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M. S. Roberson
University of Iowa, msr14@cornell.edu

M. W. Wolfe
Case Western Reserve University, mwolfe2@kumc.edu

T. T. Stumpf
University of Missouri, Columbia

D. L. Hamernik
University of Missouri, Columbia, dhamernik2@unl.edu

Andrea S. Cupp
University of Nebraska-Lincoln, acupp2@unl.edu

See next page for additional authors

Authors

M. S. Roberson, M. W. Wolfe, T. T. Stumpf, D. L. Hamernik, Andrea S. Cupp, L. A. Werth, N. Kojima, Roger J. Kittok, H. E. Grotjan, and J. E. Kinder kinder.15@osu.edu

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Steady-State Amounts of α- and Luteinizing Hormone (LH) β-Subunit Messenger Ribonucleic Acids Are Uncoupled from Pulsatility of LH Secretion during Sexual Maturation of the Heifer[1]

M. S. ROBERSON,[3] M. W. WOLFE,[4] T. T. STUMPF,[5] D. L. HAMERNIK,[7] A. S. CUPP,[6] L. A. WERTH,[6] N. KOJIMA,[6] R. J. KITTOK,[6] H. E. GROTJAN,[6] and J. E. KINDER[2,6]

Department of Animal Science[6] and Department of Veterinary Science,[7] University of Nebraska-Lincoln Lincoln, Nebraska 68583-0908

ABSTRACT

Our primary objective for this study was to determine whether steady-state amounts of α- and LH β-subunit mRNAs in the anterior pituitary are altered during sexual maturation in the bovine female. A secondary objective was to determine whether 17β-estradiol (E_2) alters amounts of LH subunit mRNAs before onset of puberty. Heifers (7 mo old) were assigned to one of three treatments: 1) ovariectomized (OVX, n = 16); 2) OVX and administered E_2 (OVXE, n = 16); or 3) ovary-intact (INTACT, n = 20). Pituitaries were collected at an estimated 120 days before onset of puberty (prepuberty) or 25 days before onset of puberty (peripuberty). Six INTACT heifers were used to determine time of puberty during the experimental period, and their pituitaries were collected 40 h after administration of prostaglandin $F_{2\alpha}$ (postpubertal INTACT group). Relative amounts of mRNAs for LH subunits in each pituitary were determined by Northern analysis and scanning densitometry. Amounts of α- and LH β-subunit mRNAs were lower in pituitaries of INTACT heifers and OVXE heifers, regardless of stage of sexual maturation, than in those of OVX heifers. Amounts of α-subunit mRNA were similar in OVXE and INTACT heifers regardless of stage of sexual maturation. Amounts of LH β-subunit mRNA did not change during sexual maturation in heifers in the INTACT group. Concentrations of E_2 were higher and LH β-subunit mRNA were lower in heifers from the prepubertal OVXE group than in heifers in all other treatment groups. We conclude that steady-state amounts of α- and LH β-subunit mRNAs are not limiting during sexual maturation in the bovine female. However, when concentrations of E_2 are elevated during the prepubertal period to those typical of the follicular phase of the bovine estrous cycle, levels of LH β-subunit mRNA are specifically suppressed.

INTRODUCTION

Studies with bovine females [1–5] provide evidence to support the gonadostat hypothesis originally developed for the rat [6]. According to this hypothesis, sensitivity of the hypothalamic-pituitary axis to negative feedback effects of 17β-estradiol (E_2) decreases as sexual maturation progresses. The decline in negative feedback effects of E_2 upon the hypothalamic-pituitary axis of the heifer has been associated with a decline in hypothalamic and pituitary receptor populations for E_2 [3] and an increase in the pulsatile release of LH [5].

