Pharmaceutical Research, Vol. 7, No. 10, 1990

Research Article

Growth Hormone (GH) Secretory Dynamics in Animals Administered Estradiol Utilizing a Chemical Delivery System[1]

William J. Millard,[2,4–6] Teresa M. Romano,[2] Nicholas Bodor,[3–5] and James W. Simpkins[2,4,5]

Received February 22, 1990; accepted April 12, 1990

We have utilized a redox chemical delivery system (CDS) for the brain targeting of estradiol (E_2) to ascertain its effects on GH secretory patterns in adult intact male rats. The E_2-CDS (1.0 mg/kg) dissolved in 20% hydroxypropyl-cyclodextrin (HPCD), E_2 (1.0 mg/kg) alone in 20% HPCD, or 20% HPCD was administered intravenously. GH secretory profiles, plasma steroid levels, and anterior pituitary levels of hormones were determined 1 week following steroid injection. Whereas E_2 in HPCD and HPCD treatment did not alter masculine GH secretory patterns, animals administered the E_2-CDS displayed disrupted GH patterns with attenuated individual pulse amplitudes and significantly elevated GH baseline levels. Moderate pituitary hyperplasia was evident only in the E_2-CDS group of animals. Plasma testosterone (T) concentrations were reduced in only the E_2-CDS group. T replacement reduced E_2-CDS-associated pituitary hyperplasia and preserved the masculine GH secretory profiles, with only a slight reduction in individual GH peak amplitudes being observed. T replacement did not prevent the increase in pituitary and plasma levels of PRL associated with E_2-CDS treatment but did block both the increase in pituitary GH content and the hyperplasia associated with prolonged E_2 exposure. E_2 given alone induced a significant increase in both GH and PRL in the pituitary without establishment of pituitary hyperplasia or elevated plasma PRL levels. These data indicate that E_2-CDS is an effective mode of steroid administration. Changes in GH secretory dynamics, pituitary levels of GH, and degree of hyperplasia are dependent upon the chemical design of the delivery system for E_2. Concomitant T therapy can prevent some of the changes in GH secretion associated with high-dose E_2 exposure.

KEY WORDS: growth hormone; anterior pituitary; estradiol; chemical delivery system; episodic hormone secretion.

INTRODUCTION

Using the chronically cannulated rat model we and others have previously demonstrated that physiological growth hormone (GH) secretion is both episodic and sex dependent (1–9). Adult male rats display a low-frequency, high-amplitude pattern of GH secretion, whereas females exhibit a high-frequency, low-amplitude GH profile (1–9). Computer analysis of these GH secretory profiles indicate that females generally display twice as many GH pulses as adult males, with individual pulses occurring approximately every hour in females and every 2.5–3 hr in males. Further, GH peak amplitudes are generally two to three times higher in males than females. Characteristic of the masculine GH secretory profile is the prolonged basal periods of GH secretion, where circulating GH levels remain at or below the assay sensitivity (<5.0 ng/ml) for up to 1 hr. In females, however, basal GH secretion both is shortened and contains higher GH levels compared to males.

It is apparent that gonadal steroids play a critical role in the expression of the GH secretory pattern and that these agents act at the level of the hypothalamus and anterior pituitary to modulate GH secretion (10–14). In adult male rats chronic exposure to estradiol (E_2) via either daily subcutaneous injections or E_2-filled Silastic subcutaneous implants feminizes the GH secretory profile by reducing individual GH pulse amplitudes and elevating GH baseline levels (6,7). Conversely, chronic testosterone replacement masculinizes GH secretory profiles in adult female animals (6,7).

In the present study we utilized a redox-chemical delivery system (CDS) to deliver E_2 preferentially to the brain following intravenous administration of E_2-CDS in an aqueous, sustained-release formulation to determine the effects of prolonged exposure to the E_2-CDS on GH secretory patterns in adult intact male rats (15). The present E_2-CDS sys-

[1] A preliminary report of this work was presented at the Second International Pituitary Congress, Palm Springs, CA, June 25–28, 1989.

[2] Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, Florida 32610.

[3] Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, Florida 32610.

[4] Center for the Neurobiology of Aging, University of Florida, Gainesville, Florida 32610.

[5] Center for Drug Design and Delivery, University of Florida, Gainesville, Florida 32610.

[6] To whom correspondence should be addressed at Department of Pharmacodynamics, Box J-487, College of Pharmacy, University of Florida, Gainesville, Florida 32610.

0724-8741/90/1000-1011$06.00/0

tem has been shown to block the postcastrational rise in luteinizing hormone as well as inhibit body weight gain in female rats (16–22) and reduce serum testosterone levels in male rats (22).

The mechanism of this CDS is based upon the interconvertible dihydropyridine ⇔ pyridinium salt carrier (15). Figure 1 schematically shows the structures and mechanisms leading to both brain-enhanced and sustained release of E_2 using this system. After administration of the E_2-CDS, the carrier system is rapidly oxidized to the corresponding quaternary pyridinium salt (E_2-Q^+), thus preventing its efflux from the brain. Although it has not been tested directly, the E_2-Q^+ moiety most likely displays very little, if any, biological activity, since C-17-substituted analogues of E_2 do not bind to the cytosolic estrogen receptor until hydrolyzed to E_2 (23). Thus, subsequent hydrolysis of the E_2-Q^+ with nonspecific esterases provides sustained release of the active species (E_2) in both the brain and the peripheral tissues. Since the E_2-Q^+ is hydrophilic, its elimination rate from the periphery is predictably much faster than from the brain.

METHODS

Animals

Adult young male (aged 3–4 months), Sprague–Dawley rats were obtained from Charles River Breeding Laboratories, Wilmington, MA. All animals were housed individually in an environmentally controlled room on a 12-hr light, 12-hr dark cycle (lights on at 0700). The animals were maintained *ad libitum* on water and rodent laboratory chow (Ralston Purina Co., St. Louis, MO).

