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The equine LH/CG β subunit combines divergent intracellular traits of the human LH β and CG β subunits

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Abstract

The pituitary LH β and placental CG β subunits are products of different genes in primates. The major structural difference between the two subunits is in the carboxy-terminal region, where the short carboxyl sequence of hLH β is replaced by a longer O-glycosylated carboxy-terminal peptide (CTP) in hCG β . In association with this structural deviation, there are marked differences in the secretion kinetics and polarized routing of the two subunits. In equids, however, the CG β and LH β subunits are products of the same gene expressed in the placenta and pituitary (eLH/CG β), and both contain a CTP. This unusual expression pattern intrigued us and led to our study of eLH/CG β subunit secretion by transfected CHO and MDCK cells. In continuous labeling and pulse chase experiments, the secretion of the eLH/CG β subunit from the transfected CHO cells was inefficient (medium recovery of 16–25%) and slow ($t_{1/2}$ >6.5 hrs). This indicated that, the secretion of the eLH/CG β subunit resembles that of hLH β rather than hCG β . In MDCK cells grown on Transwell filters, the eLH/CG β subunit was preferentially secreted from the apical side, similar to the hCG β subunit secretory route (~65% of the total protein secreted). Taken together, these data suggested that secretion of the eLH/CG β subunit integrates features of both hLH β and hCG β subunits. We propose that the evolution of this intracellular behavior may fulfill the physiological demands for biosynthesis of the eLH/CG β subunit in the pituitary as well as in the placenta.

Keywords

LH β ; CG β ; evolution; secretion; carboxyl-terminal peptide (CTP)

1. Introduction

The gonadotropins, luteinizing hormone (LH) and chorionic gonadotropin (CG), are members of the glycoprotein hormone family and each is a non-covalent heterodimer composed of a common α and a hormone-specific β subunit [1]. While LH is generically

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expressed in the vertebrate pituitary, CG is synthesized in large quantities by only primate and equid placentas [1,2] [3,4]. In primates, the LH β and CG β subunits are products of different genes, which are efficiently expressed in gonadotropes and trophoblasts, respectively [1,5,6]. In equids a single gene encodes the LH β and CG β subunits in both cell types (known in the horse as eLH/CG β) and both subunits have the same amino acid sequence [7–9]. Together with the α subunit, the eLH/CG β gene is expressed in the pituitary to synthesize eLH and in the placenta to produce eCG (also known as pregnant mare serum gonadotropin, PMSG) [9,10]. Unlike the case of the hLH β and hCG β subunits, the intracellular behavior of the eLH/CG β subunit, which is critical to the biosynthesis and secretion of the hormone, is not well characterized. The aim of the current study is to examine the secretion of eLH/CG β from transfected cells. We examined the intracellular behavior of the subunit eLH/CG β primarily in comparison to that of the hCG β subunit because both have a similar carboxy-terminal extension, which distinguishes them from the other gonadotropin subunits (see below).

The CG β genes have presumably evolved from an ancestral LH β gene by different frame shift mutations in primates and equids that resulted in the elongation of the reading frame [5,6,9]. In humans, there is more than 80% identity in the first 114 amino acid residues of the LH β and CG β subunits, and a major difference between the two subunits is the replacement of the short, hydrophobic heptapeptide at the carboxyl end of LH β with a longer hydrophilic carboxy-terminal peptide domain in CG β (known as the CTP, see Fig. 1) [1,5,6]. In the horse, the pituitary LH β and placental CG β subunits share the same amino acid sequence, differ in their carbohydrate structures and both have a CTP domain [9–11]. The extended stretch is rich with serine, threonine and proline residues and has multiple O-linked glycans; 4 in the human (h)CG β and 12 in the eLH/CG β [1,12]. Functionally, the CTP contains determinants for enhanced stability in the serum without interfering with receptor binding, and both LH and CG activate a common receptor in the ovary [1,13] [10] [14–18].