A high correlation exists between pulses of LHRH from the stalk median eminence and pulses of LH detected in systemic circulation [7]. Maintenance of steady-state amounts of gonadotropin subunit mRNAs in the pituitary is dependent upon stimulation by LHRH [8, 9]. Hence, the low frequency of LH pulses from the anterior pituitary during prepuberty may be associated with reduced amounts of LH subunit mRNAs. The primary aim of the present study was to determine whether steady-state amounts of α- and LH β-subunit mRNAs in the anterior pituitary are altered during sexual maturation in the bovine female. A second aim was to determine whether E_2 is a modulator of amounts of LH subunit mRNAs before onset of puberty.

MATERIALS AND METHODS

Experimental Protocol

Prepubertal Angus × Hereford heifers (n = 52) that were approximately 7 mo of age and 200 kg body weight at the initiation of the study were assigned at random to one of three treatments: 1) ovariectomized (OVX, n = 16); 2) OVX and administered E_2 (OVXE, n = 16); or 3) ovary-intact (INTACT, n = 20). Six heifers from the INTACT group were used to determine time of puberty within the experimental period (postpubertal INTACT). All heifers were acclimated to stanchions and human contact before initiation of the study. Feed, water, and mineral supplements were available ad libitum.

Ovariectomies were performed via high lumbar laparotomy. At the time of ovariectomy (Day 0), heifers in the OVXE groups received an ear implant containing E_2 (Compudose, Elanco, Greenfield, IN; 1 cm in length). Implants were maintained in donor animals for approximately 6 wk before transfer of the implants into heifers in the OVXE

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[2]Correspondence: James E. Kinder, A224j Animal Science, University of Nebraska, Lincoln, NE 68583-0908.

[3]Current address: Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242.

[4]Current address: Department of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

[5]Current address: Department of Animal Science, University of Missouri, Columbia, MO 65211.

treatment group. Use of donor animals helped to avoid a surge release of E_2 from implants at the time of implantation. Implants were removed and replaced with new implants in heifers from the OVXE group at 4-wk intervals throughout the study to maintain uniform circulating concentrations of E_2.

Three weeks after ovariectomy and administration of E_2 (Day 21), 8 heifers selected at random from the OVX, OVXE, and INTACT treatment groups were fitted with an indwelling jugular catheter 1 day before initiation of a 24-h serial blood collection regimen. Blood samples were collected at 15-min intervals during the 24-h period to determine the pattern and concentration of LH in serum. After the period of serial blood collection, the pituitaries of all 8 heifers that were bled in the OVX, OVXE, and INTACT groups were collected. These heifers were designated prepubertal with regard to stage of sexual maturation (estimated 120 days before onset of puberty).

The other heifers were assigned to the peripubertal group. These animals were bled to characterize their pattern of LH secretion on Days 49 and 79 after overiectomy and every 14 days thereafter until they met the following criteria: 1) Tissues were collected from heifers in the INTACT group when frequency of LH pulses reached 9 pulses/24 h (indicative of approximately 20 to 30 days before pubertal estrus); 2) For each heifer in the INTACT group that met the criteria for collection of tissue at peripuberty, a heifer from both the OVX and OVXE groups which exhibited the most rapid frequency of LH pulses was paired with INTACT heifers, and tissues were collected on the same day. These criteria were selected because it has previously been shown that heifers have an increase in frequency of LH pulses during the 50 days preceding puberty [3, 5].

Evaluation of LH pulse frequency was used to determine the time tissue collection should occur in order to standardize stage of sexual maturation (as assessed by frequency of LH pulses) in peripubertal heifers of the INTACT, OVX, and OVXE groups. In addition, these criteria were used in such a way that the time of tissue collection from peripubertal heifers coincided with the time when heifers assigned to the postpubertal INTACT group were attaining puberty.

Blood samples were collected twice weekly from INTACT heifers to assess concentrations of progesterone, which were in turn used to monitor onset of puberty (i.e., assignment to the postpubertal group). Concentrations of progesterone ≥ 1 ng/ml for two consecutive serum samples were used as a criterion to determine onset of estrous cycles (puberty). After onset of puberty, blood collection continued twice weekly to monitor changes in concentrations of progesterone during subsequent estrous cycles.

