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On the Secretion and Functions of Equine Chorionic Gonadotropin

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말의 융모성 성선자극 호르몬의 분비와 기능

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ABSTRACT

In this review we have tried to summarize that the evidence indicating that equine chorionic gonadotropin (eCG) is a member of the glycoprotein hormone, which includes luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid stimulating hormone (TSH). All of these consist of two noncovalently linked α- and β-subunits. An α-subunit of 96 amino acids common to that found in other horse glycoprotein hormones and the β-subunit of 149 amino acids identical to horse LHβ. Cells from the chorionic girdle of the equine trophoblast invade the maternal endometrium at day 36 of gestation and become established as secretory elements known as the endomerial cups. These structures, which persist for 40~60 days, produce a gonadotropin which can be found in circulation until around day 130 of gestation.

The carbohydrate content of eCG, over 40% (w/w), is highest in the glycoprotein family. The α-subunit of eCG has two N-glycosylation sites (Asn 56 and Asn 82) and its β-subunit has one (Asn 13). Analysis of a purified preparation of eCG revealed that its β-subunit consists of 149 amino acids, which was confirmed by the molecular cloning of its cDNA. There seem to be at least four to six, or even as many as 11, O-glycosylation sites on the extended C-terminal region of the eCG β-subunit. Interestingly, eCG is a unique member of this family, as it appear to be a single molecule that possesses both LH- and FSH-like activities. Using the cDNA prepared from mRNA extracted from equine placental and pituitary tissues, we cloned the cDNA of eCG α- and β-subunits and eFSH β-subunit. The mRNA expression of each subunit seems to be independently regulated, which may account for differences in the quantities of α- and β-subunits in the placenta and pituitary. Thus, eCG is a distinct molecule from the view points of its biological function and glycoresidue structures.

Recombinant eCGs including the mutants which lack oligosaccharides will be useful tools for analyzing the structure-function relationships of gonadotropins in the horse as well as other species. Similar experiments will also clarify the proposed structure and biological functions for the glycoprotein hormones. These experimental are now possible, and hopefully a resolution of the existing controversy will be forthcoming in the near future.

(Key words : Equine chorionic gonadotropin, Secretion, Structure, Function)

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Ⅰ. INTRODUCTION

Equine chorionic gonadotropin (eCG), known as pregnancy mare serum gonadotropin (PMSG), has a number of interesting and unique characteristics since it appears to be a single molecule that possesses both luteinizing hormone (LH)- and follicle-stimulating hormone (FSH)-like activities in other species than the horse (Min et al., 1994, 1996, 1997). This dual activity of eCG in heterologous species is of fundamental interest to the study of the structure-function relationships of gonadotropins and their receptors. A second unusual property of eCG is its remarkable carbohydrate content (over 40% w/w) (Min et al., 1996) which is the highest in the glycoprotein hormones, and extents its persistence in vitro. eCG is of commercial value because it is readily collected and a potent agent for induction of folliculogenesis in domestic and laboratory mammals. Passeron (1978) has published a fascinating account of the commercial purification and standardization of eCG collected annually from as many as 12,000 mares.

CG is a placental hormone that maintains the corpus luteum of pregnancy. Because human CG (hCG) is expressed at high levels soon after fertilization and implantation, it is used to detect pregnancy at early stages and to monitor the progress of pregnancy during the first trimester (Jaffe et al., 1969). CG is also produced in trophoblastic disease and in a variety of different malignancies, which makes it useful as a tumor marker in these conditions (Braunstein et al., 1973).

Members of the glycoprotein family, which includes CG, LH, FSH and thyroid stimulating hormone (TSH), comprise two noncovalently linked α- and β-subunits. The α-subunits are common to these glycoproteins and the β-subunits differ among them. CG and LH β genes are different in primates (Murphy & Martinuk, 1991). In horse, however, a single gene encodes both eCG and eLH β-subunits (Min et al., 1994). The difference between eCG and eLH lies in the structure of their glycoresidues, which are both sialylated and sulfated in LH and sialylated in CG. Information on CG has been presented as part of reviews of glycoprotein hormone structure and function (Ward et al., 1982) and placental peptide hormones (Talamantes & Ogren, 1988). Brief reviews, which include aspects of CG structure (Ward & Bousfield, 1990) and glycosylation (Kamerling et al., 1990), have appeared.