Despite the similarities between the human LH and CG β -subunits, the storage and secretion profiles of heterodimeric LH and CG differ. *In vivo*, LH is stored in secretagogue-sensitive granules [19,20]. In addition, there are data showing it is secreted from the basolateral surface of the pituitary gonadotropes [19] [21–23]. However, hCG is not stored in the trophoblasts and is constitutively released into the maternal circulation. The release of hCG is mainly through the villi at the apical side of these cells [24–27]. Since the α subunit is common to both hormones, differences in the intracellular behavior of each hormone are presumed to be related to the specific β subunit.

Substantial differences in the secretion of the hLH β and hCG β subunits, and their respective heterodimers, were shown *in vitro*, in primary pituitary and placenta cell cultures, and a variety of transfected cells ([27] [28] [29] and references therein). One of the most prominent differences is in the secretion kinetics; whereas the secretion of the hLH β subunit from transfected cells is slow and inefficient, that of the hCG β subunit is rapid and quantitative [30,31] [32] [33]. It was found that the heptapeptide of the hLH β subunit is involved in the intracellular retention of the protein and that the CTP stretch of hCG β contains determinants for secretion [15,31,32].

In recent years, Boime and his colleagues used Madin-Darby canine kidney (MDCK) cells to study polarized secretion of human gonadotropins. Using this model they identified sorting determinants in the β subunit of the human hormones [34,35]. While the secretion of the hLH β subunit and heterodimeric LH from transfected MDCK cells was mainly basolateral, hCG β and hCG were mostly routed to the apical secretion pathway [34] [35]. That study suggested the O-glycosylated CTP domain is a major factor for the secretion of hCG into the maternal blood [34].

The distinctions in the intracellular behavior of the human LH and CG β subunits seem beneficial for the corresponding hormone biosynthesis in the gonadotropes and the trophoblasts, which have different tasks and cellular properties. Here, we examined whether the secretion kinetics and routing of the eLH/CG β subunit from transfected cells are strictly hLH β - or hCG β -like, or combine characteristics of both. This is especially intriguing because the eLH/CG β subunit has a CTP domain and the gene is expressed both in the pituitary and the placenta, as part of reproductive endocrinology in mares. The data suggest that divergent characteristics of pituitary hLH β and placental hCG β subunits are combined in the eLH/CG β subunit. This implies that the secretion of the eLH/CG β subunit is distinct from that of the hLH β and hCG β subunits. We propose that equid LH/CG β subunit characteristics developed from the expression of this CTP-containing subunit gene in two organs.

2. Materials and methods

2.1 Materials

Media and reagents for tissue culture were from Biological Industries (Beit Haemek, Israel), except F-12 Ham's medium (Invitrogen Life Technologies, USA). Reagents used for cell transfection were Polyethylenimine (PEI) (Sigma, USA) and Fugene-6 (Roche, Germany). [³⁵S]-cysteine plus methionine mixture (Pro-mix) was purchased from Amersham Biosciences (UK). Antiserum against the human CG β subunit was a generous gift from Dr. I. Boime (Washington University, St. Louis, MO, USA) and the equine LH/CG β antiserum was previously raised against the eLH/CG β subunit (that also contained small amount of the equine α subunit) [36]. Antiserum raised against ovine LH β subunit was obtained by courtesy of Dr. A. Parlow (National Hormone and Pituitary Program, Torrance, CA). Normal Rabbit Serum (NRS) and Pansorbin were purchased from Calbiochem and Sigma, respectively.

2.2 Methods

2.2.1 Transfection and cell labeling—Plasmids containing the eLH/CG β cDNA and the hCG β gene were stably transfected into CHO and MDCK (strain II) cells, with Fugene-6 or polyethyleneimine [36,37] (PEI). The CHO cells expressing the human and bovine LH β subunits were described previously [38]. Each transfection was repeated twice and either pooled cells (CHO cells) or two clones (MDCK cells) resistant to G418 (0.250 mg/ml for CHO cells or 0.5 mg/ml for MDCK cells) were analyzed. The transfected cells were maintained in Ham's F-12 medium (CHO cells) or Minimum Essential medium (MEM) – Eagle's (MDCK cells) containing 5% fetal calf serum and 0.125 mg/ml (CHO) or 0.25

mg/ml (MDCK) of the neomycin analog and supplemented with penicillin (100 units/ml), streptomycin (100 µg/ml), and glutamine (2 mM).

