EXPERIMENTAL STUDY

Stimulation of pregnenolone metabolism and aromatase activity by luteinizing hormone in mouse uterus

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Abstract

In vitro metabolism of pregnenolone (P_5) as well as production of 17β -estradiol (E_2) were studied in uteri of untreated and luteinizing hormone (LH)-treated mice that had been ovariectomized (OVX) at late-diestrus stage. In the uteri of untreated mice, [3H]pregnenolone was shown to be metabolized to Δ^5 -components such as 17α -hydroxypregnenolone (17α - P_5) and dehydroepiandrosterone (DHEA), whereas LH treatment resulted in significant increases in the formation of progesterone (P_4), 17α -hydroxyprogesterone (17α - P_4), androstenedione (AD) and testosterone (T). This was assessed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The content and release of P_4 was shown to be stimulated by LH. Trilostane, an inhibitor of 3β -hydroxysteroid dehydrogenase (3β -HSD), inhibited LH-induced P_4 synthesis and its release in a dose-dependent manner. A considerable increase in [3H]estradiol formation from [3H]testosterone was recorded in LH-stimulated uterine tissue as compared with the control, indicating the stimulatory effect of LH on aromatase activity. LH-stimulation in the synthesis of P_4 and E_2 in OVX mouse uteri was mimicked by dbcAMP (cell-permeable cAMP). Incubation with LH was shown to augment the conversion of P_4 to various Δ^4 -3-oxosteroids. *In vitro* effects of LH on the synthesis and metabolism of P_4 , as well as on the stimulation of aromatase activity, were more pronounced in the uterine tissue of LH-primed OVX mice. Thus the results of the present study indicate that, under specific conditions, the uterus of the mouse behaves like steroidogenic tissue. Its prompt response to LH reveals the probable physiological relevance of the existence of LH receptors of high binding affinity in the uterine tissue of the mouse, as reported earlier.

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Introduction

Contrary to the conventional belief that luteinizing hormone (LH) and human chorionic gonadotropin (hCG) regulate only gonadal functions, several recent studies have shown that LH/hCG receptors are also present in a variety of non-gonadal tissues (1–6). Of these, the studies on the uteri of both primates and non-primates have been the most extensive. The presence of high-affinity and low-capacity binding sites for LH and hCG has been reported for the uterine tissue of pigs, rats, mice and rabbits (7–10), as well as for human endometrium and myometrium (1, 2, 11). The concentrations of uterine receptors fluctuate with the stages of reproduction. The amounts of receptors in porcine uterine tissue are higher during the luteal phase and in early pregnancy than in follicular phase of the estrous cycle (1, 12, 13). In the mouse uterus, the amounts are high during the late-diestrous stage (9), whereas in rat uterus, this occurs during the metestrous stage (14). The functional relevance of the existence of LH/hCG receptors in the mammalian

uterus is not clear, although some of the earlier studies reported regulation of increased production of progesterone (P_4) by the induction of LH (14). We have also shown augmented conversion of cholesterol to pregnenolone (P_5) in OVX mouse uteri following the induction of LH (9). This has led us to investigate whether the mouse uterus, like the ovary, is really capable of synthesizing P_4 and 17β -estradiol (E_2) in response to LH stimulation through modulation of the activity of Δ^5 - 3β -hydroxysteroid dehydrogenase (Δ^5 - 3β -HSD) and aromatase.

Materials and methods

Animals

For at least 15 days prior to use, two- to three-month-old, albino, female virgin mice (25–30 g) of an inbred strain were kept on a schedule of 12 h light:12 h darkness and were allowed to take food and water *ad libitum*. Vaginal smears were examined daily for two

consecutive 4 day estrous cycles; only the normally cycling mice were used.

