Quantitation of uncombined gonadotropin subunits within and released by the rat anterior pituitary

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Abstract. The release of uncombined gonadotropin subunits by rat anterior pituitaries during an in vitro incubation as well as intracellular concentrations were assessed. Uncombined subunits and native gonadotropins were quantitated by radioimmunoassays after samples were subjected to gel filtration on Sephadex G-100 Superfine. Small, but detectable, amounts of uncombined rat LH β subunit were released under basal conditions. GnRH increased the absolute amount of rLHβ released but did not alter the rLHβ/rLH molar ratio (∼0.02). Tissue extracts prepared in aqueous buffer (100 000 × g supernatants) and 0.5% Triton X-100 extracts of the 100 000 × g pellets from the initial homogenization (‘pellet extracts’) contained larger quantities of uncombined rLHβ as well as elevated rLHβ/rLH molar ratios (∼0.10 and ∼0.20, respectively). Significant amounts of uncombined rLHα were released and were present in both tissue and pellet extracts. However, when FSH β subunit was quantitated in tissue extracts after gel filtration, all of the immunoreactive materials eluted in the position of native rFSH (FSHβ/rFSH molar ratio < 0.0025). Pellet extracts contained significant amounts of rLHβ, rLH and rLHα but lesser amounts of rFSH suggesting that intracellular gonadotropins are not completely extracted when homogenization is performed in aqueous buffers. Thus, rat anterior pituitaries contain and release significant amounts of the uncombined α subunit, relatively small amounts of uncombined rLHβ and extremely small amounts of uncombined FSHβ, if any.

The pituitary gonadotropins LH and FSH are composed of a common α subunit and a hormone-specific β subunit (reviewed by Pierce & Parsons 1981). In the rat, and other species, the subunits of these hormones are synthesized individually (Godine et al. 1980; Counis et al. 1981, 1982). Although the secretion of the native hormones has been extensively studied, the secretion of uncombined subunits remains to be more fully characterized. Primary cultures of rat anterior pituitary cells contain and release significant amounts of the common α subunit (measured as rLHα) (Grotjan et al. 1984). Cultures also release a small amount of uncombined rLHβ (Grotjan et al. 1984) but minimal quantities of uncombined FSHβ (Grotjan et al. 1985). In the present study, levels of uncombined subunits released by rat anterior pituitaries incubated in vitro as well as those in pituitary extracts were quantitated. Similar to previous studies, small amounts of uncombined rLHβ were released or were present in pituitary extracts. However, when a heterologous FSHβ radioimmunoassay was applied to pituitary extracts after gel filtration, all the immunoreactive FSHβ was associated with the native hormone.

Materials and Methods

Experimental design

The primary objective of the studies presented herein was to quantitate the release and intracellular levels of uncombined gonadotropin subunits under basal and GnRH-stimulated conditions. Because native rLH e-
hibits appreciable cross-reactivity in the assays for rLH subunits and the uncombined rLH subunits are generally present in lower concentrations than the native hormone (Grotjan et al. 1984), the media from incubated pituitaries and pituitary extracts were subjected to gel filtration under analytical conditions prior to application of the assays. Potential changes associated with incubation were assessed by subjecting the extracts of pituitaries which had not been incubated to gel filtration and subsequent radioimmunoassay.

**Anterior pituitary incubations**

Anterior pituitaries were removed from intact, adult, male Long Evans rats killed by decapitation. They were hemi-sectioned and pre-incubated for 2 h in Eagle's Minimum Essential Medium, Earle's Salts (MEM), 1 ml/gland. The metabolic incubator was maintained at 37°C and 95% oxygen; 5% carbon dioxide was used as the gas phase. After pre-incubation, 8 hemi-pituitaries were placed in vials containing 2 ml MEM with or without 10−7 mol/l GnRH (Beckman, Palo Alto, CA) and incubated for 4 h. The tissue was removed from the medium, rinsed with MEM and extracted. The medium was clarified by centrifugation at 100 000 × g for 1 h.

