Gonadotropin and Testosterone Measurements after Estrogen Administration to Adult Men, Prepubertal and Pubertal Boys, and Men with Hypogonadotropism: Evidence for Maturation of Positive Feedback in the Male

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Extract

Nineteen male subjects were given five daily injections of 17β -estradiol and circulating levels of estradiol (E_2), testosterone (T), and gonadotropins were determined by radioimmunoassay before, during, and after the steroid course. Peak levels of E_2 attained during the 5 days of treatment ranged from 173-577 pg/ml. Four of seven normal adult men and one castrate man demonstrated suppression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) with a subsequent rise in LH (positive feedback) while E_2 levels remained elevated. A rise in T was associated with the LH increment in the four normal men. Nine pre-, early, or midpubertal boys and two men with hypogonadotropic hypogonadism displayed only gonadotropin suppression after E_2 administration. The difference in LH response to estrogen (*i.e.*, positive feedback) between the adult men with normal or elevated gonadotropin levels as compared with the endocrinologically normal boys is significant ($P < 0.01$).

Speculation

Positive feedback between estrogen and LH is present in intact adult men, although the magnitude and consistency of this response is less than in women. Testosterone in men may act like progesterone in the female rhesus monkey to blunt the magnitude of the LH rise during estrogen administration. The potential for positive feedback appears to be a maturational event occurring during puberty in men as well as in women.

Sexual differentiation of the brain and the consequent ability of the female of the species to exhibit regular ovulatory cycles exists in certain rodents and can be influenced by neonatal exposure to a number of steroid hormones. In contrast, fetal and neonatal exposure of the brain of the rhesus monkey to gonadal steroids does not appear to influence the basic hypothalamic mechanisms controlling reproduction (4).

The ability of estrogen to induce the release of LH, termed positive feedback, has been demonstrated in the intact, adult female and in the postpubertal, castrate male, and female rhesus monkey (18). Such effects of estrogen have not been found in the intact, adult male rhesus. LH release after estrogen administration has also been demonstrated in adult women (9, 11) but not in sexually immature girls (11). The onset of positive feedback appears to be a late pubertal event in the female (11). We should like to present evidence that a stimulatory luteinizing hormone response to exogenous estrogen is also a maturational event in men.

MATERIALS AND METHODS

SUBJECTS

Seven normal, adult men (ages 20-22) and one male (age 21) with the syndrome of vanishing testes (1) were hospitalized for study at the Clinical Center of the National Institutes of Health. Each patient was given five daily intramuscular injections of 10 or 15 μ g/kg body weight of 17β -estradiol (E_2) (20) and blood samples were obtained every 12-24 hr before, during, and after the estrogen course (see Table 1).

Nine endocrinologically normal boys between the ages of 7 and 18 were studied in the course of evaluation for short stature, precocious puberty, or delayed adolescence. Seven of the nine boys received E_2 injections at a dose of 10 or 15 μ g/kg body weight for 5 days in a fashion similar to the adult men. Two prepubertal patients received five daily injections at a dose of 1 to 3 μ g/kg body weight, although the plasma levels of estradiol obtained were similar to those measured in subjects who received a larger amount of steroid.

Two men, ages 20 and 23, with hypogonadotropic hypogonadism were also given the 5-day regimen of estradiol injections at a dose of 15 μ g/kg body weight/24 hr. Blood samples in the pre-, early, and midpubertal subjects were also obtained every 12-24 hr before, during, and after administration of estrogen (Figs. 4-6). Informed consent was obtained from each subject and/or his parents.

ASSAYS

Plasma FSH and LH were measured by radioimmunoassay (10), using the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP hMG) as standard material for dose interpolation. In our laboratory 1 mIU of plasma FSH and LH activity of 2nd IRP hMG is immunoreactively equivalent to 30 ng and 3.9 ng, respectively, of the pituitary standard LER-907. Samples were assayed at multiple dilutions in volumes between 50 and 300 μ l, with the sensitivity limits of the radioimmunoassay being approximately 0.5 mIU/assay tube for both FSH and LH. The intra- and interassay coefficients of variation, respectively, for FSH and LH were approximately 10% and 20%.