Chemicals

Ovine luteinizing hormone (oLH) was obtained as a gift from Dr A F Parlow, NIDDK (Lot. No. oLH-26, AFP 5551B). $\Delta^5[4,7-^3\text{H}]$ pregnenolone (sp. activity 11000 mCi/mmol), $[1,2,6,7-^3\text{H}]$ progesterone (sp. activity 6800 mCi/mmol), $[2,4,6,7-^3\text{H}]$ estradiol-17 β (sp. activity 22,500 mCi/mmol) and $[1,2,6,7-^3\text{H}]$ testosterone (sp. activity 5400 mCi/mmol) were purchased from Amersham International UK. The purity of these radiochemicals was checked by thin-layer chromatography (TLC) in a benzene/acetone (4:1, v/v) solvent system. Minimum essential medium (MEM), bovine serum albumin (BSA) and all authentic steroids were purchased from Sigma Chemical Company (St Louis, MO, USA). Highly purified P_4 and E_2 antisera were donated by Professor Gordon Niswender (Colorado State University, Fort Collins, USA). The cross-reactivities of various steroids with P_4 and E_2 antibodies were checked before their use in radioimmunoassay (RIA). Acetonitrile and methanol (Spectrochem, India) were of HPLC grade.

Tissue preparation

Female mice at diestrus stage II were ovariectomized and after 7 days they were separated into two groups. One group was primed with three i.m. injections of LH (500 ng/mouse) on alternate days, whereas those of the other group (control), which comprised twice the number of mice, received saline injections. On day 7, uteri from the mice in both groups were removed and washed with pre-chilled MEM gassed with 95% O_2 /5% CO_2 . Equal amounts of tissues (50 mg) from the control and LH-primed groups were placed in 5 ml sterile glass beakers each containing 1.0 ml MEM; these were then incubated for 4 h at $37 \pm 1^\circ\text{C}$ to study the steroid metabolism, to determine 3 β -HSD activity, to estimate P_4 and E_2 content, and to assay aromatase activity.

In vitro incubation of uterine tissue

Uterine tissues (50 mg) from saline-injected OVX mice were incubated with or without LH (500 ng/ml) in the presence of $[^3\text{H}]\text{P}_5$ (2×10^6 c.p.m., 138 pmol) and NAD (300 μM) or $[^3\text{H}]\text{P}_4$ (2×10^6 c.p.m., 222 pmol) and NADPH (5 μmol) in 1.0 ml MEM (pH 7.4) with constant shaking for 4 h. LH-primed mouse uterine tissues were also incubated similarly with LH. Incubations were terminated after placing of the beakers on ice. For P_5 metabolism, the following unlabeled steroids (15 μg each) were added to the medium as carrier steroids at the completion of incubation: P_5 , 17 α - P_5 , dehydroepiandrosterone (DHEA), P_4 , 17 α - P_4 , androstenedione (AD), testosterone (T) and E_2 . For P_4

metabolism, 17 α - P_4 , P_4 , T, E_2 , AD, 3 α -hydroxy-5 β -pregnan-20-one (3 α -OH-5 β -pregnan-20-one) and 5 β -pregnan-3 α ,17 α ,20 β -triol (5 β -3 α ,17 α ,20 β -P) were used.

Pregnenolone metabolites obtained from uterine-tissue incubations in response to LH were also fractionated by HPLC. For this purpose, uterine tissues from both saline-injected and LH-primed OVX mice were incubated without or with LH (500 ng/incubation) in the presence of unlabeled P_5 (15 $\mu\text{g}/\text{ml}$) in 1.0 ml MEM for 4 h.

For measurement of Δ^5 -3 β -HSD coupled with Δ^5 - Δ^4 isomerase (Δ^5 -3 β -HSD) activity, the procedure described by Imataka *et al.* (15) was used. In brief, uterine tissues (50 mg) were incubated with $[^3\text{H}]\text{P}_5$ and NAD without or with LH (500 ng/ml) for 4 h with constant shaking. Incubation was terminated by placing the beakers on ice. No unlabeled steroids were added as carriers.

To determine the P_4 and E_2 contents, uterine tissues, after being minced, were incubated without or with LH (500 ng/ml) for 4 h. Upon termination of incubation, tissues and media were collected separately. Steroids were extracted by the addition of 4 ml diethylether. The amounts of P_4 and E_2 were estimated by RIA according to the previous procedure (9) based on the description of Korenman *et al.* (16).