**Preparation of pituitary extracts**

Incubated or freshly obtained pituitaries were vigorously homogenized in 2 ml 0.01 mol/l sodium phosphate buffered (pH 7.3) saline (PBS) containing 1 mmol/l EDTA. After subjected to a freeze-thaw cycle, the homogenates were centrifuged at 100 000 × g for 1 h. These supernatants were designated as the tissue extracts. Because significant amounts of immunoreactive gonadotropins had previously been observed in detergent extracts of the particulate fraction of homogenates prepared in aqueous buffers (Grotjan, unpublished observation), the 100 000 × g pellet of each sample was re-homogenized in PBS-EDTA containing 0.5% (v/v) Triton X-100 and again centrifuged at 100 000 × g. These supernatants were designated as the pellet extracts.

**Gel filtration**

In order to monitor the efficacy of separation during gel filtration, samples received 5 mg each of highly purified preparations of bovine serum albumin (BSA, mol wt 67 000), ovalbumin (OV, mol wt 45 000) and ribonuclease A (RA, mol wt 13 700). They were applied to 1.5–1.6 × 90 cm columns of Sephadex G-100 Superfine (Pharmacia, Piscataway, NJ) and eluted at 4°C with 0.15 mol/l ammonium bicarbonate at 3–4 ml/h. Two-mI fractions were collected. Elution profiles were analysed for immunoreactive gonadotropins and subunits. During processing two samples derived from incubated pituitaries were lost, one medium sample and one pellet extract. In the interest of brevity, only gel filtration profiles from one set of incubated (Figs. 1–3) and one set from non-incubated pituitaries (Figs. 4 and 5) are presented.

Before utilization, each G-100 column was characterized with blue dextran (Vₖ), BSA, OV, chymotrypsinogen A (CA, mol wt 25 000), RA, 125I (included volume, Vᵢ) as well as 125I-labelled rat gonadotropins and subunits. Under the conditions utilized, iodinated rFSH-I4, rLH-I5, rLHα-C043, rLHβ-A805 and human FSHβ-2 eluted with apparent molecular weights of 52 000, 47 500, 28 000, 25 000 and 30 000, respectively.

**Radioimmunoassays**

The radioimmunoassays utilized have been described in detail previously (Grotjan et al. 1984, 1985). Their cross-reactivities with native gonadotropins were provided with the reagents. Additional characterizations were previously presented (Grotjan et al. 1984, 1985). rLH, rFSH, rLHα and rLHβ were quantitated with homologous assays and expressed in terms of rLH-DNW-8-59B (Ward et al. 1971), rFSH-I4, rLHα-C043 and rLHβ-A805 equivalents, respectively. FSHβ was quantitated in a heterologous assay utilizing anti-rFSHβ-57371658 and hFSHβ-2 as the standard and hormone for iodination. Values for this assay are expressed in hFSHβ-2 equivalents and are termed 'FSHβ' herein.

**Calculations and statistics**

Native gonadotropins (α-β dimers) and uncombined subunits were defined by their elution position during gel filtration as well as immunoreactivity. Elution profiles consistently possessed two peaks of immunoreactive rLHα and rLHβ, with the first peak corresponding to the elution position of rLH. The nadir of these two peaks was used to define the point separating cross-reacting native rLH from the uncombined subunits. Because there was not always baseline resolution, the values reported could be subject to slight errors in either direction. Nonetheless, much more reliable estimates are obtained by this approach than by applying the subunit assays directly. In Figs. 1–5, the values included in the integration for each hormone are denoted by open circles.

Molar ratios of subunits and native gonadotropins were calculated under the following assumptions: 1. the standards employed in the radioimmunoassays were relatively pure; 2. there were not differential losses of native gonadotropins and subunits during extraction and gel filtration, and 3. molecular weights of the uncombined subunits were approximately one-half those of the native hormone. Estimates for the limiting FSHβ molar ratios were calculated using the limit of detectability in the FSHβ radioimmunoassay, the average rFSH measured in the particular group under consideration, and the assumption that FSHβ, if present, would elute in approximately ten fractions during gel filtration. The latter assumption corresponds to the
typical number of fractions in which rFSH, rLH or rLH subunits typically eluted during gel filtration. Statistical significance was established by t-tests. A probability of less than 0.05 was considered statistically significant.