E_2 was measured by a radioimmunoassay (8) with a sensitivity of approximately 5 pg/assay tube and intra- and interassay coefficients of variation of 11% and 15%. T was also determined by a radioimmunoassay (3), with a sensitivity of approximately 10 pg/assay tube and intra- and interassay coefficients of variation of 8% and 12%. All samples from a given patient were analyzed in the

Table 1. Data from four men who displayed suppression and subsequent luteinizing hormone (LH) elevation with estrogen

Study			PH ²			JK ³			TS ⁴			CW ⁵		
Day	Time	Treat- ment ¹	LH, mIU/ml	Testos- terone, ng/100 ml	Estra- diol, pg/ml	LH, mIU/ml	Testos- terone, ng/100 ml	Estra- diol, pg/ml	LH, mIU/ml	Testos- terone, ng/100 ml	Estra- diol, pg/ml	LH, mIU/ml	Testos- terone, ng/100 ml	Estra- diol, pg/ml
1	AM	C ₁	9.3									8.2	655	
	PM											7.4	659	36
2	AM	C ₂	6.8			8.8			8.0				691	
	PM					8.0			8.3				677	
3	AM	C ₃	7.8			12.0			7.8			7.6	888	
	PM					9.3			6.8					
4	AM	C ₄	11.0	586	39	8.5	539	38	11.5	324	40	6.9	770	53
	PM					8.3			9.5					
5	AM	C ₅	10.3	610	59	8.5	567	31	9.5	386	59	10.2		
	PM					6.0			10.3					
6	AM	E ₁	6.8	752	51	7.3	679	33	11.0	407	55	7.6	770	
	PM					4.6			6.0					
7	AM	E ₂	4.8	492	235	5.5	266	99	6.0	206	112	4.5	576	176
	PM					4.3			5.8					
8	AM	E ₃	4.0 (N) ⁶	474	315	4.6	72	216	8.8	47	79	2.4	295	392
	PM					3.2 (N)			5.8					
9	AM	E ₄	6.3	398	360	3.7	48	279	5.5 (N)	68	208	<1.5 (N)	106	412
	PM					4.2			5.8					
10	AM	E ₅	13.5 (P) ⁶	496	175	4.9	62	258	6.5	76	268	<1.5	79	394
	PM					7.5			8.5			<1.5	88	
11	AM		10.9	670	226	6.5	170	577	10.8	143	267	5.6 (P)	139	296
	PM					8.8 (P)			11.3			1.8	103	147
12	AM		8.0	831	139	6.8	227	398	12.0 (P)	151	154	2.9	216	138
	PM					6.5			10.5					
13	AM		7.8	548	88	4.8	90	248	6.5	120	195	4.1	237	
	PM					3.5	60		6.8	30				

¹ C: control days, which includes the first day of estrogen injection, denoted as E₁. Blood samples on the days of estrogen administration (E₁, E₂, etc.) were drawn before drug administration. Subject PH received 10 µg/kg of estradiol/24 hr while the remaining three subjects received 15 µg/kg/24 hr of the steroid.

² Patient represented by Δ in Figure 1.

³ Patient represented by □ in Figure 1.

⁴ Patient represented by ○ in Figure 1.

⁵ Patient represented by ● in Figure 1.

⁶ N: nadir; P: peak points utilized to draw Figure 1.

same radioimmunoassay for gonadotropins or steroids. Since estrogen levels were determined from blood samples obtained just before each hormone injection, the E₂ values reported represent the minimum circulating blood levels.

DATA ANALYSIS

Because of the asynchronous responses in the group of adult men who displayed an increment in LH after estradiol administration, the data from each of these four men were aligned on the basis of the LH peak and the LH nadir in order that the results could be more easily visualized (Fig. 1). Data were analyzed by the unpaired Student *t*-test and are reported as the mean ($\bar{x}$) and SEM. The Fisher Exact Probability Test (16) was used to compare the results from the adult men and the immature boys. Positive feedback was defined arbitrarily as greater than a doubling of LH values from nadir to peak in the presence of continued high estrogen levels such that suppression again occurred after the LH rise; additionally, this effect had to take place well within the limits of assay sensitivity.

RESULTS

ADULT MEN

The results from four of the seven normal men who exhibited positive feedback are presented in Figure 1 and Table 1. There was

a prompt suppression of gonadotropins and testosterone in the presence of increasing plasma E₂ levels. Five to 7 days after the initiation of the estrogen course, and still in the presence of elevated E₂, there was a significant ($P < 0.01$) mean rise in LH from the nadir (3.5 ± 0.9 (SEM) mIU/ml) back to baseline (10.0 ± 1.8 mIU/ml). Since suppressive doses of estrogen eliminate or markedly reduce the circadian pattern of gonadotropin release in women (19) and men (13), the mean threefold elevation can be considered as an example of positive feedback. The decreasing estrogen levels during the latter part of the study period probably did not play a role in the LH rise, since suppression recurred after the peak. Estrogen levels were similar at peak and nadir times (Table 1) and ranged from peak values of 268–577 pg/ml. These are levels comparable with estrogen determinations obtained during the late follicular phase of the normal female menstrual cycle.