For metabolic labeling experiments, the transfected cells were grown in 6-well plates and labeled for 18 or 24 h with Pro-mix (28 µCi/ml) in cysteine- and methionine-free media, for 24 h. Cell lysate and medium samples were immunoprecipitated with antisera raised against the human CGβ or equine LH/CGβ subunits or NRS, resolved under reduced conditions on 12.5% SDS-PAGE and autoradiographed. Following densitometry analysis, the percentage of the subunit in the cell lysate and culture media of the total protein (lysate+medium) was calculated. For pulse-chase experiments, CHO cells were grown in 12-well plates until 90–95% confluence, washed twice with PBS and preincubated for 1.5 h with cysteine- and methionine-free medium, followed by a 30-min pulse labeling in cysteine and methionine-free medium containing 100 µCi/ml Pro-mix. Subsequently, the labeled cells were washed twice with medium containing unlabeled cysteine and methionine, and chased for the indicated time. Medium recovery was calculated as the percentage of the maximal protein levels in the media out of the total protein synthesis (lysate +medium). The secretion kinetics ($t_{1/2}$) were determined as the time in which half of the maximal protein levels appeared in the medium [31] [37,39].

2.2.2 Secretion from Polarized MDCK cells—MDCK clones stably expressing the hCGβ and eLH/CGβ subunit (maintained for less than 15 passages) were grown on 4.7 cm² Transwell filters (0.4 µm pore size, Corning-Costar, USA) (2×10⁶ cells/Transwell) to confluence. This methodology permitted separate access to media exposed to the apical or basolateral membrane surfaces. Cells expressing hCGβ or eLH/CGβ were metabolically labeled with [³⁵S]-cysteine (25 µCi/ml) for 24 h. Equivalent samples of apical and basolateral media were immunoprecipitated with hCGβ or eLH/CGβ antiserum, the reduced proteins resolved on 12.5% SDS-PAGE and autoradiographed. Following densitometry analysis, the total secretion into the medium of the apical and basolateral sides was combined and taken as 100%, and the distribution (%) between the two compartments was calculated. Each experiment was repeated at least three times, as indicated in the tables (in parenthesis) and figure legends. The results are expressed as mean±SD, and the data were analyzed by Student's *t* test.

3. Results

3.1 Expression and secretion of the equine LH/CGβ subunit in CHO cells

We expressed the eLH/CGβ subunit in CHO cells to examine whether the secretion of the protein is quantitative (similar to hCGβ secretion) or inefficient (analogous to LHβ subunit secretion). First, we examined the appearance of the intracellular and secreted subunits by SDS-PAGE. The cells were metabolically labeled, cell lysates and media collected, immunoprecipitated and separated on gels under reduced conditions. The eLH/CGβ subunit was recognized by the eLH/CGβ antiserum (Fig. 2A, lanes 1,2) and to a lesser extent by the hCGβ antiserum (lanes 3,4), and, as expected, was not precipitated with normal rabbit serum (NRS) (lanes 5,6). The intracellular form of the equine LH/CGβ subunit appeared as a discrete band with an apparent molecular mass of approximately 19 kDa, and the secreted

form as a diffuse, 24–35 kDa protein (Fig. 2A and Table 1). The appearance of the secreted forms resembles that of pituitary eLH β , which sometimes appears as two broad bands in Coomassie Blue-stained gels [17]. This is probably due to the heterogeneity of the single N-linked and multiple O-linked oligosaccharide structures of the eLH/CG β subunit. The difference between the migration speeds of the intra- and extra-cellular forms, which extended 7–16 kDa, is likely related to the processing of the N-linked oligosaccharide and the addition of the O-linked oligosaccharides to the CTP domain prior to secretion of the subunit, as has been shown for hCG [40]. The human CG β subunit was recognized by the hCG β antiserum (Fig. 2B, lanes 1+2) and to a much lesser extent by the eLH/CG β antisera (lanes 3+4) and was not precipitated with NRS (lanes 5+6). The appearance of hCG β as two bands is typical for expression of the subunit in CHO cells (one form bearing only one N-linked oligosaccharide, indicated as N1, and the higher molecular weight form indicated as N2 which bears two N-linked oligosaccharides) [41]. The subunit bands migrated as approximately 25 kDa and 35 kDa proteins in the lysate and medium, respectively (Table 1, calculated for the N2 form). The apparent MWs of the secreted equine and human subunits from the CHO cells are in accordance with previous studies of these subunits and imply that the proteins were glycosylated [31,36]. In the subsequent experiment we used the eLH/CG β antiserum to detect the equine subunit and the hCG β antiserum to study the behavior of the human subunit because of improved signals with the homologous antibody/subunit setup.