Results

**Molecular forms of rLH and rFSH**

As judged by gel filtration under analytical conditions, the only molecular forms of rLH and rFSH released into the medium during an in vitro incubation appeared to be the native hormones and uncombined rLH subunits (Fig. 1). No immunoreactive FSHβ was found in the subunit region even though the rFSH radioimmunoassay recognizes FSHβ (Grotjan et al. 1985). In tissue (Figs. 2 and 4) and pellet extracts (Figs. 3 and 5), both native gonadotropins and uncombined rLH subunits were also present. In addition, there appeared to be large molecular weight rLH-like materials which were recognized in the rLHβ, rLH and rLHa radioimmunoassays. These were most obvious in pellet extracts prepared from incubated pituitaries (Fig. 3). The large molecular weight rLH-like substances did not elute in the V₀ but eluted between the V₀ and native rLH.

![Figure 1](https://example.com/figure1.png)

**Fig. 1.**

Sephadex G-100 Superfine elution profile of immunoreactive rLHβ, rLH, rLHa and rFSH released into the medium during a 4-h incubation by 4 rat anterior pituitaries. Columns of 1.5–1.6 × 90 cm were eluted at 3–4 ml/h and 2 ml fractions were collected. The arrows denote the elution positions of blue dextran (V₀), bovine serum albumin (BSA), ovalbumin (OV), chymotrypsinogen A (CA), ribonuclease A (RA) and 125I (V₁), respectively. Panel A: omission of GnRH. Panel B: addition of 10⁻⁷ mol/l GnRH. The values integrated to obtain estimates of native gonadotropins and uncombined subunits (Table 1) are denoted by open circles. Similar elution profiles were obtained for two other samples (data not illustrated).
FSHβ, quantitated in selected sample, was generally below the detectable limit of the assay in pellet extracts (Figs. 3 and 5). When present in sufficient quantities to be detected, all of the immunoreactive FSHβ eluted in the position of native rFSH (Fig. 4). These observations suggest that the intracellular levels of uncombined FSHβ, at least as measured herein, are extremely low.

**Gonadotropins and rLH subunits released**

Under basal conditions, rat anterior pituitaries incubated in vitro released relatively small quantities of native gonadotropins and uncombined rLH subunits (Table 1, Fig. 1). GnRH significantly increased the release of rLHβ (P < 0.01), rLH (P < 0.05) and rFSH (P < 0.05). The increased amount of rLHα released in response to GnRH approached, but did not reach, statistical significance (P = 0.07).

In order to determine if GnRH caused the selective release of native gonadotropins and/or uncombined rLH subunits, the data were expressed as approximate molar ratios (Table 2). GnRH did not significantly alter any of the molar ratios examined except that it appeared to decrease the rLHα/rFSH molar ratio (P < 0.05).

**Intracellular gonadotropins and subunits**

In incubated pituitaries, GnRH did not significantly alter the quantities of rLHβ, rLH, rLHα or rFSH present in tissue and pellet extracts (Table 1). Similarly, the total amounts of these hormones in the incubates (medium + tissue extract + pellet extract) were not significantly altered by GnRH.

Incubated pituitaries (Table 1) contained smaller amounts of native gonadotropins and subunits than those which had not been incubated (Table 3). In previous experiments we observed that large quantities of gonadotropins are non-specifically released during the pre-incubation period (Grotjan, unpublished data) which is analogous to findings in perifused rat pituitaries (for example, FSHβ and LHβ were not detected in the peripheral circulation of animals injected with exogenous GnRH). The relative amounts of gonadotropins released in incubated pituitaries, therefore, are likely to underestimate the amounts released in vivo.

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**Fig. 2.**

Sephadex G-100 Superfine elution profile of immunoreactive rLHβ, rLH, rLHα and rFSH released in a PBS-EDTA extract (100,000 x g supernatant) of 4 rat anterior pituitaries incubated 4 h in vitro. The values integrated to obtain estimates of native gonadotropins and uncombined subunits (Table 1) are denoted by open circles. Remaining experimental details similar to Fig. 1. Similar elution profiles were obtained for two samples (data not illustrated).
Stern & Conn 1981). Thus, the difference in intracellular quantities between intact and incubated pituitaries appear to be the result of this phenomena. When expressed in relative terms (i.e. molar ratios), it appeared that the intracellular rLHp/rLH molar ratios were not markedly altered by incubation (Tables 2 and 4). There were slight changes in the relative amounts of rLHa and native gonadotropins although these followed the same general pattern: there was always an excess of uncombined rLHa, and rLH was always present in greater relative amounts than rFSH.