Aromatase activity of the uterine tissue in response to LH was determined by using the method of Chan & Tan (17). Uterine tissues (50 mg) were incubated in the absence or presence of LH (500 ng/ml) for 6 h at $37 \pm 1^\circ\text{C}$. All incubations contained 280 pmol $[^3\text{H}]\text{T}$ (2×10^6 c.p.m., sp. activity 5400 mCi/mmol).

Extraction, identification and quantification of steroids

Upon termination of incubation either with unlabeled or labeled P_5 or with labeled P_4 or T, tissues were harvested by centrifugation at 3000 *g* and homogenized in phosphate buffer (pH 7.4). Steroids were extracted with dichloromethane (DCM) (5 ml $\times$ 3) and evaporated to dryness. Dried materials were dissolved in methanol and subjected to TLC on silica gel G-coated plates (0.25 mm thickness). To obtain P_4 and P_5 metabolites, plates were developed in the solvent systems containing chloroform/diethyl ether (9:1, v/v) and benzene/acetone (4:1, v/v) respectively. For HPLC analysis, steroids were extracted with diethylether (4 ml $\times$ 3) and evaporated to dryness. Dried materials were dissolved in methanol and subjected to HPLC. To determine aromatase activity, plates were developed in the solvent systems benzene/acetone (4:1, v/v) and diisopropyl ether/petroleum spirit/acetic acid (70:30:1, v/v). Each radioactive spot was identified by (a) comparing its mobility on a thin-layer chromatogram with that of the corresponding authentic preparation,

Table 1 Crystallization and recrystallization of radioactive metabolites with respective authentic steroids, for identification.

Metabolite crystallized with:	Specific activity of crystal (c.p.m./mg crystal)			
	Original*	1	2	3
17 α -Hydroxypregnenolone	440	420	410	430
Dehydroepiandrosterone	175	170	160	150
Progesterone	380	365	370	365
17 α -Hydroxyprogesterone	135	132	140	126
Androstenedione	125	120	118	110
3 α -OH-5 β -Pregnan-20-one	500	480	470	470
Testosterone	220	200	200	180
5 β -Pregnan-3 α ,17 α ,20 β -P	300	280	270	270
17 β -Estradiol	120	100	100	80

* Specific activities of the residues before crystallization.

(b) by comparing the chemical behaviour of respective radioactive metabolites with that of authentic steroids in acetylation (18) and oxidation by 0.5% CrO₃ in 90% aqueous acetic acid solution (19), and (c) by determining the constant specific activity of crystals after repeated crystallization of radioactive metabolites with the authentic steroid preparation (20) (Table 1). Identified materials were recovered from the silica gel by extraction with chloroform/methanol (1:1, v/v), and the radioactivity in each aliquot was estimated in 3 ml of a toluene-based PPO-POPOP system in a liquid scintillation counter (LS 6000 S.C.; Beckman). Values were presented as per cent conversion of [³H]P₅ or [³H]P₄ to [³H]-labeled metabolites. For measurement of 3 β -HSD activity, results were expressed as pmol [³H]P₄ formed/mg uterine tissue protein; for determination of aromatase activity, results were expressed as pmol [³H]estradiol formed/mg uterine tissue protein. Protein was estimated by the Lowry method (21) using BSA (fraction V) as the standard.

High-performance liquid chromatography

HPLC was performed using a Model 501 pump, a UV detector, (Model 486), a Model 410 differential refractometer and a Model 740 data module (all from Waters Associates, Inc., Milliford, MA, USA). The column used was a C₁₈ μ Bondapak stainless steel reverse-phase column (3.9 mm i.d. $\times$ 30 cm; Waters Associates, Inc.). The mobile phase used was acetonitrile/water (40:60, v/v) at a constant flow rate of 1 ml/min. The absorbance of the effluent was measured at 254 nm and the column was flushed with HPLC-grade 100% methanol for 10 min before the injection of samples. Initially, the retention times of eight authentic standard steroids were recorded (Table 2) and then a chromatogram was obtained using a mixture of those eight steroid standards. The samples were injected separately and the peak and retention time for each

steroid metabolite were recorded and compared with those of corresponding authentic steroid peaks.