FSH decreased in all four individuals, but the subsequent rise was only slight. A rise and fall in testosterone levels occurred within 24 hr of the LH perturbations and appear to be related to the changes in the tropic hormone.

A patient with the syndrome of vanishing testes, low circulating testosterone, and castrate levels of gonadotropins was given 15 µg/kg of 17β-estradiol daily for 5 days (Fig. 2). In this individual, a marked suppression of gonadotropins took place with a subsequent fivefold rise in LH, from the nadir to levels well above baseline; after this rise, suppression again occurred. Testosterone

Fig. 1. Four men who exhibited gonadotropin suppression 3–4 days after the initiation of estrogen injections, followed by a luteinizing hormone (LH) rise in the presence of elevated estradiol levels. Because of asynchronous timing, the results from all individuals are aligned on the basis of the nadir and peak LH determinations. The mean nadir time was on day 4 of the estrogen course and the mean peak time occurred on day 6. The time in days with respect to nadir and peak is shown on the X axis (e.g., N-2 is 2 days before the nadir point). FSH: follicle-stimulating hormone.

measurements did not change, possibly because of maximal stimulation by LH throughout the E_2 course. The results of the study in this physiologically castrate man are qualitatively similar to those reported by Yamaji *et al.* (18) in the postpubertal, castrate, male rhesus monkey.

Three of the seven adult men showed only suppression of gonadotropins during and after the E_2 course (Fig. 3). Testosterone levels also remained suppressed in these three subjects until E_2 and/or LH levels had returned towards baseline, suggesting that the T elevations exhibited by the four men whose results are shown in Figure 1 were related to the LH increments.

The peak E_2 levels during the treatment period were not statistically different between the men who displayed positive feedback and those who did not, although the highest estrogen values were measured in the former group. Basal testosterone measurements were also similar in both groups of men. Basal estrogen levels were less than 60 pg/ml in all subjects tested in the study.

PREPUBERTAL AND PUBERTAL BOYS

Three prepubertal boys, ages 7, 11, and 13 (21), attained peak estradiol levels between 173 and 284 pg/ml during the 5-day steroid course (Fig. 4). LH and FSH values decreased in all three

boys, although the one subject with the lowest LH measurements showed only minimal decrements in this hormone. The small plasma LH increments seen in two of the boys occurred close to the limits of assay detectability and are of dubious significance.

Three early pubertal boys, ages 9, 11, and 14, attained peak estradiol levels between 181 and 400 pg/ml during estrogen administration (Fig. 5). Only suppression of gonadotropins took place, although, again, one subject with very low hormone values displayed little apparent LH decrease.

The three midpubertal boys, ages 11, 15, and 18, who received estrogen had higher basal gonadotropin levels than the younger subjects, as expected (Fig. 6). Additionally, testosterone values were easily measured and showed a prompt suppression, along with the gonadotropins. Peak estradiol values ranged between 183 and 260 pg/ml, similar to the values obtained in the other men and boys who were studied.

The existence of a stimulatory LH response in five of eight adult men as compared with none of the nine pre-, early, or midpubertal children is a statistically significant difference ($P < 0.01$). If the castrate man is excluded and the four adult responders (of seven total) are compared with the nine children (no responders), the P value is < 0.02 (16).

Fig. 2. A 21-year-old patient with elevated gonadotropin and low testosterone levels who showed a marked rise in luteinizing hormone (LH), after suppression, in the presence of elevated estradiol levels. The arrows indicate the times of estrogen injections. FSH: follicle-stimulating hormone.

Fig. 3. Three men who received 17β -estradiol injections, but who showed only suppression of luteinizing hormone (LH), follicle-stimulating hormone (not shown), and testosterone.

Fig. 4. The effect of 17β -estradiol administration (denoted by arrows) to three prepubertal boys for 5 days. LH: luteinizing hormone; FSH: follicle-stimulating hormone.