FSHβ, as judged by elution position in gel filtration and immunoreactivity, was consistently below the detectability limit of the FSHβ radioimmunoassay. In pellet extracts of incubated pituitaries this translated to a FSHβ/rFSH molar ratio of < 0.13 (Table 2), while in non-incubated pituitaries the FSHβ/rFSH molar ratio in pellet extracts was < 0.022 (Table 4). In tissue extracts as well as the composite (calculated from the total hormones measured), the FSHβ/rFSH molar ratio in non-incubated pituitaries was < 0.0025 (Table 4).

**Discussion**

Optimum conditions for the extraction of gonadotropins

There is not general agreement on the optimum conditions for the extraction of hormones from pituitary tissue (Haghi & Aoki 1981; McIntosh & McIntosh 1983). A common approach is to homogenize the tissue in a buffered salt solution such as PBS-EDTA, freeze-thaw and clarify the extract by centrifugation (Wakabayashi & Tamaoki 1965). As clearly demonstrated herein, preparation of pituitary extracts in this manner does not quantitatively solubilize the gonadotropins, because significant amounts of rLH, uncombined rLH subunits and some rFSH were present in the parti-

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**Fig. 3.**

Sephadex G-100 Superfine elution profile of immunoreactive rLHp, rLH, rLHa, rFSH and FSHβ in the pellet extract (100 000 x g supernatant of a 0.5% Triton X-100 extract of the 100 000 x g pellet of pituitaries homogenized in PBS-EDTA) of 4 rat anterior pituitaries incubated 4 h in vitro. Note that no peak of FSHβ was detected. The values integrated to obtain estimates of native gonadotropins and uncombined subunits (Table 1) are denoted by open circles. Remaining experimental details similar to Fig. 1. Similar elution profiles were obtained for two samples (data not illustrated).
Sephadex G-100 Superfine elution profile of immunoreactive rLHβ, rLH, rLHa, rFSH and FSHβ in the pituitaries of 2 intact male rats extracted with PBS-EDTA and centrifuged at 100,000 × g for 1 h. Note that all of the immunoreactive FSHβ eluted in the position of native rFSH. The values integrated to obtain estimates of native gonadotropins and uncombined subunits (Table 2) are denoted by open circles. Remaining experimental details similar to Fig. 1. Two other extracts yielded similar results (data not illustrated).

Fig. 4.

Sephadex G-100 Superfine elution profile of immunoreactive rLHβ, rLH, rLHa, rFSH and FSHβ in the pellet extract (100,000 × g supernatant of a 0.5% Triton X-100 extract of the 100,000 × g pellet of pituitaries homogenized in PBS-EDTA). Note that there was not a distinguishable peak of immunoreactive FSHβ. The values integrated to obtain estimates of native gonadotropins and uncombined subunits are denoted by open circles. Remaining experimental details similar to Fig. 1. Two other extracts yielded similar results (data not illustrated).

Fig. 5.

culate fraction after centrifugation at 100,000 × g (Tables 1 and 3). It is possible that a larger percentage of the gonadotropins might be present in supernatants prepared by centrifugation at slower speeds. Whether the gonadotropins present in the 100,000 × g pellets were precipitated or were bound within membrane vesicles (present in the tissue and/or formed as a result of homogenization) cannot be fully deduced from the present experiments. However, one would anticipate that the gonadotropins would be fully soluble in PBS-EDTA. The observations that the pelleted gonadotropins could be solubilized by including 0.5% Triton X-100, a non-ionic detergent, in the PBS-EDTA and that the molar ratios of uncombined rLH subunits were elevated (perhaps denoting less mature forms) in pellet extracts supports the view that the hormones were membrane-
Table 1.
Quantities of uncombined gonadotropin subunits and native gonadotropins within and released by rat anterior pituitaries incubated in vitro (mean ± SEM).