Steroid RIA

Steroid samples were incubated with phosphate-buffered saline (pH 7.4) containing 0.1% gelatin, P₄ or E₂ antiserum and [³H]P₄ or [³H]E₂ for 18 h at 4 °C. Bound and free [³H]P₄ or [³H]E₂ were separated by using dextran-coated charcoal. An aliquot of bound [³H] P₄ or [³H] E₂ was added separately to 3 ml of toluene-based scintillation fluid containing PPO and POPOP and then counted in a liquid scintillation counter. The sensitivity of the RIA for P₄ was 16 pg/mg uterine tissue protein or 12 pg/ml medium; for E₂, the sensitivity was 10 pg/mg uterine tissue protein or 6 pg/ml medium. The intra-assay coefficients of variation for P₄ and E₂ were 7% and 6% and the interassay coefficients of variation were 9% and 8% respectively. Results were expressed as pg steroid content/mg uterine tissue protein and pg steroid released/ml medium.

Statistical calculations

Data were analyzed by one-way ANOVA followed by Scheffe's simultaneous confidence test (22). All values

Table 2 Retention time of authentic steroids during HPLC.

Steroid	HPLC retention time (min)
Testosterone (T)	4.1
Dehydroepiandrosterone (DHEA)	5.2
17 α -Hydroxypregnenolone (17 α -P ₅)	6.1
17 α -Hydroxyprogesterone (17 α -P ₄)	7.4
Pregnenolone (P ₅)	8.5
Androstenedione (AD)	10
Deoxycorticosterone (DOC)	12.6
Progesterone (P ₄)	15.1

Elution time with acetonitrile/water (40:60) mobile phase at a flow rate of 1 ml/min.

Table 3 Metabolism of pregnenolone in the uterine tissue of ovariectomized (OVX) mice incubated with luteinizing hormone (LH). Values presented are percentages conversion of [^3H]P₅ to [^3H]-labeled metabolites. Values are the means $\pm$ S.E.M. of four incubations. Uterine tissues from saline-injected OVX and LH-primed OVX (LH-PR) mice were incubated with [^3H]P₅ (2×10^6 c.p.m., 138 pmol) and NAD (300 μmol) in the presence or absence of LH (500 ng/ml) for 4 h at $37 \pm 1^\circ\text{C}$.

Metabolite	OVX	OVX + LH	LH-PR OVX+LH
17 α -Hydroxypregnenolone (17 α -P ₅)	6.4 $\pm$ 0.47	0.60 $\pm$ 0.05*	0.112 $\pm$ 0.01 [†]
Dehydroepiandrosterone (DHEA)	6.05 $\pm$ 0.4	1.27 $\pm$ 0.1*	0.55 $\pm$ 0.05 [†]
Progesterone (P ₄)	0.25 $\pm$ 0.02	9.5 $\pm$ 0.5*	19.5 $\pm$ 1.0 [†]
17 α -Hydroxyprogesterone (17 α -P ₄)	0.34 $\pm$ 0.02	3.1 $\pm$ 0.5*	6.75 $\pm$ 0.6 [†]
Androstenedione (AD)	0.30 $\pm$ 0.05	6.5 $\pm$ 0.64*	9.5 $\pm$ 0.64 [†]
Testosterone (T)	0.27 $\pm$ 0.02	4.8 $\pm$ 0.40*	10.0 $\pm$ 0.8 [†]
Unknown-I (polar)	1.21 $\pm$ 0.07	1.24 $\pm$ 0.08	1.1 $\pm$ 0.1
Unknown-II (polar)	1.27 $\pm$ 0.41	1.97 $\pm$ 0.1	1.87 $\pm$ 0.1
Unknown-III (non-polar)	0.41 $\pm$ 0.04	0.77 $\pm$ 0.05	0.45 $\pm$ 0.11
Unknown-IV (non-polar)	1.25 $\pm$ 0.09	1.8 $\pm$ 0.07	1.4 $\pm$ 0.06

* $P < 0.01$ compared to OVX.

