present in both the median eminence (Lesniak et al. 1988) and the pituitary (Rosenfeld et al. 1984) in rats. Consequently, it is possible that IGF-I may alter the effectiveness of oestradiol negative feedback at both the medial basal hypothalamus and pituitary. Whether these effects are limited to the adolescent period or if IGF-I can affect LH secretion throughout the lifespan, as suggested from data on rats (Hiney et al. 1991, Kanematsu et al. 1991), must be determined.

The GH axis, including IGF-I, has specific metabolic effects in addition to its mitogenic effects (Moller et al. 1992, Turkalj et al. 1992). Although increments in body weight were not affected by IGF-I in the present study, the possibility remains that the facilitatory effects of IGF-I on LH secretion during late puberty may not be mediated through the direct effects of IGF-I on LHRH neurons and pituitary gonadotrophs. Rather, the effect may be the result of changes in metabolism and a subsequent increase in the availability of metabolic fuels which are known to be critical for sustaining LH secretion and normal reproductive function (Cameron & Nosbisch 1991).

In summary, the GH axis may play a role in primate sexual development. Previous research suggests that during the adolescent period between menarche and first ovulation, the GH-IGF-I axis may enhance ovarian sensitivity to LH stimulation (Wilson et al. 1989, Wilson & Tanner 1994). The present study indicates that once LH secretion is initiated at the onset of puberty, further increases in LH are regulated by hypersensitivity to oestradiol negative feedback whose eventual decrease is the result of developmental increases in IGF-I release. Consequently IGF-I may enhance oestradiol secretion during adolescence both at the level of the ovary but also at the hypothalamic-pituitary unit by increasing LH release in the face of low levels of oestradiol. Since it is likely that oestradiol regulates the increase in GH-IGF-I activity during the adolescent period (Harris et al. 1985, Wilson & Tanner 1993), these data suggest that oestradiol-induced increases in IGF-I secretion may be an important determinant of continued sexual maturation. However, the important factor is not simply an elevation in oestradiol but rather IGF-I. Consequently, those neuroendocrine factors which regulate developmental increases in GH and IGF-I would ultimately be important in setting the tempo of puberty.

Acknowledgements

The technical assistance of K Chikazawa, E Seres and B Mueller is greatly appreciated. The IGF-I was provided as a gift by Genentech Inc. All hormone assays were done in the Yerkes Assay Laboratory at Emory University. This work was supported by NIH HD 16305 and in part, RR 00165. The Yerkes Primate Research Center is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

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Received 27 June 1994
Revised manuscript received 18 October 1994
Accepted 26 October 1994