Drugs

E_2-CDS (3 hydroxy-17β-[[1-methyl-1,4-dihydropyridin-3-yl)-carbonyl]oxy]estra-1,3,5 (10)-triene (estradiol 17-(1,4)-dihydrotrigonellate) was synthesized as previously described (16–18). The 3, 17β-dinicotinate ester of estradiol was prepared by refluxing 17β-estradiol with either nicotinoyl chloride or nicotinic anhydride in pyridine and then hydrolyzed to the estradiol-17 monoester with potassium bicarbonate in 95% methanol. The estradiol-17 monoester was quartenized with methyl iodide, and the E_2-CDS was prepared by reduction of the quaternary salt with $Na_2S_2O_4$.

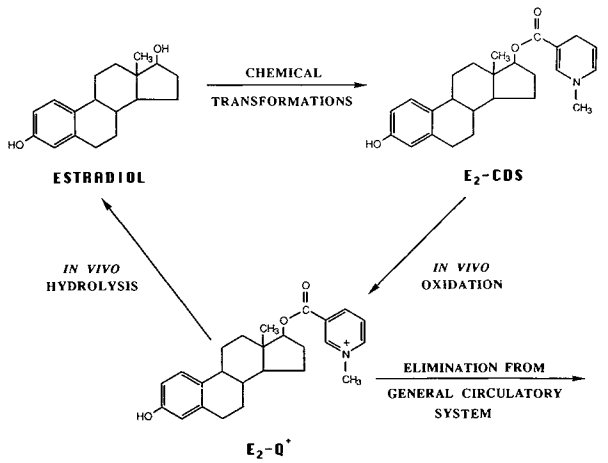

Fig. 1. Schematic representation of the synthesis and distribution of the estradiol-chemical delivery system. The estradiol-chemical delivery system (E_2-CDS) is oxidized to its quaternary form, E_2-Q^+, which is locked into the brain and quickly eliminated from peripheral tissues. Estradiol (E_2), the biologically active steroid moiety, is then released from the E_2-Q^+ by nonspecific hydrolysis.

The E_2-CDS and 17β-estradiol (Steraloids, Wilton, NH) were dissolved in 20% hydroxypropylcyclodextrin (HPCD) on the day of injection and administered intravenously through the intraatrial cannula at a dose of 1.0 mg/kg. The vehicle (20% HPCD) served as the control injection.

Subcutaneous implants of testosterone were prepared following previously described procedures (24,25). Medical-grade Silastic tubing (od, 3.18 mm; id 1.57 mm; Dow Corning Corp, Midland, MI) was cut into 15-mm lengths and packed with testosterone (Steraloids, Wilton, NH). The ends were sealed with Medical Adhesive Silicone Type A (Dow Corning Corp., Midland, MI) and permitted to dry. Capsules were incubated for 48 hr in 0.01 *M* phosphate-buffered saline prior to subcutaneous implantation along the back. Implantation of the steroid-filled capsules was performed in animals lightly anesthetized with methoxyflurane (Metofane, Pitman-Moore, Washington Crossing, NJ).

Experimental Procedures

To facilitate blood sampling from undisturbed, unrestrained animals, a Silastic catheter was positioned in the right atrium under pentobarbital (Butler Co., Columbus, OH) anesthesia (40 mg/kg, sc) following previously described procedures (26,27).

After recovery from the surgical procedures (usually within 1 week) animals were administered through the atrial catheter either the E_2-CDS (1.0 mg/kg) dissolved in 20% HPCD, E_2 (1.0 mg/kg) in 20% HPCD, or 20% HPCD as the control injection. An additional group of E_2-CDS animals received two 15-mm testosterone (T)-filled capsules. Previous studies have shown that two 15-mm T-filled implants were sufficient to normalize plasma T levels (32,33). The implants were positioned 48 hr prior to drug treatment.

Five days after drug treatment animals were transferred to special sampling chambers and permitted to adapt to their new environment for 48 hr prior to monitoring GH secretory profiles. One day prior to GH profile sampling a 0.5-ml blood sample was withdrawn via the jugular catheter between 0900 and 1000 for plasma gonadal steroid level determinations by radioimmunoassay (RIA). On the day of experimentation, blood samples (0.3 ml) were removed at 15-min intervals for 8 hr (0800–1600). At each sampling time the blood was immediately centrifuged and the plasma collected for GH by RIA. Red cells were resuspended in heparinized saline (40 units/ml) and returned to each respective animal after the next blood sample.

At the end of the study animals were sacrificed by decapitation, and anterior pituitary glands removed and weighed. Anterior pituitary glands were placed in 1.0 ml cold 0.1 *M* sodium borate, pH 9.0, the tissue was homogenized by sonication (30 sec) and centrifuged, and the resultant supernatant was frozen at −35°C for later assay of GH, PRL, and TSH. At the time of pituitary harvesting trunk blood was

collected, and the serum separated and frozen at −35°C for PRL and TSH determinations by RIA.

During the course of this study additional HPCD- and E_2-CDS-treated animals were prepared as described above except that they did not have GH secretory patterns determined; instead they were sacrificed, and their hypothalami removed for the determination of somatostatin and GRF mRNA levels (to be reported separately). However, the pituitary and trunk blood was collected, processed, and assayed for anterior pituitary hormones along with the tissue and serum from all other animals. Statistical analysis of the data determined that there were no significant differences in the two subsets of data from the HPCD- or E_2CDS-treated animals either within a group or between the four groups of experimental animals. Therefore, the additional HPCD- and E2-CDS-treated animals were added to their respective groups and incorporated into the data in Tables III and IV.