<table>
<thead>
<tr>
<th>GnRH1</th>
<th>Medium</th>
<th>N</th>
<th>Tissue extract</th>
<th>N</th>
<th>Pellet extract (%)2</th>
<th>N</th>
<th>Total</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>rLHβ</td>
<td>−</td>
<td>3</td>
<td>22.2 ± 4.2</td>
<td>3</td>
<td>40.7 ± 6.0 (61 ± 7)</td>
<td>2</td>
<td>66.6 ± 2.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>28.0 ± 4.7</td>
<td>3</td>
<td>27.4 ± 7.9 (54 ± 3)</td>
<td>4</td>
<td>65.3 ± 12.7</td>
<td>2</td>
</tr>
<tr>
<td>rLH</td>
<td>−</td>
<td>3</td>
<td>526 ± 79</td>
<td>3</td>
<td>476 ± 214 (39 ± 8)</td>
<td>2</td>
<td>1170 ± 312</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>534 ± 72</td>
<td>3</td>
<td>424 ± 244 (37 ± 9)</td>
<td>3</td>
<td>1460 ± 500</td>
<td>2</td>
</tr>
<tr>
<td>rLHa</td>
<td>−</td>
<td>3</td>
<td>149 ± 38</td>
<td>3</td>
<td>205 ± 96 (45 ± 9)</td>
<td>2</td>
<td>429 ± 129</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>115 ± 12</td>
<td>3</td>
<td>146 ± 46 (48 ± 3)</td>
<td>3</td>
<td>382 ± 62</td>
<td>2</td>
</tr>
<tr>
<td>rFSH</td>
<td>−</td>
<td>3</td>
<td>571 ± 62</td>
<td>3</td>
<td>50 ± 14 (7 ± 1)</td>
<td>2</td>
<td>705 ± 140</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>541 ± 59</td>
<td>3</td>
<td>38 ± 15 (6 ± 1)</td>
<td>3</td>
<td>870 ± 189</td>
<td>2</td>
</tr>
<tr>
<td>FSHβ</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>= 0 (ND)</td>
<td>2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>= 0 (ND)</td>
<td>3</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

1 Denotes the addition (+) or omission (−) of 10⁻⁷ mol/l GnRH.
2 The values in brackets indicate the percentage of total hormone found in pellet extracts.
* Significantly different (P < 0.05) from the corresponding samples incubated in the absence of GnRH.
ND: not determined.

Table 2.
Approximate molar ratios of uncombined gonadotropin subunits and native gonadotropins within and released by rat pituitaries incubated in vitro (mean ± SEM).

<table>
<thead>
<tr>
<th>GnRH1</th>
<th>Medium</th>
<th>N</th>
<th>Tissue extract</th>
<th>N</th>
<th>Pellet extract</th>
<th>N</th>
<th>Composite</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>rLHβ/</td>
<td>−</td>
<td>3</td>
<td>0.09 ± 0.01</td>
<td>3</td>
<td>0.20 ± 0.06</td>
<td>2</td>
<td>0.12 ± 0.03</td>
<td>2</td>
</tr>
<tr>
<td>rLH</td>
<td>+</td>
<td>2</td>
<td>0.11 ± 0.02</td>
<td>3</td>
<td>0.19 ± 0.05</td>
<td>3</td>
<td>0.09 ± 0.01</td>
<td>2</td>
</tr>
<tr>
<td>rLHα/</td>
<td>−</td>
<td>3</td>
<td>0.55 ± 0.08</td>
<td>3</td>
<td>0.85 ± 0.02</td>
<td>2</td>
<td>0.73 ± 0.02</td>
<td>2</td>
</tr>
<tr>
<td>rLH</td>
<td>+</td>
<td>2</td>
<td>0.44 ± 0.05</td>
<td>3</td>
<td>0.97 ± 0.25</td>
<td>3</td>
<td>0.56 ± 0.11</td>
<td>2</td>
</tr>
<tr>
<td>rLHa/</td>
<td>−</td>
<td>3</td>
<td>0.27 ± 0.05</td>
<td>3</td>
<td>0.76 ± 0.03</td>
<td>2</td>
<td>0.45 ± 0.03</td>
<td>2</td>
</tr>
<tr>
<td>(rLH + rFSH)</td>
<td>+</td>
<td>2</td>
<td>0.22 ± 0.02</td>
<td>3</td>
<td>0.86 ± 0.20</td>
<td>3</td>
<td>0.34 ± 0.05</td>
<td>2</td>
</tr>
<tr>
<td>rLHa/</td>
<td>−</td>
<td>3</td>
<td>0.52 ± 0.12</td>
<td>3</td>
<td>7.67 ± 1.61</td>
<td>2</td>
<td>1.19 ± 0.13</td>
<td>2</td>
</tr>
<tr>
<td>rFSH</td>
<td>+</td>
<td>2</td>
<td>0.43 ± 0.03</td>
<td>3</td>
<td>8.09 ± 0.66</td>
<td>3</td>
<td>0.89 ± 0.05</td>
<td>2</td>
</tr>
<tr>
<td>FSHβ/</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>= 0 (&lt;0.10)</td>
<td>2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>= 0 (&lt;0.13)</td>
<td>3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>rFSH/</td>
<td>−</td>
<td>3</td>
<td>1.11 ± 0.13</td>
<td>3</td>
<td>0.12 ± 0.02</td>
<td>2</td>
<td>0.61 ± 0.04</td>
<td>2</td>
</tr>
<tr>
<td>rLH</td>
<td>+</td>
<td>2</td>
<td>1.02 ± 0.03</td>
<td>3</td>
<td>0.12 ± 0.02</td>
<td>3</td>
<td>0.62 ± 0.08</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Denotes the addition (+) or omission (−) of 10⁻⁷ mol/l GnRH.
* Significantly different (P < 0.05) from the corresponding samples incubated in the presence of GnRH.
ND: not determined.
bound. Detergents at these concentrations have previously been demonstrated to release the contents of pituitary secretory granules (Moriarty et al. 1983) and other microsomes (Redman & Cherian 1972; Kreibich & Sabatini 1974). Although detergents in the homogenization buffer can potentially dissociate rLH (Keel & Grotjan 1987), it would appear that adding low concentrations to the homogenization buffer may yield more quantitative extraction of the gonadotropins from pituitary tissue.

