Research Article

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

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We have utilized a redox chemical delivery system (CDS) for the brain targeting of estradiol (E2) to ascertain its effects on GH secretory patterns in adult intact male rats. The E2-CDS (1.0 mg/kg) dissolved in 20% hydroxypropyl-cyclodextrin (HPCD), E2 (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 E2 in HPCD and HPCD treatment did not alter masculine GH secretory patterns, animals administered the E2-CDS displayed disrupted GH patterns with attenuated individual pulse amplitudes and significantly elevated GH baseline levels. Moderate pituitary hyperplasia was evident only in the E2-CDS group of animals. Plasma testosterone (T) concentrations were reduced in only the E2-CDS group. T replacement reduced E2-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 E2-CDS treatment but did block both the increase in pituitary GH content and the hyperplasia associated with prolonged E2 exposure. E2 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 E2-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 E2. Concomitant T therapy can prevent some of the changes in GH secretion associated with high-dose E2 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 (E2) via either daily subcutaneous injections or E2-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 E2 preferentially to the brain following intravenous administration of E2-CDS in an aqueous, sustained-release formulation to determine the effects of prolonged exposure to the E2-CDS on GH secretory patterns in adult intact male rats (15). The present E2-CDS sys-
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-17$\beta$-([1-methyl-1,4-dihydropyridin-3-yl]-carbonyl)oxy)lestra-1,3,5 (10)-triene (estradiol 17-(1,4)-dihydrotrigonellate) was synthesized as previously described (16–18). The 3, 17$\beta$-dicinocinate ester of estradiol was prepared by refluxing 17$\beta$-estradiol with either nicotinyl 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 quantized with methyl iodide, and the $E_2$-CDS was prepared by reduction of the quaternary salt with Na$_2$S$_2$O$_4$.

The $E_2$-CDS and 17$\beta$-estradiol (Steraloids, Wilton, NH) were dissolved in 20% hydroxypropylcyclodextrin (HPCD) on the day of injection and administered intravenously through the inatrial 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 $-35^\circ$C for later assay of GH, PRL, and TSH. At the time of pituitary harvesting trunk blood was