A marked difference in the secretion between the eLH/CG β and the hCG β subunits was observed (Fig. 2A and B). The equine LH/CG β subunit was mostly detected in the intracellular fraction and only some 25% of the total protein was recovered in the medium (Table 2). In contrast, the human CG β subunit was efficiently secreted, because about 80% was recovered in the medium (Table 2), as previously reported [32,41]. The inefficient secretion of the eLH/CG β subunit was analogous to the secretion of the human and bovine LH β subunits, that were readily detected in the lysate, but barely detectable in the conditioned medium of metabolically labeled transfected CHO cells (Fig. 2C).

3.2 Expression and secretion of the equine LH/CG β subunit in MDCK cells

To address whether the poor secretion yield of the equine LH/CG β subunit is unique to CHO cells or not we examined the secretion efficiency from MDCK cells (Fig. 3). The intracellular form of the equine LH/CG β subunit migrated similarly in the MDCK and CHO cells as a protein with an apparent molecular mass of 19–20 kDa (Fig. 3 and Table 1). The secreted form of the equine subunit expressed in the MDCK clones, migrated as a discreet band at an apparent molecular weight of about 34 kDa, which differed from the heterogeneous appearance in the culture media of the expressing CHO cells (24–35 kDa; see Table 1 and Fig. 2). It is well known that different tissues and cell lines decorate the same glycoprotein with different populations of glycans. For example the horse pituitary eLH β appears as a very broad band covering the molecular weight range of 20–25 kDa, while the eCG β subunit band ranges from 30–100 kDa [42]. The recovery from the medium for the eLH/CG β subunit was 17% of the total protein in MDCK (Fig. 3A). This low yield contrasted with 80% for the hCG β subunit, which was efficiently secreted from both cell lines (Fig. 3B). Taken together, these experiments suggested that there are marked differences in the secretion efficiency of the hCG β and the eLH/CG β subunits. This was

observed in both cell lines, implying that the difference in the secretion extent of the hCG β subunit and the eLH/CG β subunits was not a cell-line specific phenomenon.

3.3 Secretion kinetics of the eLH/CG β subunit

It is well documented that the secretion kinetics of the hLH β and hCG β subunits markedly differ [32,41] and that the hCG β -CTP contains determinants for secretion that are not fully characterized [15,32,41]. We next performed pulse chase experiments to compare the secretion rate of equine LH/CG β with that of human CG β , as both these subunits possess a CTP that share some similarities, but otherwise differ (Fig. 1). The secretion half-life of the *de novo* synthesized equine LH/CG β subunit is 6.6 hr, which is about 4 times slower than the 1.5 hr $t_{1/2}$ of the human CG β subunit (Fig. 4). Consistent with the continuous labeling experiments, the recovery from medium for human CG β subunit (63%) was about 4 times higher than that of the equine LH/CG β subunit (16%) (Fig. 4). Whereas the secretion of the hCG β subunit is fast and quantitative, that of the eLH/CG β is slow and inefficient. This suggests that despite the presence of the CTP, the secretion kinetics eLH/CG β subunit from CHO cells resemble those of the LH β subunit, which is well documented [41] [32].