Molecular forms of the gonadotropins
Evidence from cell-free translations of rat anter-
or pituitary mRNAs suggests that rLH and rFSH are synthesized from pre-subunits which have their leader sequences cleaved co-translationally (Godine et al. 1980; Counis et al. 1981, 1982). However, large molecular weight forms of the gonadotropins which generally elute in the V₀ during gel filtration have been observed in extracts prepared from intact pituitary tissue (Liu & Jackson 1981; Chowdhury et al. 1982). In the present study, no discernible peaks of immunoreactive gonadotropins were found in the V₀. It should be noted that all samples were subjected to ultracentrifugation prior to gel filtration which substantially reduces or eliminates immunoreactive materials eluting in the V₀ (Grotjan, unpublished observation). Nonetheless, tissue and pellet extracts consistently contained large molecular weight rLH-like materials eluting between the V₀ and native rLH. The significance of large molecular weight rLH-like substances has not been determined but they could simply be aggregated gonadotropins and/or subunits (Liu & Jackson 1981; Weintraub et al. 1985). Such aggregates would not be recognized in cell-free translation studies because the products are analysed under denaturing conditions.

Table 3.
Quantities of uncombined gonadotropin subunits and native gonadotropins in pituitary extracts of intact male rats (mean ± SEM).

<table>
<thead>
<tr>
<th>Hormone/pituitary, ng</th>
<th>Tissue extract</th>
<th>Pellet extract</th>
<th>Total</th>
<th>Percent in pellet extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>rLHβ</td>
<td>241 ± 42</td>
<td>87 ± 17</td>
<td>328 ± 54</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>rLH</td>
<td>4990 ± 400</td>
<td>826 ± 34</td>
<td>5810 ± 390</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>rLHα</td>
<td>1770 ± 2</td>
<td>574 ± 162</td>
<td>2340 ± 300</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>rFSH</td>
<td>1980 ± 240</td>
<td>227 ± 67</td>
<td>2210 ± 300</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>FSHβ</td>
<td>~0</td>
<td>~0</td>
<td>~0</td>
<td>~0</td>
</tr>
</tbody>
</table>

Table 4.
Approximate molar ratios of uncombined gonadotropin subunits and native gonadotropins in pituitary extracts of intact male rats (mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Tissue extract</th>
<th>Pellet extract</th>
<th>Composite</th>
</tr>
</thead>
<tbody>
<tr>
<td>rLHβ/rLH</td>
<td>0.10 ± 0.01</td>
<td>0.21 ± 0.05</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>rLHα/rLH</td>
<td>0.72 ± 0.05</td>
<td>1.42 ± 0.42</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>rLHα/(rLH + rFSH)</td>
<td>0.52 ± 0.04</td>
<td>1.07 ± 0.26</td>
<td>0.59 ± 0.02</td>
</tr>
<tr>
<td>rLHα/rFSH</td>
<td>1.89 ± 0.20</td>
<td>5.08 ± 0.13</td>
<td>2.16 ± 0.14</td>
</tr>
<tr>
<td>FSHβ/rFSH</td>
<td>~0 (&lt; 0.0025)</td>
<td>~0 (&lt; 0.022)</td>
<td>~0 (&lt; 0.0023)</td>
</tr>
<tr>
<td>rFSH/rLH</td>
<td>0.40 ± 0.02</td>
<td>0.28 ± 0.09</td>
<td>0.38 ± 0.03</td>
</tr>
</tbody>
</table>