The present work is an attempt to summarize recent literature. Throughout this review the data obtained from our laboratory have been used to illustrate some findings that we believe are critical in the understanding of the structrue and function of the CG.

Ⅱ. SECRETION OF CG

1. Source of CG Secretion

Domestic species with an epitheliochorial placenta include cattle, sheep, pigs and horses. While this type of placenta is associated with the least invasive type of placentation, the equine placenta is different from the epitheliochorial placenta of other domestic species because it has a discrete area consisting of a subpopulation of highly invasive trophoblast cells. This subpopulation of trophoblast cells differentiates between days 25 and 36 of pregnancy to form the chorionic girdle, a discrete narrow (9-mm) band of specialized, avascular tissue encircling the spherical conceptus (Ginther, 1979, 1992). Trophoblasts of the chorionic girdle are unique in that, by day 35, cells of the chorionic girdle adhere the uterine epithelium and begin to invade the endometrial wall (Allen & Moor, 1972; Enders & Liu, 1991). Within a 48-h period, the girdle cells aggressively migrate through the uterine epithelium into the endometrial

stroma where they form distinct nodules. At this point, the girdle cells are no longer attached to the placenta but are part of a distinct and highly differentiated tissue type buried within the endometrial stroma. These nodules are called "endomerial cups". The source of gonadotropin is the specialized trophoblast cells of the chorionic girdle, which attach, invade and phagocytose the maternal epithelium. The active cells (cup cells) produce gonadotropin, PMSG, from approximately day 40 to day 100 of pregnancy (Vagnoni et al., 1995; Min et al., 1996) (Fig. 1).

In humans, during pregnancy, trophoblasts differentiate in a multistep process that converts cytotrophoblasts into syncytiotrophoblasts (Ringler & Strauss, 1990). Cytotrophoblasts are mitotically active, mononucleated cells that fuse to form nondividing, mutinucleated syncytiotro phoblasts. As the placenta grows and matures, there is increased formation of syncytiotrophoblast cells and associated production of CG. In models of trophoblast differentiation *in vitro*, the onset of CG production appears to parallel the formation of the multinucleated cells (Kliman et al., 1986). The factors governing cell fusion and differentiation have not been identified, although a role for direct cell-cell interactions has been suggested by experiments involving induction of CG biosynthesis by co-culture of cytotrophoblasts with choriocarcinoma cells (Hochberg et al., 1991).

2. Pattern of CG Secretion

The first apperance of horse CG coincides with the migration of the specialized chorionic cells into the endometrium. The pattern of the presence of CG inserum is shown in Fig. 2 and, in general, the peak values can be observed between days 55 and 70 of gestation (Holtan et al., 1975; Nett et al.,

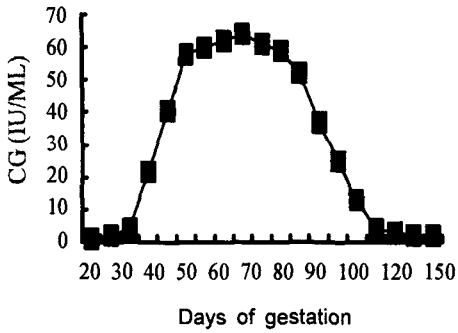

Fig. 2. The pattern of appearance of eCG in equine serum.