HYPOGONADOTROPIC MEN

Two men with hypogonadotropic hypogonadism, ages 20 and 23, were also tested with 5 days of estradiol injections. The results, shown on Figure 7, demonstrated FSH and LH suppression without notable increments, while circulating estradiol remained elevated (between 300 and 400 pg/ml).

There were no significant group differences in the peak estrogen levels attained between any of the groups of subjects studied. The only side effects experienced by any of the patients were a mild degree of breast tenderness and/or the appearance of a small nubbins of breast tissue at the end of the study period. These manifestations resolved in all individuals.

DISCUSSION

The data from five of eight adult men with normal or elevated gonadotropin levels (Fig. 1 and Table 1) show that the development of a basic central nervous system control mechanism for reproduction, once thought to be unique to the female, may be similar for men and women. Support for such a thesis exists in the study of Stearns et al. (17), who demonstrated a positive feedback effect of progestins upon LH in five estrogen-primed, castrate men. Additionally, Seyler and Reichlin (15) have recently presented data which suggests that estrogens may stimulate endogenous luteinizing hormone-releasing factor (LRF) secretion in men.

Fig. 5. Results from three early pubertal boys given estradiol protocol. LH: luteinizing hormone; FSH: follicle-stimulating hormone.

Fig. 6. Three midpubertal boys were given $15 \mu\text{g/kg}$ of 17β -estradiol for 5 days. LH: luteinizing hormone; FSH: follicle-stimulating hormone.

Fig. 7. Hormone measurements in two patients with hypogonadotropism given estrogen injections (arrows) for 5 days. LH: luteinizing hormone; FSH: follicle-stimulating hormone.

Administration of higher doses of estrogen to intact adult men may be necessary to effect a positive feedback response of greater magnitude in all subjects, as testosterone, like progesterone in the female rhesus, may diminish the stimulatory LH effect of 17β -estradiol (5). Recently, Santen (13) has presented evidence that testosterone in men may have a progesterone-like effect on the nature of the spontaneous LH pulse released by the pituitary gland.

Further evidence for dose-related effects of estrogen in causing an LH surge was provided by Reiter, Kulin, and Hamwood (11). Four adult women who achieved peak E_2 levels between 100 and 200 pg/ml during 5 days of estrogen administration exhibited LH responses very similar to those of the four adult men studied above; in other women, higher levels of E_2 after a 5-day estrogen course were associated with greater LH stimulatory responses after initial gonadotropin suppression (11).

The present work suggests a difference between the rhesus monkey and man regarding the ability of estrogen to release LH in some intact, adult males. Brain mechanisms which control reproductive function between the sexes may differ, then, not only among mammalian orders, but even among primate species. Although the rodent has been a useful animal for investigations in reproductive biology, increasing attention is being focused on the primate for studies in this field (2).

Although only five of eight men with normal or elevated levels of gonadotropins responded to the estrogen protocol with a rise in LH, while estrogen levels remained elevated, none of the nine pre-, early, or midpubertal boys displayed such an effect after the administered steroid. Additionally, two patients with hypogonadotropism exhibited only suppression of FSH and LH, a response similar to the younger subjects. This latter finding is consistent with either a specific hypothalamic abnormality in hypogonadotropic hypogonadism, or with a maturation arrest of the normal pubertal process (14).

The high level of significance ($P < 0.01$) between a stimulatory LH response in five of eight adult men compared with none of nine pre-, early, or midpubertal children suggests that the adult and immature male respond differently to exogenously administered estradiol. This difference may be due to a maturational event in the male similar to the phenomenon which allows positive feedback and consequent ovulation in the female (11).

Whether the maturational aspects of the positive feedback phenomenon resides in the hypothalamus or pituitary or in both sites is unresolved. The onset of puberty is associated with increased LH release after exposure to exogenous LRF, although

even the prepubertal child is responsive to this stimulus (12). The fact that none of the six early- or midpubertal boys responded to estrogen with an LH rise suggests that limited pituitary responsiveness to endogenous LRF is not the reason for an absent LH rise after estrogen administration to the younger subjects. Thus, a hypothalamic locus for the maturation of positive feedback is likely.

The relationship between the development of positive feedback potential in the male to the appearance of a stimulatory gonadotropin response to clomiphene citrate (6) remains unclear. Clomiphene responsiveness of the adult type does appear to be a late pubertal event in boys (7), and could be a process which involves both pituitary and hypothalamus.