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A heterologous radioimmunoassay was constructed and characterized to quantify FSHβ (Grotjan et al. 1985). In addition, the rFSH radioimmunoassay employed strongly reacts with FSHβ (Grotjan et al. 1985). Neither of these assays detected significant amounts of immunoreactive FSHβ eluting in the range of gonadotropin subunits when pituitary extracts had been subjected to gel filtration. In contrast, we have observed detectable levels of FSHβ in extracts of cultured rat anterior pituitary cells greater than can be accounted for by the cross-reaction of rFSH in the FSHβ assay (Grotjan et al. 1985). The reason for this difference between intact pituitary tissue and cultured cells is not readily apparent. Unfortunately, immunoreactive FSHβ is not present in sufficient concentrations in extracts of cultured pituitary cells to be analysed by the gel filtration system used herein.

Relative amounts of uncombined gonadotropin subunits released and present in pituitary extracts

A variety of glycoprotein hormone secreting tissues 1. produce and release an excess of the common α subunit; 2. produce and release minimal quantities of uncombined β subunits, and 3. respond to stimulation by releasing larger absolute amounts of uncombined subunits (see Grotjan et al. 1984 for references). In this study, rat hemi-pituitaries were incubated in vitro in order to compare their responses with those obtained with primary cultures. Under these conditions, the tissue is subjected to a minimum of mechanical or enzymatic disruptions.

Similar to rat anterior pituitary cell cultures (Grotjan et al. 1984), incubated rat anterior pituitaries (present study) produce and release an excess of the common α subunit. GnRH increases the absolute amount of rLHα released but does not appear to alter the rLHα/rLH or rLHα/rFSH molar ratios. Pituitary cells cultured in the absence of GnRH exhibited considerably higher rLHα/rLH molar ratios in the medium than incubated pituitaries. Perhaps a portion of this difference is due to the removal of negative feedback inhibition during the 4-6 day culture period. Both cultures (Grotjan et al. 1984) and rat anterior pituitaries incubated in vitro (present study) release a small amount of uncombined rLHβ. In both cases, GnRH increased the absolute amount of rLHβ released but did not appear to alter the percentage of uncombined rLHβ found in the medium (i.e. did not alter the rLHβ/rLH molar ratio). Pituitaries from intact, adult, male rats appear to have slightly higher intracellular rLHβ/rLH molar ratios than those found in cultures.

The FSHβ radioimmunoassay was not applied to medium samples subjected to gel filtration because of the extremely low levels of FSHβ observed in tissue extracts. Any uncombined FSHβ in the medium would have likely been derived from dissociation of native FSHβ rather than secretion per se. Furthermore, if there was uncombined FSHβ in the medium it should have reacted in the rFSH assay. On the basis of these considerations, it is assumed that incubated pituitaries, like cultures (Grotjan et al. 1985), release extremely small quantities of uncombined FSHβ, if any.

Recent studies have suggested that the subunits of the glycoprotein hormones combine early in the biosynthetic process (Hoshina & Boime 1982; Magner & Weintraub 1982). The data presented herein, especially with regard to rFSH, are consistent with this hypothesis and it appears that almost all of the gonadotropin β subunits exist in association with α subunits. Thus, α-β dimerization must be an efficient process in the pituitary.

If rLH and rFSH are synthesized in the same cell type (Childs et al. 1983) and if the two gonadotropins are synthesized, stored and secreted by similar mechanisms, then the question arises as to why some uncombined rLHβ, but not uncombined FSHβ, is released and is present intracellularly. Perhaps the difference is attributable to the relative stability of the native hormones. It is known that rLH is fairly easy to dissociate (Ward et al. 1971; Keel & Grotjan 1987). Thus, it is possible that the uncombined rLHβ found in the medium may not have been released in the uncombined form but may have been derived from the dissociation of native rLH.

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References


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