Hormone Assay

All plasma samples for CLUSTER analysis of episodic GH secretion were assayed in triplicate. All other plasma and/or pituitary samples were assayed in duplicate and in appropriate dilutions for GH, PRL, and TSH by RIA using materials supplied by Dr. A. F. Parlow and the National Hormone and Pituitary Program (NIDDK, Baltimore, MD). Unknown values are expressed as nanograms per milliliter in terms of the respective NIDDK reference preparation (rat GH-RP-2, rat PRL-RP-3, or rat TSH-RP-1). Values for unknowns were derived from the 10–90% inhibition portion of the respective standard curve.

The range of GH assay detectability in 25 μl undiluted plasma was 2.5–320 ng/ml. To minimize nonspecific effects of plasma in the GH RIA, 25 μl plasma from hypophysectomized rats was added to each GH standard, reference and nonspecific binding tube in the assay. Undetectable plasma GH levels were assigned the assay sensitivity (2.5 ng/ml).

The range of PRL and TSH assay detectability in 50 μl undiluted plasma was 0.25–50 and 25–2500 ng/ml, respectively. Plasma samples containing undetectable PRL or TSH were assigned the respective assay sensitivity (0.25 for PRL and 25 ng/ml for TSH). Plasma samples with PRL levels greater than 50 ng/ml or TSH levels greater than 2500 ng/ml were appropriately diluted in EDTA phosphosaline buffer and assayed at a volume of 50 μl.

The intraassay coefficients of variation for the GH, PRL, and TSH RIA were 4.7, 4.9, and 4.7%, respectively. Interassay coefficients of variation for GH, PRL, and TSH were 8.6, 9.8, and 9.9%, respectively.

Blood concentrations of the gonadal steroids testosterone and estradiol were assayed in duplicate by RIA using commercial kits supplied by Diagnostic Products Corp (Los Angeles, CA). The assay sensitivity of the testosterone assay was 0.2 ng/ml and 20 pg/ml for estradiol. Previous studies from our laboratory have established that primary antiserum to estradiol shows less than 0.3% cross-reactivity to the E_2-Q^+ (28).

Data Analysis

Identification and analysis of the individual GH peak amplitudes, basal or trough levels, and mean integrated plasma GH concentrations were performed using the CLUSTER program developed by Veldhuis and Johnson (29). The variance model used in applying this statistical program was based on the actual variation of the individual samples (samples were assayed in triplicate). The definition of a cluster size was set at one for identifying peaks and three for trough levels. The t statistic was set at 2.00 for testing both increases and decreases in plasma GH concentrations.

Statistical analysis of all data except for the blood gonadal steroid concentrations was performed using one-way analysis of variance (ANOVA) and Scheffe's multiple-range test (30). Homogeneity of variance was tested prior to each ANOVA, and whenever possible, analysis was performed on the untransformed data. When the variances were found to be unequal, individual hormone values were transformed to their natural logarithms prior to statistical analysis. This transformation normalized the variances and allowed for ANOVA analysis. Significance was set at $P \leq 0.05$.

Because log transformation of the serum gonadal steroid values did not normalize these data, they were analyzed by the Kruskal–Wallis nonparametric one-way ANOVA and multiple-comparisons test (31). Significance was set at $P \leq 0.05$.

RESULTS

GH Secretory Profiles in HPCD-Treated Animals

HPCD-treated adult male rats displayed the typical masculine GH secretory pattern where individual GH pulses were interspersed with prolonged baseline periods of low GH levels (at or below 5.0 ng/ml) for periods up to an hour (see Fig. 2 and Table 1). The episodes of GH secretion either consisted of a single peak or were multiphasic, with two or more defined GH peaks (Fig. 2). Peak amplitudes generally exceeded 150 ng/ml rGH-RP-2 and, on occasion, reached levels exceeding 500 ng/ml (mean, 174.6 ng/ml; Table 1).

Effects of E_2 Alone in HPCD on GH Secretory Dynamics

Male rats given 1.0 mg/kg E_2 in 20% HPCD displayed normal male GH secretory patterns 1 week after receiving the steroid (Fig. 3 and Table 1). Both the high-amplitude GH secretory episodes and the prolonged periods of basal GH secretion were observed (see Table 1) even in the face of a significant reduction in plasma T levels (Table II). On the other hand, E_2 was completely cleared from the plasma 1 week following injection as evidenced by the undetectable plasma levels (Table II).

GH Secretory Profiles in Animals Administered E_2-CDS

In contrast to both HPCD- and E_2-treated animals, adult male rats displayed disrupted masculine GH secretory patterns 1 week after receiving 1.0 mg/kg E_2-CDS (Fig. 4). Although mean plasma GH levels were not affected, individual GH secretory peaks were somewhat blunted, although not significantly, and baseline levels significantly elevated in these animals (Table I). No significant increase in pulse frequency (number of GH pulses/8 hr) was observed in E_2-CDS-treated animals. Associated with this alteration in GH secretory profiles in E_2-CDS-treated males was a significant