Heifers assigned to the postpubertal INTACT group were fitted with indwelling jugular catheters during the luteal phase (as determined by concentrations of progesterone) of the first or second estrous cycle following onset of puberty. Subsequently, heifers were treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 25 mg; The UpJohn Co., Kalamazoo, MI) via a jugular catheter to regress the corpus luteum. Blood samples were collected at 15-min intervals for 24 h beginning 14 h after administration of $PGF_{2\alpha}$. Blood samples collected during this time were used to determine concentrations of LH during the follicular phase of the estrous cycle, when concentrations of E_2 were relatively high and concentrations of progesterone were low. Pituitaries were collected from heifers assigned to the postpubertal INTACT group at 40 h after administration of $PGF_{2\alpha}$.

Tissues were removed from heifers in all treatment groups after exsanguination. Pituitaries were collected within 15 min after exsanguination. Anterior pituitaries were hemisected, transferred to separate vials, frozen in liquid nitrogen, and stored at −70°C until amounts of mRNAs or heterogeneity of gonadotropic isoforms [10] was determined.

RIAs

All blood samples were allowed to clot at room temperature and were stored at 4°C for 24 h. Serum was collected after centrifugation at 1 520 × *g* for 15 min and stored at −20°C until assayed for LH, progesterone, and E_2. Concentrations of LH in serum samples collected at 15-min intervals were determined by an RIA [5, 11] using an antiserum against ovine LH (TEARAOLH #35), highly purified ovine LH (LER-1056-C2) as radiolabeled tracer, and NIH-LH-B7 as standard. Intra- and interassay coefficients of variation were 2.8% and 8.8%, respectively. Concentrations of progesterone in serum samples collected twice weekly were determined by RIA [12]. Intra- and interassay coefficients of variation were 4.0% and 12.0%, respectively. Concentrations of E_2 in serum samples pooled within each serial blood collection period were determined by RIA [12]. Concentrations of E_2 were determined in a single assay with an intraassay coefficient of 2.1%.

The pattern of LH secretion was characterized by determination of mean concentrations (ng/ml), frequency (pulses/24 h), and amplitude (ng/ml) of LH pulses through use of algorithms designed for this purpose (Pulsar software modified for the IBM-PC for J.F. Gitzen and V.D. Ramirez, Urbana, IL). Data reported are for variables of LH secretion from samples collected during the 24 h immediately before collection of tissue.

Northern Analysis of Total Cellular RNA

After pituitaries were collected, the posterior portion was separated from the anterior pituitary and discarded. The anterior pituitaries were frozen in liquid nitrogen. Weights of frozen hemipituitaries were determined. Total cellular RNA (tcRNA) was isolated by centrifugation through a cesium chloride gradient [13]. No greater than a 12% tissue weight/homogenization buffer volume was used. Pellets of tcRNA from each hemipituitary were subsequently resus-

TABLE 1. Mean concentrations of E_2 and LH as well as amplitude and frequency of LH pulses.

Treatment[a]	Mean E_2 (pg/ml)	Mean LH (ng/ml)	Amplitude (ng/ml)	Frequency (pulses/24 h)
Prepubertal OVX	1.7[b]	2.4[b]	3.4[b]	23.5[b]
Peripubertal OVX	2.2[b]	3.4[c]	2.3[b]	30.1[c]
Prepubertal OVXE	14.7[c]	0.6[d]	0.0[e]	0.0[d]
Peripubertal OVXE	6.7[d]	1.9[b]	3.8[b]	21.1[b]
Prepubertal INTACT	2.9[b]	0.6[d]	3.2[b]	4.4[e]
Peripubertal INTACT	4.9[b]	0.6[d]	4.0[b]	9.6[f]
Postpubertal INTACT	12.2[c]	1.9[b]	2.8[b]	26.6[bc]
Pooled SEM	0.7	0.2	0.4	1.0

[a]Treatments: prepubertal: pituitaries collected from heifers approximately 120 days before attainment of puberty; peripubertal: pituitaries collected from heifers approximately 25 days before attainment of puberty: OVX: ovariectomized; OVXE: ovariectomized and administered E_2; INTACT: ovary-intact; postpubertal: pituitaries collected from heifers assigned to the postpubertal intact group 40 h after administration of $PGF_{2\alpha}$ at first or second estrous cycle postpuberty.