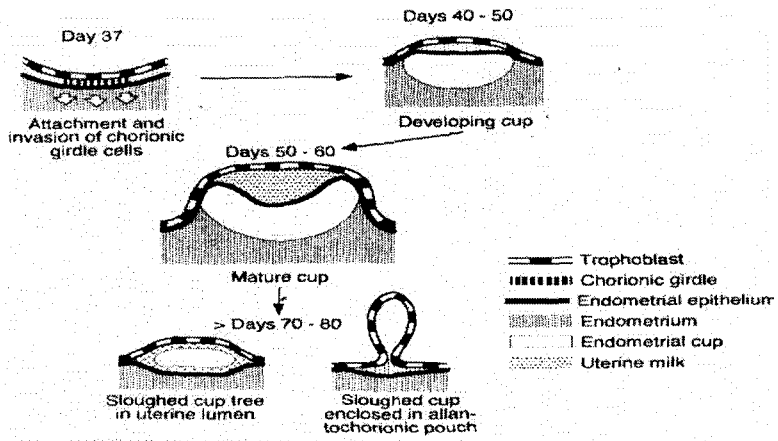

Fig. 1. Morphogenesis and demise of the endometrial cups in the mare (Reproductive biology of the mare. McNaughton and Gunn, Ann Arbor, MI, 1979).

1975).

The hormone levels decline gradually until approximately day 130, although CG has been found after day 200 of gestation (Spincemaille et al., 1975). There is pronounced individual variation between mares in the amounts of CG found in serum (Holtan et al., 1975; Murphy et al., 1985; Nett et al., 1975). RIAs that employ polyclonal antiserum have resulted in minima in the range of 300 ng/ml serum about day 40 of gestation. Maxima of as much as 35 ug/ml, or 2 orders of magnitude greater, have been found at the peak of secretion, day 50~70 (Bello et al, 1989; Martinuk et al, 1990). The pattern of apperance of CG in horse serum has been correlated with gross and histological changes in the endometrial cups (Fig. 1).

In humans, after fertilization, the embryo produces hCG at a very early stage in development (Ohlsson et al., 1989). hCG has been detected at the 6 to 8-cell stage using *in situ* hybridization techniques (Bonduelle et al., 1988), and is secreted from blastocysts by 7 days after fertilization *in vitro* (Hay & Lopata, 1988). hCG can be detected in maternal serum near the time of implantation, and it is routinely used to detect pregnancy (Fig. 3). The cellular mechanisms that lead to the onset of CG gene transcription are not understood. However,

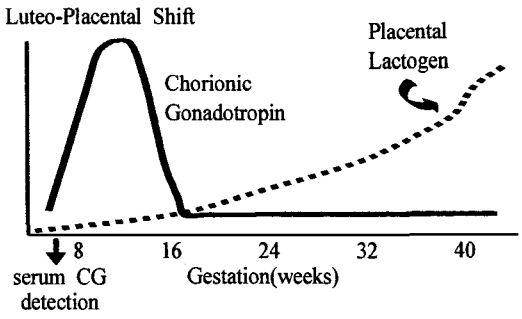

Fig. 3. Placental hormone secretion during pregnancy in human.

it is likely that the CG α- and β-genes are among the first embryo-specific genes to be transcribed. The mechanisms that result in deactivation of CG gene expression after the first trimester are also unknown.

3. Factors Regulating CG Synthesis and Secretion

Little is known about the factors that control the synthesis and secretion of the equine CG. CG secretion is likely to be a constitutive function of chorionic girdle cells (Murphy & James, 1976). Serum levels of CG in the horse do not undergo episodic changes as do pituitary gonadotropins, further suggesting a constant mode of secretion. Injection of GnRH had no effect on CG concentrations over 4 h (Thompson et al., 1982). Steroid feedback may not be important for the regulation of CG secretion. In pony mares, treatment with diethylstilbesterol, a potent synthetic estrogen, or with androgens, had no apparent effect on CG concentrations in blood samples taken weekly.