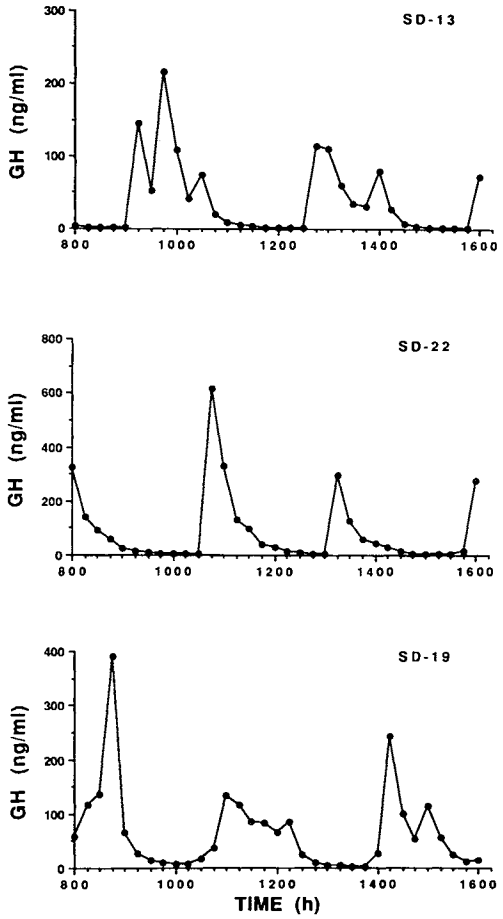

Fig. 2. GH secretory profiles in three individual randomly chosen HPCD control adult male Sprague–Dawley rats. HPCD-treated adult males displayed masculine episodic GH secretion patterns with high-amplitude pulses and low baseline levels. Animals were sampled 1 week following HPCD administration and at 15-min intervals for 8 hr. Samples were assayed in triplicate for GH by RIA.

elevation in plasma E_2 levels and a severe reduction in plasma T (Table II).

Effects of T Replacement on GH Secretion in E_2-CDS-Treated Animals

When plasma T levels were normalized (Table II) in E_2-CDS-treated rats, the male typical GH secretory pattern was again observed. T-replaced, E_2-CDS-treated animals displayed low-frequency GH surges and prolonged periods of low basal GH secretion (Fig. 5 and Table I). Individual GH peak amplitudes were slightly but not significantly reduced in these animals (Table 1).

Effects of Steroid Treatment on Pituitary and Plasma Hormone Levels

The administration of E_2-CDS induced a moderate but significant increase in anterior pituitary weight which was reversed by concomitant T replacement (Table III). E_2 in HPCD did not affect the size of the anterior pituitary relative to HPCD-treated control animals. Because the steroid treatment induced a change in pituitary size, both the content (μg/pituitary) and the concentration (μg/mg wt) of GH, PRL, and TSH have been presented. It is clear that E_2, whether administered alone in HPCD or complexed to the CDS, significantly increased GH and PRL content in the pituitary without affecting TSH content (see Table III). However, when expressed in terms of hormone concentration GH levels were not significantly elevated in animals given E_2-CDS but remained higher in those animals given E_2 in HPCD. PRL concentrations remained elevated in all three groups of animals compared to HPCD-treated animals. A significant reduction in pituitary TSH concentration was observed in E_2-CDS-treated male rats.

A significant elevation in plasma PRL levels was observed in E_2-CDS-treated animals (Table IV). Despite the reversal of pituitary enlargement observed by T replacement in E_2-CDS-treated male rats (see Table III), T replacement did not lower circulating plasma PRL levels (Table IV). Neither E_2 in HPCD, E_2-CDS, nor concomitant T replacement in E_2-CDS-treated animals resulted in an alteration in circulating TSH levels (Table IV). However, plasma TSH levels were significantly higher in T-replaced E_2-CDS-treated animals compared to those animals receiving E_2 in HPCD.

DISCUSSION

The regulation of moment-to-moment GH secretion is accomplished by a complex interaction of the two neuropeptides, somatostatin and GH-releasing hormone (GHRH). A number of studies have clearly established that somatostatin

Table I. GH Secretory Pattern Parameters and Mean Plasma Levels in Gonadal Steroid-Treated Intact Adult Male Rats[a]

Group	No. of peaks/8 hr	Peak amplitude (ng/ml)	Baseline level (ng/ml)	Mean plasma level (ng/ml/min^{-1})
HPCD (10)	2.30 ± 0.15	174.6 ± 45.0	5.0 ± 0.8	42.3 ± 7.1
E_2CDS (12)	2.67 ± 0.36	87.9 ± 17.7	22.6 ± 3.7*	39.3 ± 6.2
E_2CDS + T (5)	2.00 ± 0.45	98.6 ± 17.9	7.4 ± 2.8	29.2 ± 2.7
E_2 (6)	2.33 ± 0.21	246.7 ± 127.4	8.9 ± 1.9	44.7 ± 18.5

[a] Values are the mean ± SE; numbers in parentheses indicate sample size. Prior to statistical analysis (one-way ANOVA and Scheffe's multiple-range test) individual data points were normalized by ln(x) transformation.

* $p < 0.05$ vs HPCD-, E_2CDS + T-, and E_2-treated animals.

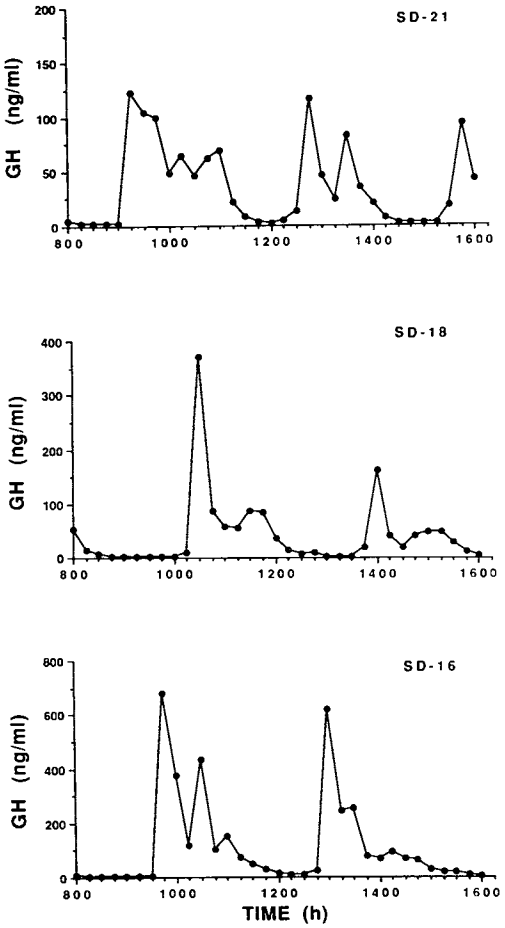

Fig. 3. GH secretory profiles in three individual randomly chosen adult male rats given 1.0 mg/kg E_2 alone in HPCD. One week following administration of E_2 adult male rats displayed masculine episodic GH secretion patterns similar to those of HPCD-treated animals (Fig. 2). Both high-amplitude GH pulses and low-GH baseline levels were consistently observed in these animals.