SUMMARY

Exogenous estradiol given for 5 days effected initial suppression and then elevation of luteinizing hormone (LH) and testosterone in four of seven normal men and one physiologically castrate man. Nine pre-, early, or midpubertal boys and two adult men with hypogonadotropic hypogonadism exhibited only LH suppression after a similar estrogen course. Positive feedback between estrogen and the hypothalamic pituitary unit exists in men as well as in women, and may be a maturational event in both sexes.

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Galactose	insulin
glucose	liver
glycogen	newborn

Glycogen Regulation in Isolated Perfused Near Term Monkey Liver

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Extract

Glycogen metabolism was studied in the isolated perfused liver of the monkey conceptus at 90% of gestation using an *in situ* recirculating perfusion system. Net uptake of glucose and galactose and the activities of the enzymes, glycogen synthetase and phosphorylase, were studied in response to varied perfusate composition. Synthetase activity was expressed as %I, the percentage of total synthetase activity in the active form. Perfusate glucose concentrations as high as 700 mg/100 ml did not lead to net glucose uptake or to an increase in the baseline %I synthetase (4 ± 1 , mean $\pm$ SEM). In the presence of 300 mg/100 ml glucose, insulin at 10^{-7} M increased %I to 8 ± 2 , and galactose > 75 mg/100 ml increased %I to 8 ± 1 . The combination of galactose, glucose, and insulin increased %I to 40 ± 5 . With this latter combination, synthetase activity was proportional to perfusate glucose concentration above 100 mg/100 ml. Phosphorylase activity was diminished by either galactose or insulin, and phosphorylase activity was lowest in the group receiving galactose, glucose, and insulin. Galactose was taken up by all livers, but net glucose uptake was not observed under any condition; net hexose uptake was observed in perfusions with galactose. Glycogen levels did not vary significantly with varied perfusate composition during the 30-min perfusion periods.

Speculation

We speculate that galactose may be uniquely important for neonatal liver glycogen synthesis, and that its action is mediated through reciprocal changes in the activities of the enzymes, glycogen synthetase and phosphorylase. If liver glycogen is important for acute neonatal glucose homeostasis, then galactose may also be essential for maintaining circulating glucose concentration by ensuring glycogen synthesis during feeding.

The infant has a continuing obligatory requirement for glucose (7, 23). During feeding, this requirement is met by exogenous glucose, the excess carbohydrate being stored principally as glycogen. During fasting, the liver derives glucose both from

gluconeogenesis and from mobilization of stored carbohydrate, principally liver glycogen (1, 23). Since gluconeogenesis is limited in the infant (1, 15), liver glycogen synthesis and mobilization may be particularly important for maintaining circulating glucose levels.

Glycogen metabolism in liver is regulated by reciprocal changes in the activities of the rate-limiting enzymes of glycogen synthesis and degradation, glycogen synthetase and glycogen phosphorylase, respectively. The isolated adult rat liver regulates the activities of these two enzymes directly in response to circulating glucose concentration as well as in response to hormones (4, 12, 13, 20). The relative physiologic contributions of glucose and hormonal regulation of glycogen metabolism *in vivo* are unknown.

In a previous study with isolated, perfused newborn monkey liver (11), we provided evidence that glucose mobilization occurred in response to hypoglycemia or glucagon. However, net glucose uptake and activation of glycogen synthetase did not occur in response to hyperglycemia. This lack of response to hyperglycemia suggested to us that the infant primate liver may be limited in its ability to store glucose as glycogen in the presence of hyperglycemia, such as might occur in the portal vein during feeding. We also considered the possibility that other dietary constituents might regulate glycogen synthesis directly.

In an companion study (28), we have provided evidence that galactose as well as glucose regulates glycogen cycle enzyme activities and promotes glycogen synthesis in the isolated perfused liver of the adult rat. Galactose and glucose form lactose, the principal dietary carbohydrate in milk (6). In human milk, lactose provides 40% of the total calories (19). Galactose-metabolizing enzymes are well developed at birth (6). Because of the abundance of galactose in the newborn period, and the known enzymatic pathways linking galactose, glucose, and glycogen metabolism, we questioned whether galactose might play a major role in glycogen metabolism of the newborn primate liver.

In the present study we provide evidence that the isolated perfused liver of the near term monkey regulates glycogen enzyme activities in response to a combination of galactose, glucose, and insulin. We speculate that galactose may play a unique role in glycogen synthesis during the newborn period.