[†] $P < 0.01$ compared to OVX + LH.

were expressed as the mean $\pm$ S.E.M. A probability level of $P < 0.01$ was considered as significant.

Results

When saline-injected OVX mouse uterine tissue was incubated with [^3H]P₅ without any hormonal stimulation, 10 metabolites were obtained by TLC (Table 3). Of these, six were identified and four remained unidentified. Of the identified metabolites, 17 α -P₅ and DHEA were found to be the major ones (Table 3). Conversion of P₅ to 17 α -P₅ was 6.4% and to DHEA was 6.05%. The formation of P₄ was conspicuously less (0.25%), indicating that the Δ^5 -pathway was dominated in unstimulated OVX mouse uterine tissue. The production of 17 α -P₄, AD and T was negligible. There was also less formation of unidentified metabolites, of which two were polar and two non-polar components. In contrast, when uterine tissue of saline-injected OVX mice was incubated in the presence of LH, P₄ was found to be the major metabolite. Tritiated P₅ recovery in such tissues was approximately 21% of the total radioactivity added; 9.5% of the radioactivity was co-migrated as P₄, 3.1% as 17 α -P₄, 4.8% as T, and 6.5% as AD. The formation of 17 α -P₅ and DHEA was found to be significantly ($P < 0.01$) low compared with the OVX. The levels of unidentified metabolites remained almost unaltered. Interestingly, the rate of conversion of P₅ to Δ^4 steroid metabolites was significantly higher when OVX mice were initially primed with LH for three doses and uterine tissues were further incubated with LH. In these cases, conversion to P₄ and 17 α -P₄ was found to be 19.5% and 6.63% respectively of total radioactivity.

Metabolites of P₅ identified by TLC were confirmed after HPLC fractionation. The retention times (Table 2) and peaks of individual standard steroids in HPLC were marked as references. In the chromatogram for the saline-injected OVX samples, only three peaks of

absorbance were coincident with the retention times and peaks for authentic DHEA, 17 α -P₅ and P₅ respectively (Fig. 1A). In addition, there were three unidentified small peaks. In the chromatogram for OVX + LH samples, six peaks were obtained; the retention times and peaks of four of these were found to coincide with those of T, 17 α -P₄, P₅ and P₄ (Fig. 1B). Comparison of the absorbances of the peaks revealed the existence of greater amounts of P₄ than of any other metabolite in OVX + LH samples. Interestingly, in uterine tissue samples of LH-primed mice incubated with LH, nine peaks were obtained; of these, five were shown to coincide with peaks for T, 17 α -P₄, P₅, AD and P₄ respectively (Fig. 1C) and the remaining four were unidentified. Peaks identified as AD and P₄ were shown to be greater than any other peaks in the chromatogram. It is noteworthy that a peak for DHEA was not observed in LH-stimulated samples (Fig. 1B and C).

For measurement of Δ^5 -3 β -HSD activity, uterine tissue was incubated with [^3H]P₅ in the presence of NAD for 4 h. The rate of formation of P₄ was significantly high ($P < 0.01$) in the uterine tissue of OVX mice incubated with LH *in vitro*, and, when LH-primed tissues were incubated in the presence of LH, the production of P₄ was more pronounced (Fig. 2). From the radioactivity of P₄, the calculated activity of Δ^5 -3 β -HSD was 0.63 pmol/mg protein for OVX, 6.5 pmol/mg protein for OVX + LH and 10.7 pmol/mg protein for LH-primed OVX + LH mouse uterine tissue.