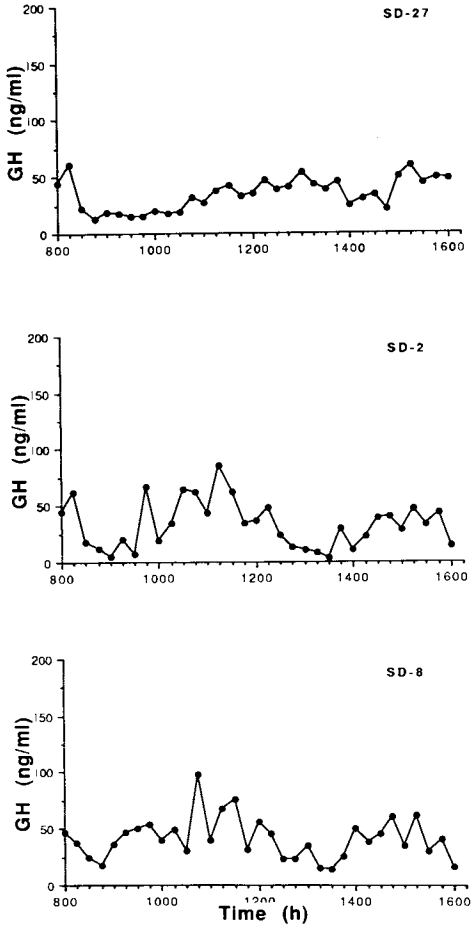

Fig. 4. GH secretory profiles in three randomly chosen individual adult male rats given 1.0 mg/kg E_2-CDS. In contrast to HPCD- and E_2-treated animals, intact male rats given E_2-CDS showed disrupted GH secretory profiles with both diminished individual GH peak amplitudes and elevated basal GH levels. Animals were sampled 1 week following E_2-CDS administration.

is responsible for the low interpeak baseline GH levels and GHRH is primarily responsible for the spontaneous surges of GH secretion (1–4). As a consequence of the interplay between these two peptides, GH is released from the anterior pituitary in an intermittent fashion, with the pattern of hormone secretion being established as sex dependent in the rat (1,3,6,7). Previous studies have clearly demonstrated that the sex-dependent GH secretory pattern observed in adult rats is modulated by gonadal steroids (1,7,10,12,13).

Table II. Plasma Steroid Levels in Steroid-Treated Male Rats[a]

Group	Estradiol (pg/ml)	Testosterone (ng/ml)
HPCD (10)	<20	2.2 ± 0.2
E_2CDS (12)	59.8 ± 9.7*	<0.2**
E_2CDS + T (8)	69.9 ± 4.9*	2.3 ± 0.2
E_2 (6)	<20	1.2 ± 0.3***

[a] Values are the mean ± SE; numbers in parentheses indicate sample size. Statistical significance was determined via the Kruskal–Wallis nonparametric one-way ANOVA and multiple-range test.

* $P < 0.05$ vs HPCD- and E_2-treated animals.

** $P < 0.05$ vs HPCD- and E_2-CDS + T-treated animals.

*** $P < 0.05$ vs HPCD-, E_2-CDS-, and E_2-CDS + T-treated animals.

With respect to E_2, prolonged exposure (1–8 weeks) to this steroid hormone, whether by daily subcutaneous injections or steroid-filled Silastic implants, severely elevates trough or basal GH levels and reduces individual GH pulse amplitudes in male rats (1,7). The results of the present study, utilizing a chemical delivery system (CDS) which provides a sustained release of E_2 in the brain (15–22), confirm the effects of prolonged E_2 exposure on physiological GH secretion in the rat (1,7).

A significant elevation of basal GH secretion was observed in E_2-CDS-treated animals 1 week after administration of 1.0 mg/kg of steroid–carrier complex. These data imply that estrogens act to alter either the synthesis or the release of somatostatin from the hypothalamus (32,33) or they may interfere with the effects of the inhibitory peptide on somatotrope function at the level of the anterior pituitary. The latter effect has recently been demonstrated as evidenced by the observation that the addition of 1 n*M* somato-