One hypothesis has been put forth to explain the long-term regulation of eCG: there is a maternal cell-mediated immunological reaction to paternal antigens in the fetal chorionic cells, resulting in cytolysis of the cup cells and consequent abrogation of CG sunthesis and secretion (Antczak & Allen, 1989). Further evidence for the hypothesis can be found. It was suspected that fetal component of the endometrial cups expresses the paternal major histocompatibility complex (MHC) antigens (Antczak & Allen, 1989). Some of the factors that regulate hCG are listed in Table 1. Because of the similarity in the structures and biological activities of LH and CG, GnRH has been investigated as a potential paracrine regulator of CG production in the placenta and low affinity GnRH receptors have also been demonstrated (Iwashita et al., 1986). GnRH is present in both cytotrophoblasts and syncytiotrophoblasts (Kelly et al., 1991), raising the possibility

Table 1. Modulators of CG synthesis and secretion

Increases CG synthesis and secretion	Decreases CG synthesis and secretion
GnRH	Inhibin
EGF	Progesterone
Activin	GnRH antagonists
Glucocorticoid	RU 486
DHEA	Dopamine
Retinoic acid	Transforming growth
Interleukin-6	factor-β
Interleukin-1	
cAMP	
Phorblo ester	
Methotrexate	

of autocrine as well as paracrine regulation. GnRH stimulates the release of CG in a dose-dependent fashion from term placenta *in vitro*, and a GnRH antagonist blocks this effect (Siler-Khodr et al., 1983, 1986). Epidermal growth factor (EGF) stimulates CG levels in both choriocarcinoma cell line and in primary cultures derived from first trimester placenta (Ritvos et al., 1988). Further CG secretion is synergistically stimulated by EGF and cAMP in JEG-3 cells, suggesting that divergent signaling pathways may act to increase CG production. Interleukins also stimulate CG release in dispersed trophoblasts from first trimester placenta. Trophoblast-derived interleukin (IL-1) and tumor necrosis factor α stimulate IL-6 release, which in turn activates the release of CG (Matsuzaki et al., 1992). This pathway is distinct from the Gnrh-mediated pathway in that transforming growth factors-β inhibited the interleukin effects on CG release without altering stimulation by GnRH.

Ⅲ. Functional roles of eCG

1. Primary Structures of Subunits

Members of the glycoprotein hormone family, which includes LH, FSH, TSH and CG, consist of two noncovalently linked α- and β-subunits. Within a given species, the α-subunit is identical, except for differences in the oligosaccharides, whereas the β-subunit is hormone-specific and contains the determinant domains for receptor specificity (Pierce & Parsons, 1981). Both subunits are required for these glycoprotein hormones and combination of the subunits is essential for the expression of their biological activities (Pierce & Parsons, 1981) (Fig. 4). Its molecular weight has been estiamted to be about 45 ~ 65 kDa, 17 kDa for the α-subunit and 44 kDa for the β-subunit (Combarnous et al., 1981). The amino-acid sequences of both eCG subunits have been determined (Sugino et al., 1987). They are exactly identical to those of eLH subunits, containing an α-subunit of 96 amino acids and β-subunit of 149 amino acids (Bousfield et al., 1985, 1987). There are five disulfide bonds in the α-subunit and six in the β-subunit (Bousfield et al., 1987; Sugino et al., 1987).

Using the cDNA prepared from mRNA extracted from equine placental and pituitary tissues, we cloned the eCG α- and β-subunits cDNA and eFSH β-subunit cDNA (Fig. 5A).

The cDNA fragment with 387 bp predicted for the eCG α-subunit was amplified. A cDNA band of 524 bp for the eCG β-subunit was also amplified using a set of eCG β primers. Computer-aided sequence data analysis showed that these encoded

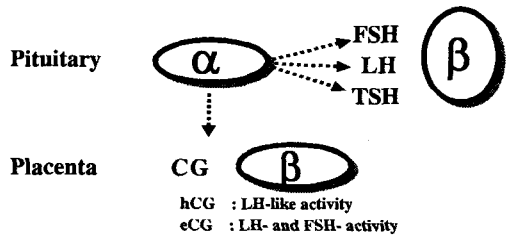

Fig. 4. Combination of subunits in the glycoprotein hormones.