The addition of LH during the incubation of uterine tissue from saline-injected OVX mice greatly augmented ($P < 0.01$) the tissue content (synthesis) of P₄ as well as its release (content in the medium) in comparison with the OVX control (Table 4). Both the synthesis and the release of P₄ were found to be more pronounced ($P < 0.01$) when LH-primed uterine tissues were incubated in the presence of LH in the medium (Table 4). Trilostane, an inhibitor of 3 β -HSD,

was shown to inhibit LH-induced P_4 synthesis and release (almost in a dose-dependent manner) in LH-primed OVX mouse uterine tissue (Fig. 3). LH-stimulated P_4 synthesis and release were found to be mimicked by dbcAMP. At a dose of 0.1 mM, dbcAMP

Figure 2 Conversion of [3 H]pregnenolone to [3 H]progesterone by the uterine tissues of saline-injected OVX and LH-primed (LH-PR) OVX mice. Details of LH priming are given in the text. Uterine tissues were incubated without or with LH (500 ng/ml) for 4 h in presence of [3 H]pregnenolone (2×10^6 c.p.m., 138 pmol). Each value is the mean $\pm$ S.E.M. of four incubations. * $P < 0.01$ compared with OVX and OVX + LH.

Table 4 Luteinizing hormone (LH) stimulation of progesterone content and release. Uterine tissues from saline-injected ovariectomized (OVX) and LH-primed OVX (LH-PR) mice were incubated with or without LH for 4 h at $37 \pm 1^\circ\text{C}$.

System	Progesterone*	
	Content (pg/mg uterine protein)	Released (pg/ml medium)
OVX (C)	850 ± 40	699 ± 30
OVX + LH (C + LH)	$1200 \pm 44^\dagger$	$980 \pm 30^\dagger$
LH-PR OVX + LH	$3060 \pm 100^\dagger$	$2500 \pm 110^\dagger$

* Values are means $\pm$ S.E.M. ($n = 6$).

$^\dagger P < 0.01$ compared to OVX and OVX + LH.

significantly ($P < 0.01$) stimulated the synthesis and release of P_4 in saline-injected OVX mouse uterine tissue relative to the control (Fig. 4).

In the uteri of saline-injected OVX mice, the tissue content of E_2 and its release in the medium were very low. The addition of LH was shown to augment ($P < 0.01$) both the synthesis and the release of E_2 . A further increase was observed in the tissue from LH-primed mice incubated with LH in the medium (Table 5). Both of these processes were shown to be mimicked by dbcAMP (Fig. 5). A higher dose of dbcAMP was found to be more effective.

When uterine tissue from saline-injected OVX mice

Figure 1 HPLC of pregnenolone (P_5) metabolites of mouse uterine tissue. Eluent system: acetonitrile/water (40:60, v/v). The identities of the fractionated metabolites are indicated on the peaks; unidentified ones are labeled 'u'. HPLC patterns of the metabolites for saline-injected ovariectomized (OVX) mouse uterine tissue (A), with OVX + LH (500 ng/ml) in the medium (B), and with LH-primed OVX incubated with LH (500 ng/ml) in the medium (C). Details of LH priming and the full nomenclature of the steroid metabolites are given in the text.

Figure 3 Effects of trilostane on LH-stimulated (500 ng/ml) progesterone synthesis (tissue) and release (medium) by the LH-primed OVX mouse uterine tissue. Details of LH priming are given in the text. Uterine tissues were incubated for 1 h with trilostane prior to the addition of LH, and incubated for 4 h. Each value is the mean $\pm$ S.E.M. of six incubations. * $P < 0.01$ compared with control (0).

Figure 4 Effects of dbcAMP on progesterone synthesis and release by saline-injected OVX mouse uterine tissue. Tissues were incubated with graded doses of dbcAMP for 4 h. Each value is the mean $\pm$ S.E.M. of six incubations. * $P < 0.01$ compared with the control (0).

was incubated with [3 H]T for 6 h in the presence of LH, a considerable increase in [3 H]E₂ formation was noticed relative to the control (Fig. 6). Furthermore, the formation of [3 H]E₂ was significantly higher when LH-primed OVX mouse uterine tissue was incubated with LH. These findings indicate that LH stimulates aromatase activity (Fig. 6).

Saline-injected OVX mouse uterine tissue was found to convert [3 H]P₄ to several metabolites (listed in Table 6). The Δ^4 -3-oxosteroid metabolites obtained from P₄ comprised 17 α -P₄, T and AD. Small amounts of 3 α -OH-5 β -pregnan-20-one, 5 β -3 α ,17 α ,20 β -P and E₂ were also found (Table 6). *In vitro* LH stimulation of such tissue significantly increased the production of all of the Δ^4 -3-oxosteroids and E₂, but failed to alter the concentrations of 3 α -OH-5 β -pregnan-20-one and 5 β -3 α ,17 α ,20 β -P. When LH-primed uterine tissue was incubated in the presence of LH, no significant increase in the formation of metabolites was observed (Table 6).