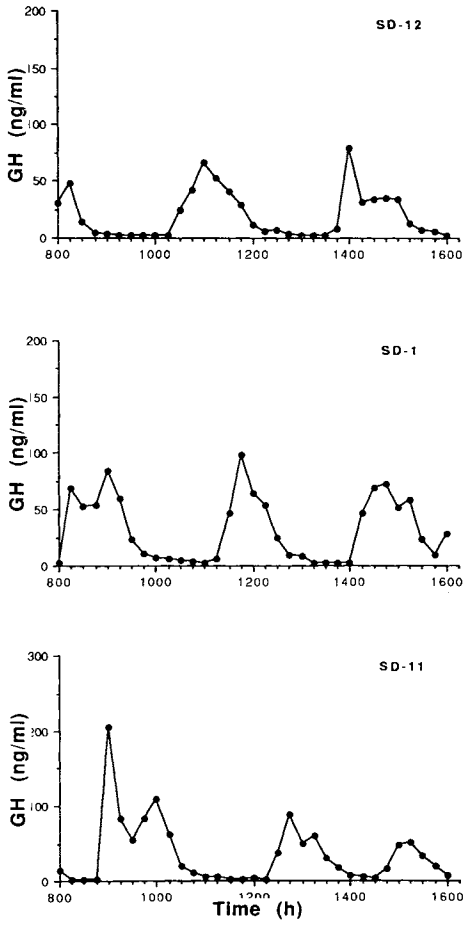

Fig. 5. The effects of testosterone replacement on GH secretory profiles of three randomly chosen intact male rats given 1.0 mg/kg E_2-CDS. Testosterone replacement prevented the alteration in GH secretory patterns observed in E_2-CDS-treated animals (cf. Fig. 4) 1 week following drug administration. Low-frequency individual GH surges were observed as well as prolonged basal or trough periods where GH levels remained near the sensitivity of the GH RIA (2.5 ng/ml). However, there was an attenuation of individual GH peak amplitudes in the T-replaced E_2-CDS-treated animals.

statin to E_2-treated pituicytes *in vitro* was ineffective in inhibiting GH secretion (34).

The observation that concomitant T replacement, in the face of elevated plasma E_2 levels, normalized baseline GH levels in E2-CDS-treated rats indicates that, in addition to the possible direct effects of E_2 on somatostatin regulation of GH secretion, inhibition of T secretion by the testes plays a significant role in the elevated GH trough levels observed in E_2-CDS-treated animals. These data support the notion that T is integral to the efficacy of somatostatin in modulating basal GH secretion (1,5–7,35). Further, T increases median eminence concentrations of somatostatin and somatostatin mRNA and sensitizes the somatotroph to the inhibitory actions of the peptide on GH secretion (32–34).

With respect to the effects of prolonged E_2 exposure on individual GH pulse amplitudes, the present data conflict with previous findings in castrate male rats or intact male rats exposed to E_2 for periods of up to 8 weeks, in which a significant reduction in peak amplitudes has been observed (1,7). We found that, although GH peaks were somewhat dampened (≈45%), the reduction did not reach statistical significance in E_2-CDS-treated animals 1 week after steroid exposure. We have previously found that in adult intact male rats a 1-week exposure to E_2 using Silastic steroid-filled capsules gave variable results, with some rats showing a reduction in GH peak amplitudes and no effect in other animals (7; our unpublished observations). A consistent reduction in GH pulse amplitudes has been observed after 2 weeks of E_2 exposure (7). Perhaps, allowing the animals to be exposed to E_2-CDS for a period longer than 1 week may induce a significant reduction in GH peak amplitudes.

Further, we did not observe any increase in individual GH pulse frequency in intact male rats given E_2-CDS in the present study. This may not be surprising given the fact that we observed a concomitant increase in basal GH with a decrease in individual peak amplitudes, which may have diminished the GH excursions enough to not be recognized by cluster analysis.

It appears unlikely that any residual testicular function may have provided a counterregulatory effect on the individual GH pulse amplitudes, since normalization of circulating testosterone levels by concomitant testosterone replacement in E_2-CDS-treated male rats did not significantly influence the GH peak amplitudes in the present study. In fact, individual GH peak amplitudes remained somewhat dampened in T-replaced E_2-CDS-treated animals compared to HPCD-treated controls and similar to those of animals given E_2-CDS. This implies that chronic exposure to E_2 interferes with GHRH-induced GH secretion, a phenomenon that has

Table III. Anterior Pituitary (AP) Weight and Hormonal Changes in Steroid-Treated Male Rats[a]

		Content (μg/pituitary)			Concentration (μg/mg wt)		
	AP wt (mg)	GH	PRL	TSH	GH	PRL	TSH
HPCD (19)	10.7 ± 0.3	430.1 ± 38.8	12.7 ± 1.2	641.9 ± 43.0	40.3 ± 3.7	1.2 ± 0.1	60.0 ± 3.6
E_2CDS (22)	15.1 ± 0.5*	606.9 ± 51.3**	31.3 ± 2.0***	670.7 ± 54.1	39.7 ± 2.7	2.0 ± 0.1***	46.0 ± 4.7*
E_2CDS + T (8)	11.7 ± 0.7	455.6 ± 39.4	24.2 ± 2.8***	692.2 ± 52.1	38.6 ± 1.9	2.1 ± 0.2***	59.4 ± 3.5
E_2 (10)	11.1 ± 0.5	660.5 ± 51.6**	20.1 ± 2.6***	788.0 ± 65.7	59.6 ± 3.5****	1.8 ± 0.2***	72.8 ± 6.9

[a] Values are the mean ± SE; numbers in parentheses indicate sample size. Prior to statistical analysis (one-way ANOVA and Scheffe's multiple-range test) individual data points were normalized by ln(x) transformation.

* $P < 0.05$ vs HPCD-, E_2CDS + T-, and E_2-treated animals.

** $P < 0.05$ vs HPCD- and E_2CDS + T-treated animals.

*** $P < 0.05$ vs HPCD-treated animals.

**** $P < 0.05$ vs HPCD-, E_2CDS-, and E_2CDS + T-treated animals.

Table IV. Plasma PRL and TSH in Steroid-Treated Male Rats[a]

Group	PRL (ng/ml)	TSH (ng/ml)
HPCD (21)	7.0 ± 0.7	432.7 ± 44.6
E_2CDS (22)	50.6 ± 4.8*	384.2 ± 65.6
E_2CDS + T (8)	55.3 ± 7.9*	683.1 ± 143.8**
E_2 (10)	9.8 ± 3.9	255.2 ± 33.5

[a] Values are the mean ± SE; numbers in parentheses indicate sample size. Prior to statistical analysis (one-way ANOVA and Scheffe's multiple-range test) individual data points were normalized by ln(x) transformation.