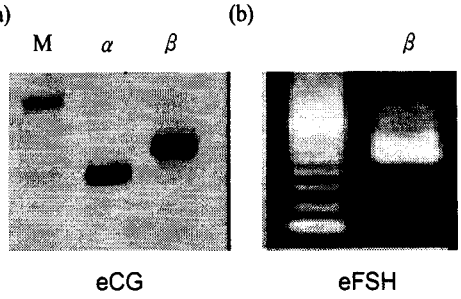

Fig. 5. PCR amplification of eCG α- and β-subunits, and eFSH β-subunit cDNA. (a) PCR amplification of the eCG α- and β-subunits. The PCR products amplified from equine placenta cDNA with each primer were analyzed by 1.0% agarose electrophoresis. The major fragments that comprised 387bp (eCG α) and 524 bp (eCG β), were amplified. M: marker. (b) PCR production of eFSH β-subunit from equine pituitary cDNA.

the eCG α-subunit including the signal peptide and mature protein. The nucleotide and deduced amino acid sequences of eCG α- and β-subunits in equine placenta are shown in Fig. 6A and B.

Comparisions of deduced amino acid sequences of eCG α- and β-subunits are shown in Fig. 7A (Fiddes & Goodman, 1979; Chin et al., 1981; Godine et al., 1982; Erwin et al., 1983; Bello et al., 1989; Golos et al., 1991) and Fig. 7B (Fiddes & Goodman, 1980; Crawford et al., 1986; Leigh & Stewart, 1990; Min et al., 1994; Simula et al., 1995).

The similarity of amino acid and nucleotide sequences of glycoprotein hormone α-and β-subunit is shown in Table 2 and Table 3. eCG α-subunit is very similar to that of the other species (74~84% at the nucleotide and 69~80% at the amino acid level). The β-subunits of the glycoprotein hormones also appear to be highly conserved with the porcine (Kato & Hirai, 1989), bovine (Virgin et al., 1985), and human (Talmadge et al., 1984) LH β-subunits, with an overall homology of 80%. The nucleotide sequence of the marmoset exhibits lower homology with the eCG β-subunit (73.7%). The analysis between the first 110 amino acids of eCG β and hCG β demonstrates a 66% homology. But, homology between the primary structures of the C-terminal tails was much lower.

We also cloned the cDNA of eFSH β-subunit from equine pituitary using the mixed primers designed from the nucleotide sequence of human, rat, and bovine FSH β-subunits (Fig. 5B). Sequence data analysis showed that cDNA encoded the eFSH β-subunit including the signal peptide region consists of 18 amino acids and mature protein of 111 amino acids (Fig. 8). The homologies of nucleotide sequences of eFSH β-subunit with bovine, human, rat, porcine and ovine one are 92.7, 93.2, 87.7, 95.0, 91.9%, respectively.

2. Secondary Structure

The eCG α-subunit has 10 half-systeine residues, the same number present in all known mammalian glycoprotein hormones (Fig. 7A). eCG β-subunit contains 12 half-cysteine residues (Fig. 7B), which is the usual number found in glycoprotein β-subunits (Bousfield et al., 1987; Sugino et al., 1987). The chem ical assignment of the disulfide pairings has been extensively studied (Ryan et al., 1988). Lapthorn et al. (1994) demonstrated the three-dimensional structure of hCG. The α-subunit contained a similar cystine knot, and for the amino-acid sequence to fit disulfide linkage 7~31, 10~60, 28~82, 32~84 and 59~87 were necessary. The disulfide pairings of β-subunit are 9~57, 23~72, 26~110, 34~88, 38~90 and 93~100. Of the linkages not previously predicted (α 10~60, 28~82, 32~84; β 9~57, 38~90), all are involved in the cystine knots (Chen & Puett, 1991) and a short form of hCG β consisting of residues 8-100 associates only weakly with α-subunit (Puett