Discussion

In the present study, there is a clear evidence that uterine tissue of the mouse is capable of synthesizing P₄ from the precursor P₅. Progesterone (P₄) is subse-

quently metabolized to C₁₉ steroids, particularly the 17-oxo ones, which, in turn, lead to final conversion to E₂. The study of Δ^5 -3 β -HSD activity *in vitro* substantiates the Δ^4 -pathway of P₅ metabolism. The most significant findings are the stimulatory role of LH in *in vitro* synthesis and metabolism of P₄ as well as the activation of aromatase in mouse uterine tissue.

In OVX mouse uterine tissue under basal conditions, i.e. without LH stimulation, P₅ was metabolized primarily to 17 α -P₅ and DHEA via the Δ^5 -pathway. When such tissues were incubated in the presence of LH, or when LH-primed OVX uterine tissues were incubated further with LH, the Δ^5 -pathway was replaced by the Δ^4 -pathway, resulting in the formation of P₄ along with other Δ^4 metabolites. This was observed using TLC and was further substantiated by HPLC. The increased activity of Δ^5 -3 β -HSD when stimulated by LH, using P₅ as the substrate, also lends support to the view that there is a shift from the Δ^5 - to the Δ^4 -pathway in mouse uterine tissue. The findings that trilostane, an inhibitor of Δ^5 -3 β -HSD, effectively blocked P₄ synthesis and release from P₅ in uterine tissue agrees with a previous report on gonads,

Table 5 Luteinizing hormone (LH) stimulation of 17 β -estradiol content and release. Uterine tissue from saline-injected ovariectomized (OVX) and LH-primed OVX (LH-PR) mice were incubated with or without LH for 4 h at 37 $\pm$ 1 $^{\circ}$ C.

System	17 β -Estradiol*	
	Content (pg/mg uterine protein)	Released (pg/ml medium)
OVX (C)	230 $\pm$ 13	160 $\pm$ 5
OVX + LH (C + LH)	840 $\pm$ 70 [†]	610 $\pm$ 31 [†]
LH-PR OVX + LH	2270 $\pm$ 150 [†]	1750 $\pm$ 110 [†]

* Values are means $\pm$ S.E.M. (n = 6).

[†] P < 0.01 compared to OVX and to OVX + LH.

in which LH/hCG was shown to exert a steroidogenic influence through modulation of Δ^5 -3 β -HSD (23).

From the present study, as well as from some earlier reports (8, 9, 11), it is clear that the mechanism by which LH exerts its stimulatory effect in the mouse uterus involves its initial binding with an LH-specific receptor in the uterine cell membrane and the activation of adenylate cyclase activity to produce cAMP which, in turn, stimulates the synthesis of P₄. The high rate of conversion of cholesterol to P₅ in LH-stimulated OVX mouse uterine tissue, reported previously by us (9), not only indicates the earlier action of LH on uterine mitochondrial steroidogenesis but also

justifies the availability of P₅ within the cells of this organ for subsequent conversion to C₁₉ steroids. The present study furnishes ample evidence that dbcAMP mimics the action of LH for both synthesis and release of P₄ by the OVX mouse uterus. This finding corroborates the earlier observations, in which hCG was reported to stimulate P₄ synthesis in metestrus rat uterus via a cAMP-dependent pathway (10).

Another aspect of LH action was the significant augmentation of aromatase activity in the OVX mouse uterus. This is supported by a high rate of metabolism of aromatizable androgen (T to E₂) and by enhanced synthesis and release of E₂ under LH stimulation. That the mammalian uterus possesses several steroidogenic enzymes participating in the conversion of steroid precursors to metabolites has been documented (10, 24–27), but it is not clear how the activities of such enzymes are regulated. The increased conversion of labeled T to labeled E₂ found in the present study and also that which occurs in the stromal cells of human endometrium, reported recently (28), amply indicate that another locus of LH action in the uterine steroidogenic cascade is the regulation of aromatase activity in addition to that of cytochrome P-450_{sc} (9) and Δ^5 -3 β -HSD activities.