* $P < 0.05$ vs HPCD- and E_2-treated animals.

** $P < 0.05$ vs E_2-treated animals.

been clearly documented both *in vivo* and *in vitro* (10–13,34,36). Although a definitive site of action of estrogens on GHRH-induced GH secretion has not been firmly established, the somatotrope appears to be a major target where the steroid reduces the secretory capacity and sensitivity of somatotrophs to GHRH (12,13) as well as the potency of GHRH to elicit GH secretion (34). Whether E_2 also alters the synthesis and release of GHRH from hypothalamic neurons has not been firmly established.

It should be noted that alteration of the GH secretory patterns occurs only in animals in which the steroid was chemically designed for prolonged or sustained release of the steroid. This is evidenced by the fact that adult male rats administered a dose of E_2 (1.0 mg/kg) in the vehicle (HPCD) showed no alteration in their GH secretory patterns at 1 week. Since these animals were not sampled at earlier time points, we cannot be assured that this dose of E_2 did not disrupt GH secretory patterns for a period after steroid treatment. Clearly, data from the present study indicate that these animals were exposed to E_2 for a sufficient period of time to affect the T production but it was not sufficient to alter GH secretory dynamics.

It is known that following the iv administration of E_2 in dimethyl sulfoxide (DMSO) and at doses (≈2.0 mg/kg) higher than those used in the present study (1.0 mg/kg), E_2 is virtually cleared from the plasma within 24 hr of injection (17,20). Similarly, E_2 in HPCD at a dose of 1.0 mg/kg is cleared from the plasma within 24 hr of injection (our unpublished data). We have previously shown that despite the rapid clearance of E_2 following the administration of E_2-CDS, residual E_2 can be observed in the plasma for at least 3 weeks (28). The source of this E_2 is likely the brain, since brain levels of E_2 exceed plasma E_2 levels by 41-fold at 7 days after E_2-CDS administration (28). Further, our calculations indicate that there is sufficient E_2 and E_2-Q^+ in the brains of rats at 7 days post-E_2-CDS administration to account for all of the steroid present in the plasma. After hydrolysis of E_2-Q^+ to liberate E_2, the free steroid can redistribute down a large concentration gradient from the brain to the plasma. Thus, the high concentrations of E_2 in the brain at 7 days post-E_2-CDS injection or the modest elevation in plasma E_2 could account for the feminization of GH secretory patterns observed in the present study.

Pituitary enlargement or hyperplasia as a result of the prolonged exposure to E_2 may also be causative to the alteration of GH secretory patterns in these animals by an alteration of sensitivity to both somatostatin and GHRH (24). There is evidence that hyperplastic pituitaries develop abnormal responses to dopamine (37).

Associated with this time- and dose-related enlargement (hyperplasia) of the anterior pituitary gland is an increase in both pituitary and plasma PRL levels (12,38). These phenomena (increase in pituitary weight and PRL levels within the pituitary and associated hyperprolactinemia) were observed in E_2-CDS-treated animals, indicating a proliferative effect of estrogens on lactotrophs. However, recent evidence indicates that the population of somatotrophs and a population of non-PRL- or non-GH-secreting cells are also increased following E_2 exposure (12).

T replacement blocked both the pituitary hyperplasia and the increase in pituitary GH content associated with E_2-CDS treatment but it did not prevent the increase in pituitary PRL levels or associated hyperprolactinemia. It is possible that concomitant T replacement may selectively block the known proliferative effects of E_2 on both somatotrophs and the non-PRL- and non-GH-secreting cells (12) without affecting lactotroph proliferation. This, in effect, could reduce the enlargement of the pituitary gland without affecting the associated hyperprolactinemia. The identity of this population of non-PRL- and non-GH-secreting cells remains unknown at present. It is unlikely that they are thyrotrophs as evidenced by the fact that prolonged E_2 exposure did not alter the pituitary content of TSH. Further, when expressed as pituitary concentration, a significant reduction in TSH levels was observed in E_2-CDS-treated male rats.

Ho and co-workers (12) have proposed that the effects on somatotroph function may be time dependent, with early effects favoring the expression of somatotroph function and later effects directed toward lactotroph function. This proposal, in part, may explain the significant elevation in both pituitary content and concentration of GH in animals given E_2 in HPCD. However, it does not explain the observation that both pituitary PRL content and concentration were elevated in these animals despite an absence of pituitary hyperplasia or hyperprolactinemia.

One problem that we are still faced with is attempting to discern whether the steroids act at the level of the pituitary and/or within the hypothalamus to alter GH secretion. Despite our documentation of high levels of brain E_2 following E_2-CDS administration (15–19,21,22,28), the persistence of plasma E_2 at levels of 50–60 pg/ml prevents us from ascribing the E_2 effect specifically to the brain or the anterior pituitary. Further studies with lower doses of E_2-CDS, at which plasma E_2 levels are not increased, are needed. Additionally, assessment of pituitary responsiveness to GHRH and somatostatin is essential to help discern the role of the anterior pituitary in theE_2-CDS alteration of GH secretion.

ACKNOWLEDGMENTS

This work was supported by PHS Grants HD22199 (W.J.M.) and HD22540 (J.W.S.).