					-24 Met	Asp	Tyr	Tyr	-20 Arg	Lys	His	Ala	Ala	Val	Ile	Leu
		AGG	AGA	GCT	ATG	GAT	TAC	TAC	AGA	AAA	CAT	GCA	GCT	GTC	ATC	CTG
		-10										1				
Ala	Thr	Leu	Ser	Val	Phe	Leu	His	Ile	Leu	His	Ser	Phe	Pro	Asp	Gly	Glu
GCC	ACA	TTG	TCC	GTG	TTT	CTG	CAT	ATT	CTC	CAT	TCC	TTT	CCT	GAT	GGA	GAG
				10										20		
Phe	Thr	Thr	Gln	Asp	Cys	Pro	Glu	Cys	Lys	Leu	Arg	Glu	Asn	Lys	Thr	Phe
TTT	ACA	ACG	CAG	GAT	TGC	CCA	GAA	TGC	AAG	CTA	AGG	GAA	AAC	AAG	TAC	TTC
							30									
Phe	Lys	Leu	Gly	Val	Pro	Ile	Tyr	Gln	Cys	Lys	Gly	Cys	Cys	Phe	Ser	Arg
TTC	AAA	CTG	GGC	GTC	CCG	ATT	TAC	CAG	TGT	AAG	GGC	TGC	TGC	TTC	TCC	AGA
40										50						
Ala	Tyr	Pro	Thr	Pro	Ala	Arg	Ser	Arg	Lys	Thr	Met	Leu	Val	Pro	Lys	Asn
GCG	TAC	CCC	ACT	CCA	GCA	AGG	TCC	AGG	AAG	ACA	ATG	TTG	GTC	CCA	AAG	AAC
			60										70			
Ile	Thr	Ser	Glu	Ser	Thr	Cys	Cys	Val	Ala	Lys	Ala	Phe	Ile	Arg	Val	Thr
ATC	ACC	TCA	GAA	TCC	ACA	TGC	TGT	GTG	GCC	AAA	GCA	TTT	ATC	AGG	GTC	ACA
						80										90
Val	Met	Gly	Asn	Ile	Lys	Leu	Glu	Asn	His	Thr	Gln	Cys	Tyr	Cys	Ser	Thr
GTG	ATG	GGA	AAC	ATC	AAG	TTG	GAG	AAC	CAC	ACC	CAG	TGC	TAT	TGC	AGC	ACA
Cys	Tyr	His	His	Lys	Ile	***										
TGC	TAT	CAC	CAC	AAG	ATT	TAA	AGG	TTT	CAC	CAA	GTG					

Fig. 6A. Nucleotide and deduced amino acid sequences of eCG α -subunit cDNA. The amino acid sequence deduced from the gene is shown above the nucleotide. The eCG α -subunit contains an open reading frame encoding 120 amino acids including a signal peptide of 24 amino acids. Amino acids are numbered from the first amino acid of the mature protein as+1.