[b-f]Numbers with differing superscripts within columns differ ($p < 0.05$).

pended in 10 mM TRIS-HCl (pH 7.6), 1 mM EDTA, and 1% *N*-lauryl sarcosine and extracted three times with chloroform:butanol (4:1). Total cellular RNA was precipitated by adding 3 M sodium acetate until the concentration of the solution was 0.3 M, followed by 2 volumes of absolute ethanol [14]. Precipitates of tcRNA were maintained under ethanol at −70°C until electrophoretic separation. Concentrations of tcRNA were estimated by absorbance spectrophotometry at 260 nm, and purity was estimated by the absorbance ratios of A260/A280 and A260/A230.

Five micrograms of tcRNA from individual hemipituitaries were separated by electrophoresis in 1% agarose gels containing formaldehyde. Within each gel, tcRNA samples from heifers in each treatment group were represented. Total cellular RNA isolated from cerebellum and tcRNA from steer pituitaries were used as negative and positive controls, respectively. Size-separated tcRNA was subsequently transferred to nitrocellulose filters by capillary action and baked at 80°C for 2 h.

The cDNA probes (generously provided by Dr. Richard Maurer, University of Iowa, Iowa City, IA) to bovine α- [14] and LH β-subunits [15] were cloned into pSP65. The α- and LH β-subunit cDNAs were linearized with restriction endonuclease digestion (*Eco*RI for α-subunit; *Xba* I for LH β-subunit). Riboprobes were labeled to high specific activity with ^{32}P α CTP (NEN DuPont, Boston, MA) using a Riboprobe II transcription kit and SP6 polymerase (Promega, Madison, WI) according to the manufacturers' instructions. Furthermore, heterologous oligonucleotide probes to rat α-tubulin (NEN DuPont; nucleotides +958 to +987 of rat cDNA) were 3′ end-labeled (3′ End Labeling Kit; NEN DuPont) to high specific activity with ^{35}S-labeled α-ATP according to the manufacturer's instructions. All radiolabeled RNA and oligonucleotide probes were isolated in a NENSORB 20 nucleic acid purification cartridge (NEN DuPont).

Total cellular RNA bound to nitrocellulose filters was hybridized (10^6 total cpm/ml hybridization buffer for each probe) to α-subunit, LH β-subunit, and α-tubulin probes for 48 h at 60°C. All radiolabeled probes used in the hybridization reactions were generated from a single riboprobe transcription or 3′ end-labeling reaction. After hybridization, nitrocellulose filters were washed at room temperature (three washes; 20 min/wash) in double-strength SSC (3 M NaCl, 0.3 M sodium citrate [pH = 7.4]) and 0.1% SDS and in 0.2-strength SSC at 60°C (2 washes; 60 min/wash). Specific radiolabeled bands were visualized [13] following autoradiography (4 h for α-subunit; 8 h for LH β-subunit; 72 h for α-tubulin). The relative total density of each band (OD × mm^2) was determined by image analysis using a Visage 110 image analyzer (Eastman Kodak, Rochester, NY).

Statistical Analysis

Effect of treatment and stage of sexual maturation on characteristics of pulsatile secretion of LH and relative steady-state amounts of α-subunit, LH β-subunit, and α-tubulin mRNAs were analyzed by analysis of variance [16]. Differences in treatment means were determined by Fischer's Least Significant Difference test [16]. Changes in frequency of LH pulses in heifers in the postpubertal INTACT group that occurred over the 50 days before puberty were analyzed by regression analysis [16]. The resulting prediction equation was used to estimate number of days before puberty for heifers in the peripubertal INTACT group [3].