3.4 Secretion route of the eLH/CG β subunit from MDCK cells

Boime and his colleagues showed that both hCG β subunit and heterodimeric hCG were predominantly secreted from the apical compartment of polarized MDCK cells, while the LH β subunit and LH were mainly released from the basolateral side [34] [35]. We next exploited the MDCK model to compare the secretion route of eLH/CG β with that of the hCG β subunit. MDCK clones expressing the eLH/CG β and hCG β subunits were grown on Transwell filters to confluence and then metabolically labeled for either 24 hr (eLH/CG β) or 6 hr (hCG β). The media were collected separately from the apical and basolateral compartments, immunoprecipitated and subjected to SDS-PAGE. The secretion of the eLH/CG β subunit from polarized MDCK cells was mostly from the apical side (Fig. 5A). The apical/basolateral distribution of the eLH/CG β and the hCG β subunits were nearly identical (~65/35%, Fig. 5B). The data suggest that routing of both eLH/CG β and hCG β subunits is similar in the MDCK model. About 2/3 of the synthesized protein was secreted from the apical side and 1/3 from the basolateral side of the cells.

4. Discussion

Our study suggests that the secretion of the eLH/CG β subunit from transfected cells was slow and inefficient, and that the subunit was secreted mainly from the apical side of MDCK cells. These results indicate that the intracellular behaviors of the CTP-extended β subunits of CG in humans and horses are not identical, despite their overall structural and functional similarities. This eLH/CG β anomaly compared to the hLH β and hCG β subunits is possibly related to curious distinctions between the expression sites of LH and CG in primates and equids, as further discussed below.

Slow and inefficient secretion is typical of the monomeric human glycoprotein hormone β subunits expressed in the pituitary (i.e., the LH β , FSH β and TSH β subunits). The determinants for intracellular retention of the hLH β subunit are not precisely known. Studies

with hLH β lacking the heptapeptide at the carboxy-terminus showed that this hydrophobic sequence contributes to the subunit retention [31] [32]. In contrast, the hydrophilic, O-glycosylated hCG β -CTP improves subunit secretion efficiency when fused to the hLH β and hFSH β subunits [15,31,32]. For example, Muyan et al. showed that fusing the hCG β -CTP to the hLH β subunit slightly improved the secretion kinetics from transfected CHO cells as compared to the unmodified hLH β or the subunit variant lacking the heptapeptide [32]. The secretion yield for unmodified and truncated hLH β subunit variants, was dramatically improved in the presence of the CTP (from about 7% to 30%; [32]). Despite the absence of the continuous heptapeptide stretch and the presence of a CTP domain in the eLH/CG β subunit, the data of the current study suggests that the protein was poorly secreted. The pulse-chase results imply that the secretion kinetics of eLH/CG β secretion are similar to those documented for the hLH β subunits rather than to those of the hCG β subunit [32,41], and particularly closer to those of the LH β -CTP chimera (lacking the heptapeptide) shown by Muyan et al. [32]. The results of the current eLH/CG β study are in accordance with the notion that the heptapeptide of the hLH β subunit contributes to the slow and inefficient secretion of the subunit, but it is not a discrete retention signal [32,41]. It is likely that additional determinants for retention exist in the hLH β prior to the heptapeptide and in the eLH/CG β subunit.

The CG β subunit is produced by the syncytiotrophoblast in humans and by specialized invasive trophoblast cells of the progenitor chorionic girdle that form the endometrial cups in the horse (reviewed in [1,3,43–45]). CG secretion appears to occur mainly through the villi of the human syncytiotrophoblast and also the equine endometrial cup cells [1,3,46–48]. It was shown that the CTP, and particularly the O-linked oligosaccharides of the domain, route the hCG β subunit through the apical side of polarized MDCK cells [34]. In contrast, the hLH β subunit, which lacks the CTP, and a hCG β subunit lacking the O-glycans in the CTP domain, were secreted mainly from the basolateral side of the cells [34]. In a previous study, we showed that a hCG β variant which included a CTP-like peptide lacking O-glycans in place of the natural CTP domain was secreted predominantly through the basolateral side of transfected MDCK cells, further supporting the role of the O-glycosylated CTP in apical secretion [38]. Here we observed that the secretion direction of the eLH/CG β and hCG β subunits from MDCK cells is similar, although we did not map the determinants for apical direction of the eLH/CG β subunit. Based on the conservation of the cysteine residues and the overall similarities between the eLH/CG β and hCG β subunits as related to the length, sequence and O-glycosylation, it is likely that, similar to the CTP of the hCG β subunit, the CTP domain of the horse subunit contains information for potential apical secretion. Studies with a mutated eLH/CG β subunit lacking the CTP could address this issue; basolateral secretion of such a truncated eLH/CG β subunit would support this prediction.