				-20 Met	Glu	Thr	Leu	Gln	Gly	Leu	Leu	Leu	Trp	-10 Met	Leu	Leu	Ser	Val	Gly	Gly	Val
			GCA	CCG	AGG	ATG	GAG	ACG	CTC	CAG	GGG	CTG	CTG	CTG	TGG	ATG	CTG	CTG	AGT	GTT	GG(	GGG	GTC
		+1											10										20
Trp	Ala	Ser	Arg	Gly	Pro	Leu	Arg	Pro	Leu	Cys	Arg	Pro	Ile	Asn	Ala	Thr	Leu	Ala	Ala	Glu	Lys
TGG	GCA	TCC	AGG	GGG	CCA	CTG	CGG	CCA	CTG	TGC	CGG	CCC	ATC	AAC	GCC	ACT	CTG	GCT	GC]	GAG	AAG
										30										40	
Glu	Ala	Cys	Pro	Ile	Cys	Ile	Thr	Phe	Thr	Thr	Ser	Ile	Cys	Ala	Gly	Tyr	Cys	Pro	Ser	Met	Val
GAG	GCC	TGC	CCC	ATC	TGC	ATC	ACC	TTC	ACC	ACC	AGC	ATC	TGT	GCC	GGC	TAC	TGC	CCC	AG(	ATG	GTG
							50										60				
Arg	Val	Met	Pro	Ala	Ala	Leu	Pro	Ala	Ile	Pro	Gln	Pro	Val	Cys	Thr	Tyr	Arg	Glu	Leu	Arg	Phe
CGG	GTG	ATG	CCA	GCT	GCC	CTG	CCG	GCC	ATT	CCC	CAG	CCA	GTG	TGC	ACC	TAC	CGT	GAG	CT(	CGC	TTT
					70										80						
Ala	Ser	Ile	Arg	Leu	Pro	Gly	Cys	Pro	Pro	Gly	Val	Asp	Pro	Met	Val	Ser	Phe	Pro	Val	Ala	Leu
GCT	TCC	ATC	CGG	CTC	CCC	GGC	TGC	CCG	CCT	GGT	GTG	GAC	CCC	ATG	GTC	TCC	TTC	CCC	GT(	GCC	CTC
			90										100								
Ser	Cys	His	Cys	Gly	Pro	Cys	Gln	Ile	Lys	Thr	Thr	Asp	Cys	Gly	Val	Phe	Arg	Asp	Gln	Pro	Leu
AGT	TGT	CAC	TGC	GGG	CCC	TGC	CAG	ATC	AAG	ACC	ACT	GAC	TGC	GGG	GTT	TTC	AGA	GAC	CA(	CCC	TTG
	110										120										130
Ala	Cys	Ala	Pro	Gln	Ala	Ser	Ser	Ser	Ser	Lys	Asp	Pro	Pro	Ser	Gln	Pro	Leu	Thr	Ser	Thr	Ser
GCC	TGT	GCC	CCC	CAG	GCC	TCC	TCT	TCC	TCT	AAG	GAT	CCC	CCA	TCC	CAA	CCT	CTC	ACA	TC(	ACA	TCC
									140									149			
Thr	Pro	Thr	Pro	Gly	Ala	Ser	Arg	Arg	Ser	Ser	His	Pro	Leu	Pro	Ile	Lys	Thr	Ser	***		
ACC	CCA	ACT	CCT	GGG	GCC	AGC	AGA	CGT	TCC	TCT	CAT	CCC	CTC	CCA	ATA	AAG	ACT	TCT	TG/	ACT	AC

Fig. 6B. Nucleotide and deduced amino acid sequences of eCG β-subunit cDNA. The amino acid sequence deduces from the gene is shown above the nucleotide. The eCG β -subunit contains an open reading frame encoding 169 amino acid including a signal peptide of 20 amino acid. Amino acids are numbered from the first amino acid of the mature protein as +1.

			1	* *		* **	
Equine	MDYYRKHAAV	ILATLSVFLH	ILHSFPDGEF	TTQDCPECKL	RENKYFFKLG	VPIYQCKGCC	36
human	MDYYRKYAAI	FLVTLSVFLH	VLHSAPDV--	--QDCPECTL	QENPFFSQPG	APILQCMGCC	32
R.monkey	MDYYRKYAAV	ILVTLSVFLH	ILHSFPDGEF	TMQDCPECKP	RENKFFSKPG	APIYQCMGCC	36
Marmoset	MDYYRKYAAI	ILITLSVFLH	ILHSLPDGEF	TAEECPECKL	KENKYFSRLG	SPIYQCMGCC	36
Rat	MDYYKRYAAV	ILVMLSMFLH	ILHSLPDGDF	IIQGCPECKL	KENKYFSKLG	APIYQCMGCC	36
Mouse	MDYYRKYAAV	ILVMLSMFLH	ILHSLPDGDF	IIQGCPECKL	KENKYFSKLG	APIYQCMGCC	36
Bovine	MDYYRKYAAV	ILTILSLFLQ	ILHSFPDGEF	TMQGCPECKL	KENKYFSKPD	AAIYQCMGCC	36
Sheep	MDYYRKYAAA	ILAILSLFLQ	ILHSFPDGEF	TMQGCPECKL	KENKYFSKPD	APIYQCMGCC	36
			**		*	* *	
	FSRAYPTPAR	SRKTMLVPKN	ITSESTCCVA	KAFIRVTVMG	NIKLENHTQC	YCSTCYHHKI	96
	FSRAYPTPLR	SKKTMLVQKN	VTSESTCCVA	KSYNRVTVMG	GFKVENHTAC	HCSTCYYHKS	92
	FSRAYPTPVR	SKKTMLVQKN	VTSESTCCVA	KSLTRVMVMG	SVRVENHTEC	HCSTCYYHKF	96
	FSRAYPTPLR	SQKTMLVPKN	VTSESTCCVA	KAYTKATVMG	NIRVENHTEC	HCSTCYHHKF	96
	FSRAYPTPAR	SKKTMLVPKN	ITSEATCCVA	KAFTKATVMG	NARVENHTEC	HCSTCYYHKS	96
	FSRAYPTPAR	SKKTMLVPKN	ITSEATCCVA	KAFTKATVMG	NARVENHTEC	HCSTCYYHKS	96
	FSRAYPTPAR	SKKTMLVPKN	ITSEATCCVA	KAFTKATVMG	NVRVENHTEC	HCSTCYYHKS	96
	FSRAYPTPAR	SKKTMLVPKN	ITSEATCCVA	KAFTKATVMG	NVRVENHTEC	HCSTCYYHKS	96