RESULTS

Concentrations of E_2

Concentrations of E_2 in serum increased ($p < 0.05$) during sexual maturation in INTACT heifers (Table 1). Administration of E_2 to heifers in the peripubertal OVXE group resulted in concentrations of E_2 similar ($p > 0.05$) to those in peripubertal INTACT heifers. Concentrations of E_2 were higher ($p < 0.05$) in heifers in the prepubertal OVXE group than in prepubertal INTACT heifers. Concentrations of E_2 at 40 h after administration of $PGF_{2\alpha}$ in heifers assigned to the postpubertal INTACT group were similar ($p > 0.05$) to those in heifers in the prepubertal OVXE group (Table 1).

Pattern of LH Secretion

A linear increase ($p < 0.05$; $r^2 = 0.53$) in frequency of LH pulses occurred over the 50 days before puberty in heifers assigned to the postpubertal INTACT group. This relationship could be expressed by the following equation: frequency of LH pulses = 13.11 − 0.18 (days prepuberty). On the basis of this regression equation, mean estimated number of days to puberty for peripubertal INTACT heifers was 4.33 ± 1.8 (Mean ± SEM) days at the time tissues were collected (range = 0.11–10.90 days). A cautionary interpretation of this relationship is necessary because approximately 47% of the variation in days to puberty was unaccounted for by frequency of LH pulses. Regardless, frequency of LH pulses detected in peripubertal INTACT heifers immediately before tissues were collected is characteristic of the pulse frequency in heifers within 25 days of puberty [3, 5].

Frequency of LH pulses was greater ($p < 0.05$) in ovariectomized heifers and intact heifers bled during the follicular phase (postpubertal INTACT) than in heifers in the prepubertal OVXE, prepubertal INTACT, and peripubertal INTACT groups (Table 1). Furthermore, frequency of LH pulses was greater ($p < 0.05$) in heifers in the peripubertal OVXE and INTACT groups than in heifers in the prepubertal OVXE and INTACT groups. Amplitude of LH pulses was relatively constant throughout the experimental period in heifers receiving all treatments, with the exception of heifers in the prepubertal OVXE group (Table 1). Pulsatile release of LH was abolished in heifers in the prepubertal OVXE group, and thus amplitude of LH pulses could not be calculated. Mean concentrations of LH increased ($p < 0.05$) during the treatment period in heifers in the OVXE group. However, mean concentrations of LH did not change ($p > 0.05$) during sexual maturation in INTACT heifers. Mean concentrations of LH were greater ($p < 0.05$) in ovariectomized heifers compared to heifers in all other treatment groups regardless of stage of sexual maturation.

Concentrations of mRNAs

Concentrations of tcRNA isolated from pituitaries were not affected by treatment ($p > 0.05$) and averaged 1.38 ± 0.04 μg tcRNA/mg pituitary^{-1}. Absorbance ratios of A260/A280 and A260/A230 were similar ($p > 0.05$) among pituitary tissues from heifers of the different groups and averaged 1.74 ± 0.01 and 2.40 ± 0.02, respectively. All tcRNA preparations contained obvious and discrete ribosomal RNA