The ability of the O-glycosylated CTP domain to orient CG to the apical side was proposed to be a mechanism that evolved to direct hCG to the villi at the apical face of the trophoblasts and secretion into the maternal circulation to ensure sufficient levels to delay luteolysis at early stages of gestation in primates [34]. In the horse, the trophoblast-derived endometrial cups are embedded in the endometrium. The eCG-secreting cup cells are surrounded by capillaries, are not polarized and no clear apical and basolateral sides are defined [49]. Equine CG reaches the maternal blood stream primarily via a complex of

lymph sinuses, which develop beneath each cup, and the persistence of high blood concentrations between 40 and 120 days of gestation synergize with continuing release of pituitary FSH to stimulate the development of accessory luteal structures in the maternal ovaries to help maintain the pregnant state [50–54]. Hence, it appears that as part of the different placentation in equids and primates, various strategies evolved to supply high levels of CG to the maternal circulation when necessary. These include secretion through apical side of the cells in primates and release into an extensive lymph drainage system beneath the endometrial cups in the horse and other equids.

In conclusion, the primate LH β /CG β gene cluster contains a single LH β gene and several CG β genes/pseudogenes, and clear distinctions exist between the β subunits of LH and CG. The differences include, the predominant site of expression (pituitary vs. placenta), protein structure at the carboxy-terminus, O-glycosylation and circulatory survival. Apparently, these differences are advantageous to allow steroidogenesis, aimed to achieve conception (LH) and in such a case to maintain early gestation (CG) in humans. In the horse, the situation is different, and LH β and CG β are products of the same gene expressed in the pituitary and placenta and both subunits possess the CTP extension. Our data raise the question as why the intracellular behavior of the eLH/CG β subunit integrates characteristics that differ between hLH β and hCG β subunits. We propose that this is related to the presence of the CTP in both eLH β and CG β subunits and the dual organ biosynthesis of the subunits in the horse. In humans, the marked difference in the secretion of the two hormones evolved in association to the efficient expression in a single organ, LH β in the pituitary and CG β in the placenta, and the restricted presence of the CTP to the placental subunit. We hypothesize that the eLH/CG β subunit combines intracellular characteristics of both hLH and hCG β subunits in relation to the biosynthesis of the LH/CG β subunit in two organs and the presence of the CTP domain in the pituitary and placenta proteins.

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Abbreviations

The abbreviations used are

CTP	carboxyl-terminal peptide
CHO	Chinese hamster ovary
MDCK	Madin-Darby canine kidney
h	human
e	equine
NRS	normal rabbit serum

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Highlights

- Whereas hLH β and hCG β subunits are products of different genes expressed in the pituitary and placenta, respectively, a single gene encodes the eLH β and eCG β subunits (eLH/CG β) in both organs
- Unlike hCG β , but similar to the hLH β subunit, the eLH/CG β secretion from transfected cells is poor
- The eLH/CG β subunit is predominantly secreted from the apical side of MDCK cells, similar to the hCG β case
- The secretion of the eLH/CG β subunit integrates properties of hLH β and hCG β and this may relate to its biosynthesis in both placenta and pituitary

Figure 1. Comparison of hCGβ, hLHβ, and eLH/CGβ sequence C-terminal peptides

Alignment of the carboxy-terminal regions of the three subunits is shown. The sequence beyond the conserved Cys¹¹⁰ residue is marked as CTP. The dots identify O-glycosylated residues of the hCGβ and eLH/CGβ subunits, and the heptapeptide sequence of the hLHβ subunit is boxed.