REFERENCES

1. J-O. Jansson, S. Eden, and O. Isaksson. *Endocrinol. Rev.* 6:128–150 (1985).

2. J. B. Martin and W. J. Millard. *J. Anim. Sci.* **63** (Suppl 2):11–26 (1986).
3. O. G. P. Isaksson, J.-O. Jansson, R. G. Clark, and I. C. A. F. Robinson. *NIPS* **1**:44–47 (1986).
4. W. J. Millard. In D. R. Campion, G. J. Hausman, and R. J. Martin (eds.), *Animal Growth Regulation,* Plenum Press, New York, 1989, pp. 237–255.
5. W. J. Millard, J. A. Politch, J. B. Martin, and T. O. Fox. *Endocrinology* **119**:2655–2660 (1986).
6. W. J. Millard, D. M. O'Sullivan, T. O. Fox, and J. B. Martin. In W. F. Crowley Jr. and J. Hoefler (eds.), *The Episodic Secretion of Hormones,* Churchill Livingstone, New York, 1987, pp. 287–304.
7. W. J. Millard, T. O. Fox, T. M. Badger, and J. B. Martin. In R. J. Robbins and S. Melmed (eds.), *Acromegaly a Century of Scientific and Clinical Progress,* Plenum Press, New York, 1987, pp. 139–150.
8. R. G. Clark, G. Chambers, J. Levin, and I. C. A. F. Robinson. *J. Endocrinol.* **111**: 27–35 (1986).
9. R. G. Clark, L. M. S. Carlsson, and I. C. A. F. Robinson. *J. Endocrinol.* **114**:399–407 (1987).
10. K. Y. Ho, D. A. Leong, Y. N. Sinha, M. L. Johnson, W. S. Evans, and M. O. Thorner. *Am. J. Physiol.* **250**:E650–E654 (1986).
11. K. Y. Ho, W. S. Evans, R. M. Blizzard, J. D. Veldhuis, G. R. Merriam, E. Samojlik, R. Furlanetto, A. D. Rogol, D. L. Kaiser, and M. O. Thorner. *J. Clin. Endocrinol. Metab.* **64**:51–57 (1987).
12. K. Y. Ho, M. O. Thorner, R. J. Krieg Jr., S. K. Lau, Y. N. Sinha, M. L. Johnson, D. A. Leong, and W. S. Evans. *Endocrinology* **123**:1405–1411 (1988).
13. W. S. Evans, R. J. Krieg, E. R. Limber, D. L. Kaiser, and M. O. Thorner. *Am. J. Physiol.* **249**:E276–E280 (1985).
14. S. M. Gabriel, W. J. Millard, J. I. Koenig, T. M. Badger, W. R. Russell, D. M. Maiter, and J. B. Martin. *Neuroendocrinology* **50**:299–307 (1989).
15. N. Bodor, H. H. Farag, and M. E. Brewster III. *Science* **214**:1370–1372 (1981).
16. J. W. Simpkins, J. McCornack, K. S. Estes, M. E. Brewster, E. Shek, and N. Bodor. *J. Med. Chem.* **29**:1809–1812 (1986).
17. K. S. Estes, M. E. Brewster, J. W. Simpkins, and N. Bodor. *Life Sci.* **40**:1327–1334 (1987).
18. W. R. Anderson, J. W. Simpkins, M. E. Brewster, and N. Bodor. *Life Sci.* **42**:1493–1502 (1988).
19. J. W. Simpkins, W. R. Anderson, R. Dawson Jr., A. Seth, M. Brewster, K. S. Estes, and N. Bodor. *Physiol. Behav.* **44**:573–580 (1988).
20. D. K. Sarkar, S. J. Friedman, S. S. C. Yen, and S. A. Frautschy. *Neuroendocrinology* **50**:204–210 (1989).
21. J. W. Simpkins, W. R. Anderson, R. Dawson Jr., and N. Bodor. *Pharm. Res.* **6**:592–600 (1989).
22. W. R. Anderson, J. W. Simpkins, M. E. Brewster, and N. Bodor. *Endocrinol. Res.* **14**:131–148 (1988).
23. L. Janocko, J. M. Larner, and R. B. Hochberg. *Endocrinology* **114**:1180–1186 (1984).
24. E. R. Smith, D. A. Damassa, and J. M. Davidson. *Meth. Psychol.* **3**:259–279 (1977).
25. P. S. Kalra and S. P. Kalra. *Endocrinology* **106**:390–397 (1980).
26. G. S. Tannenbaum and J. B. Martin. *Endocrinology* **98**:562–570 (1976).
27. W. J. Millard, J. B. Martin Jr., J. Audet, S. M. Sagar, and J. B. Martin. *Endocrinology* **110**:540–550 (1982).
28. M. H. Rahimy, N. Bodor, and J. W. Simpkins. *J. Steroid Biochem.* **33**:179–187 (1989).
29. J. D. Veldhuis and M. L. Johnson. *Am. J. Physiol.* **250**:E486–E493 (1986).
30. G. Keppel. *Design and Analysis: A Researcher's Handbook,* Prentice Hall, Engelwood Cliffs, NJ, 1973.
31. M. Hollander and D. A. Wolfe. *Nonparametric Statistical Methods,* Wiley and Sons, New York, 1973.
32. D. S. Gross. *Am. J. Anat.* **158**:507–519 (1980).
33. F. Baldino Jr., S. Fitzpatrick-McElligott, T. M. O'Kane, and I. Gozes. *Synapse* **2**:317–325 (1988).
34. P. Hertz, M. Silbermann, L. Even, and Z. Hochberg. *Endocrinology* **125**:581–585 (1989).
35. J.-O. Jansson and L. A. Frohman. *Endocrinology* **120**:1551–1557 (1987).
36. D. 1. Schulman, M. Sweetland, G. Duckett, and A. W. Root. *Endocrinology* **120**:1047–1051 (1987).
37. A. I. Esquifino, C. Agrasal, R. W. Steger, J. J. Fernandez-Ruiz, A. G. Amador, and A. Bartke. *Life Sci.* **45**:199–206 (1989).
38. J. Gorski. In R. B. Jaffe (ed.), *Prolactin,* Elsevier, New York, 1981, pp. 57–83.