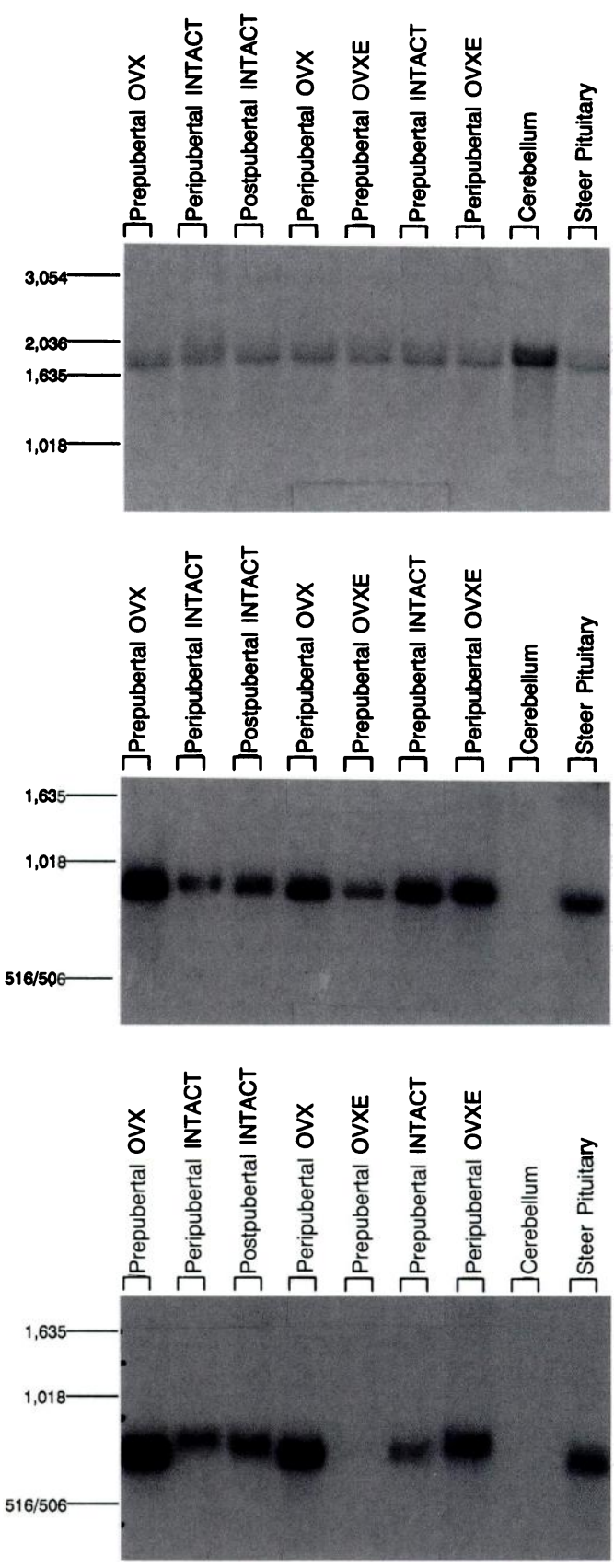

FIG. 1. Representative autoradiogram from Northern analysis following hybridization to α-tubulin (top panel), α-subunit (middle panel) and LH β-subunit (bottom panel) radiolabeled probes. Within each Northern analysis, 5 μg of tcRNA from hemipituitaries of heifers in each treatment (see text for details of treatments), from cerebellum, and from steer pituitaries were electrophoresed through a denaturing agarose gel. Relative molecular size is based upon a 1-kb DNA standard (Bethesda Research Laboratories, Gaithersburg, MD) and is indicated at the left of each panel.

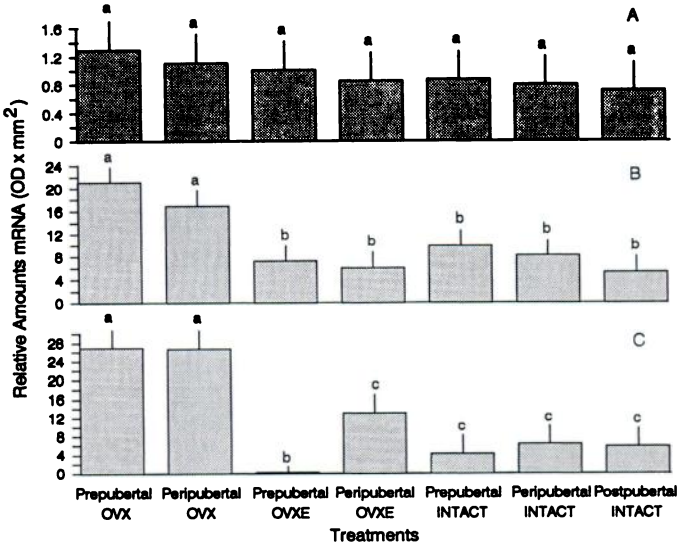

FIG. 2. Relative steady-state amounts of (A) α-tubulin, (B) α-subunit and (C) LH β-subunit mRNAs. Prepubertal: pituitaries collected from heifers approximately 120 days before puberty; peripubertal: pituitaries collected approximately 25 days before puberty; OVX: ovariectomized; OVXE: ovariectomized and administered E_2; INTACT: ovary-intact; postpubertal: pituitaries collected 40 h after administration of $PGF_{2\alpha}$ from heifers assigned to the postpubertal intact group. Letters differing between treatments within mRNA species differ ($p < 0.05$).