Figure 5 Effects of dbcAMP on 17 β -estradiol synthesis and release by saline-injected OVX mouse uterine tissue. Tissues were incubated with graded doses of dbcAMP for 4 h. Each value is the mean $\pm$ S.E.M. of six incubations. * P < 0.01 compared with the control (0).

Figure 6 Conversion of [³H]testosterone to [³H]estradiol in the uterine tissues of saline-injected OVX- and LH-primed OVX mice. Details of LH priming are given in the text. Tissues were incubated in the presence of [³H]testosterone (2 $\times$ 10⁶ c.p.m., 280 pmol) without or with LH (500 ng/ml) for 6 h. Each value is the mean $\pm$ S.E.M. of four incubations. * P < 0.01 compared with OVX and OVX + LH.

Table 6 Metabolism of progesterone in the uterine tissue of ovariectomized (OVX) mice incubated with luteinizing hormone (LH). Values presented are percentages conversion of [^3H]P₄ to [^3H]-labeled metabolites. Uterine tissue from saline-injected OVX and LH-primed OVX (LH-PR) mice were incubated with [^3H]P₄ (2×10^6 c.p.m., 222 pmol) and NADPH (5 μmol) in the presence or absence of LH (500 ng/ml) for 4 h at $37 \pm 1^\circ\text{C}$. Values are the means $\pm$ S.E.M. of four incubations.

Metabolite	OVX	OVX+LH	LH-PR OVX+LH
17 α -Hydroxyprogesterone (17 α -P ₄)	3.62 $\pm$ 0.24	5.04 $\pm$ 0.25*	5.04 $\pm$ 0.33
Androstenedione (AD)	3.8 $\pm$ 0.08	5.2 $\pm$ 0.08*	4.99 $\pm$ 0.5
Testosterone (T)	3.87 $\pm$ 0.10	5.47 $\pm$ 0.27*	6.24 $\pm$ 0.27
3 α -Hydroxy-5 β -pregnan-20-one (3 α -OH-5 β -pregnan-20-one)	0.56 $\pm$ 0.05	0.49 $\pm$ 0.04	0.58 $\pm$ 0.04
5 β -Pregnan-3 α ,17 α ,20 β -triol (5 β ,3 α ,17 α ,20 β -P)	0.45 $\pm$ 0.06	0.515 $\pm$ 0.05	0.662 $\pm$ 0.06
17 β -Estradiol (E ₂)	0.52 $\pm$ 0.054	0.85 $\pm$ 0.036*	1.1 $\pm$ 0.2

* $P < 0.01$ compared to OVX.

Progesterone metabolism by the mouse uterus is not unexpected, since there are several earlier reports showing that mammalian endometrial tissue can metabolize P₄ (25, 26, 29–31). The influence of the E₂ metabolism of P₄ by this tissue is primarily directed towards the formation of some inactive metabolites (24). In the present study, very small amounts of inactive metabolites of P₄, namely 3 α -OH-5 β -pregnan-20-one and 5 β -3 α -17 α ,20 β -P, have been identified in both LH-stimulated and -unstimulated uterine tissues. Such small amounts may be due to the absence of endogenous E₂ in the OVX mouse. What is interesting, here, is that, under LH stimulation, P₄ metabolism in the uterine tissue of OVX mice is channeled towards the steroid-synthesizing pathway, with the formation of aromatizable androgen through 17 α -P₄ and AD.

Finally, it can be concluded that mouse uterus participates in the synthesis and metabolism of P₄ in the same way as the mammalian ovary, and that it is a potential organ for the synthesis of E₂ through aromatase. It is significant that, as in mammalian ovary, LH exerts its stimulatory influence in these processes. It remains to be determined whether there is any role for follicle-stimulating hormone in uterine steroidogenesis.

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