Figure 2. Expression and secretion of the equine LH/CGβ subunit and the human CGβ and LHβ subunits from CHO cells

Cells expressing the different subunits were incubated for 24 h in the presence of [³⁵S]cysteine plus methionine. Cell lysate (L) and medium samples (M) were immunoprecipitated, and the reduced and heated proteins were resolved on 12.5% SDS-PAGE. A immunoprecipitation (IP) of the equine LH/CGβ (eLH/CGβ) subunit with equine LH/CGβ antiserum (anti-eLH/CGβ, lanes 1+2), hCGβ antiserum (anti-hCGβ, lanes 3+4), and normal rabbit serum (NRS, lanes 5+6). B. IP of the human CGβ (hCGβ) subunit with human CGβ antiserum (anti-hCGβ, lanes 1+2), equine LH/CGβ antiserum (anti-eLH/CGβ, lanes 3+4), and normal rabbit serum (NRS, lanes 5+6). C IP of the human (lanes 1+2; hCGβ antiserum) and bovine (lanes 3+4; ovine LHβ antiserum) LHβ subunits.

Figure 3. Expression and secretion efficiency of the equine LH/CG β and the human CG β subunits from MDCK cells

MDCK clones expressing the equine LH/CG β (A) or the human CG β (B) subunits were incubated for 24 h in the presence of [35 S]cysteine plus methionine. Cell lysate (L) and medium samples (M) were immunoprecipitated, and the reduced and heated proteins were resolved on 12.5% SDS-PAGE. Medium recovery was calculated as the percent of the subunit recovered in the media of the total protein (lysate +media). Data are presented as the mean $\pm$ S.D, and are calculated by densitometry measurements. n=4 (hCG β) and n=12 (eLH/CG β).

Figure 4. Kinetics of secretion of equine LH/CGβ and human CGβ subunits

CHO cells expressing the β subunits were pulse-labeled for 30 min, chased for the indicated times (h) in medium containing non-radioactive methionine and cysteine, immunoprecipitated with eLH/CGβ (eLH/CGβ subunit) or anti-hCGβ (hCGβ subunit) antiserum, and prepared as described under “Experimental Procedures”. Recovery was calculated as the maximal percentage of protein appearing in the media. $t_{1/2}$ is the time in which half of the maximal protein percentage appears in the medium. Data are presented as mean ± S.D and are calculated by densitometry measurements (n=3).

Figure 5. Secretion routing of the eLH/CGβ and hCGβ subunits from MDCK cells

A, cells expressing the β subunits were grown on Transwell filters to confluence and labeled for 24 h with [³⁵S]cysteine plus methionine. The media were collected separately from the apical and basolateral compartments and immunoprecipitated with eLH/CGβ or hCGβ antiserum and NRS. The proteins were resolved on 12.5% SDS-PAGE and autoradiographed. *B*, the relative percentage secretion of the eLH/CGβ and hCGβ subunits into the apical (*filled bars*) and basolateral (*empty bars*) compartments were quantified by densitometry of the autoradiographs. The total secretion into the medium of the apical and basolateral faces were combined and taken as 100%; the bars illustrate the distribution between the two compartments. Results are shown as the mean ± S.D (n>4). *p*<0.01 was considered as significantly different (asterisks).

Table 1

Apparent molecular mass (kDa)		
subunit	Lysate	Media
eLH/CG β (n=15)	18.9 $\pm$ 1.9	24.7 – 34.6
hCG β (n=12)	25.1 $\pm$ 1.8	35.5 $\pm$ 1.8
hLH β (n=3)	16.4 $\pm$ 1.4	19.0 $\pm$ 1.4

Data are presented as the mean $\pm$ S.D except that the apparent molecular mass of the eLH/CG β in the media is presented as a range. The number of experiments is indicated in brackets.

Table 2

Secretion from transfected CHO cells: lysate and media recovery (%)		
Subunit	Lysate	Media
eLH/CG β (n=12)	74.3 $\pm$ 7.0	25.6 $\pm$ 7.0
hCG β (n=6)	17.0 $\pm$ 6.0	82.7 $\pm$ 6.0
hLH β (n=3)	86.2 $\pm$ 2.2	13.2 $\pm$ 2.2

Percentage of the subunit encountered in the cell lysate and media of the total protein (lysate+media) after a 24 h of metabolic labeling. Data are presented as the mean $\pm$ S.D and are calculated by densitometry measurements. The number of experiments is indicated in brackets.