bands of approximately 2.0 and 5.0 kb. Total cellular RNA isolated from the cerebellum did not specifically hybridize to either α- or LH β-subunit probes (Fig. 1).

Relative amounts of α-tubulin detected in tcRNA were similar ($p > 0.05$) among heifers in the different groups and did not appear to be influenced by E_2 or to be developmentally regulated in heifers (Fig. 2a). Amounts of α-tubulin were greater (3.67 OD × mm^2; $p < 0.05$) in tcRNA preparations isolated from the cerebellum compared to tcRNA preparations isolated from the pituitary.

Hybridization to either α- or LH β-subunit probes resulted in specific discrete bands. Relative amounts of α- (Fig. 2b) and LH β-subunit (Fig. 2c) mRNAs were lower ($p < 0.05$) in heifers in OVXE and INTACT groups as well as in heifers assigned to the postpubertal INTACT group, than in heifers in the OVX groups regardless of stage of development. Amounts of α-subunit mRNA were similar ($p > 0.05$) in heifers in the OVXE and INTACT groups regardless of stage of sexual maturation. Amounts of LH β-subunit mRNA were markedly lower ($p < 0.05$) in heifers from the prepubertal OVXE group compared to heifers from the pre-, peri- and postpubertal INTACT and peripubertal OVX groups (Fig. 2). Furthermore, concentrations of LH β-subunit mRNA were similar ($p > 0.05$) among INTACT heifers at the pre-, peri- and postpubertal stages.

DISCUSSION

Stimulation of gonadotropes by LHRH appears to be necessary for maintenance of steady-state concentrations of gonadotropin subunit mRNAs [8, 9]. Our original premise was that hypothalamic release of LHRH would be inhibited by negative feedback effects of E_2 during the prepubertal period. Consequently, a reduction in concentrations of LH subunit mRNAs would be observed. When frequency of LHRH and LH pulses increased as the time of puberty approached, LH subunit mRNAs were expected to increase. In the present study, E_2 from ovarian or exogenous sources reduced steady-state amounts of α-subunit mRNA 50 to 65% compared to amounts of α-subunit mRNA from OVX heifers. These results are in agreement with those from the rat [17] and ewe [13], in which E_2 at levels characteristic of late gestation were administered. However, stage of sexual maturation did not affect steady-state amounts of α-subunit mRNA in heifers receiving E_2 from endogenous or exogenous sources in the present study. Because both gonadotropes and thyrotropes express the α-subunit gene [18], observation of alterations in content of α-subunit mRNA does not necessarily reflect changes in gonadotropic activity.

Accumulation of LH β-subunit in the pituitary is thought to be the primary rate-limiting step in assembly of glycoprotein subunit dimers and in synthesis of LH [19]. Steady-state amounts of LH β-subunit mRNA were also coordinately reduced in heifers with E_2 from endogenous or exogenous sources compared to ovariectomized heifers not receiving E_2. Moreover, when serum concentrations of E_2 were similar to follicular-phase levels (12–15 pg/ml), a specific reduction in amounts of LH β-subunit mRNA occurred in heifers in the prepubertal OVXE group. These data are in agreement with previous data [17] collected after supraphysiological doses of E_2 were administered to adult female rats. When endogenous concentrations of E_2 were present, a reduction in steady-state levels of LH β-subunit mRNA was not observed in INTACT heifers during the prepubertal period, reflecting a differential effect of E_2 depending on the dose of E_2 to which the pituitary is exposed. An alternative explanation is that ovarian secretions other than E_2 are involved in modulation of amounts of LH β-subunit mRNA.

On the basis of our results, it is possible to speculate that anterior pituitary content of steady-state amounts of LH β-subunit mRNA remain constant throughout sexual maturation. Thus, sufficient LHRH is present and capable of maintaining levels of LH β-subunit mRNA. Furthermore, amounts of LH β-subunit mRNA do not appear to be a limiting factor in the occurrence of puberty.

In the present study, concentrations of E_2 in circulation were similar in heifers that were in the follicular phase of the estrous cycle (postpubertal INTACT) and in prepubertal ovariectomized heifers administered E_2 (OVXE). However, pulsatile release of LH and amounts of LH β-subunit mRNA were markedly lower in prepubertal OVXE heifers. These data support the conclusion that there is a differential effect of E_2 upon the hypothalamic-pituitary axis during sexual maturation and that it is apparent with regard to secretion

of LH and steady-state amounts of LH β-subunit mRNA. However, because concentrations of E_2 in circulation of heifers in the prepubertal OVX group were relatively high in the present study, extrapolation to ovary-intact heifers during prepuberty must be done with caution. Although speculative, one possibility is that suppression of steady-state amounts of LH β-subunit mRNA in ovariectomized heifers administered relatively high levels of E_2 is characteristic of heifers that are more than 120 days prepuberty, when pulsatile secretion of LH is greatly reduced (1 pulse of LH/24 h [5]).

Despite the fact that no change in amount of mRNAs for the subunits of LH was evident in INTACT heifers throughout sexual maturation, changes in pattern of secretion of LH did occur. Changes in frequency of LH pulses during the 50 days before puberty in heifers assigned to the postpubertal INTACT group in the present study were consistent with previous results [2, 3]. The criteria used to determine the time tissues were collected from heifers during the peripubertal period (9 LH pulses/24 h) allowed for collection of tissues during the time when heifers assigned to the postpubertal INTACT group were attaining puberty. Heifers assigned to the postpubertal INTACT group initiated corpus luteum function between 25 May and 20 July, and tissues were collected from heifers in the peripubertal INTACT group between 2 June and 28 July.

Clearly, a differential feedback of E_2 on secretion of LH occurs during the transition from the prepubertal to the postpubertal states in heifers (20–22). In mature ovariectomized cows, E_2 (3–10 pg/ml) enhances mean concentration of LH in circulation compared to that in ovariectomized cows not receiving E_2 [21]. If heifers are ovariectomized during the prepubertal period and given similar amounts of E_2 (3–10 pg/ml), secretion of LH is inhibited. The change in pattern of LH secretion during sexual maturation appears to be mediated by changes in sensitivity to negative feedback effects of E_2 at the medial basal hypothalamus [23–25]. This maturational change appears to be mediated in part by a loss in estrogen receptor populations in the hypothalamus and pituitary [3]. Pituitary responsiveness to administration of LHRH increases as puberty approaches in heifers [26] in the absence of detectable changes in concentrations of LHRH receptors [3]. In the present study, similar concentrations of E_2 in prepubertal and postpubertal heifers demonstrate the possibility of a differential effect of E_2 on pulsatile release of LH and on steady-state amounts of LH β-subunit mRNA. Endogenous E_2, however, was not limiting steady-state amounts of LH subunit mRNAs as ovary-intact heifers approached puberty. The anterior pituitary is capable of synthesis and storage of LH during prepuberty. Pituitary content of LH was greater in ovary-intact heifers, whereas amounts of mRNAs for both α- and LH β-subunits were greater in heifers from the OVX groups. There was no increase in the amount of either α- or LH β-subunit mRNA as sexual maturation progressed in ovary-intact heifers. Likewise, no increase in pituitary stores of LH occurs during sexual maturation in ovary-intact heifers [10]. This leads us to conclude that the primary rate-limiting factor modulating time of puberty in heifers is the inhibition of pulsatile LH secretion and not limited amounts of mRNAs for LH or limited stores of LH in the anterior pituitary.

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