Fig. 7A. Comparison of deduced amino acid sequences of glycoprotein hormone α-subunits. Amino acid residues are numbered from the first amino acid of the mature protein as 1. Amino acids corresponding to deleted sequences in human are shown by hyphens. Potential cysteine amino acids are indicated by asterisks.

			1 *		* *	* *	
Equine	METLQGLLLW	MLLSVGGVWA	SRGPLRPLCR	PINATLAAEK	EACPICITFT	TSICAGYCPS	40
human	MEMFQGLLLL	LLLSMGGTWA	SKEPLRPRCR	PINATLAVEK	EGCPVCITVN	TTICAGYCPT	40
Baboon	METLQGLLLW	LLLSMGGAQA	SREPLRPLCR	PINATLAAEK	EACPVCVTVN	TTICAGYCPT	40
Marmoset	MEMLQGLLLC	LLLSTGGAWA	SKEPLRPLCR	PVNAILAAEK	EGCPVCVAFN	TTICAGYCSS	40
Donkey	----------	----------	----------	----------	----------	----------	
		*		*	* *	* *	
	MVRVMPAALP	AIPQPVCTYR	ELRFASIRLP	GCPPGVDPMV	SFPVALSCHC	GPCQIKTTDC	100
	MTRVLQGVLP	ALPQVVCNYR	DVRFESIRLP	GCPRGVNPVV	SYAVALSCQC	ALCRRSTTDC	100
	MMRVLQAVLP	PVPQVVCNYR	EVRFESIRLP	GCPPGVDPMV	SVPVALSCRC	ALCRRSTSDC	100
	MVRVLQTILP	PLPQSVCNYR	ELRFTSVRLP	GCRPGVDPVV	SMPVALSCRC	GLCRRSYSDC	100
	----------	----------	----------	----------	---ALSCHC	GPCRLKTTDC	16
	*						
	GVFRDQPLAC	APQASSSSKD	PPSQPLTSTS	TPTPGASRRS	SHPLPIKTS		149
	GGPKDHPLTC	DDPRFQDSSS	SKAPPPSLPS	PSRLPGPSDT	PILPQ		145
	GGPKDHPLTC	DDPNLQASSS	SKDPPPSPPS	PSRLLEPAGT	PFLPQ		145
	GSLRDEPLGC	DYSTFQD-SS	SKDPPRNLTS	PSQLLEPADP	PLVPQ		144
	GGPRDHPLAC	APQTSSSCKD	PPSQPLTFHI	PPQLLGPADV	PLIPSQ		62

Fig. 7B. Alignment of deduced amino acid sequences of chorionic gonadotropin β-subunits. Amino acid residues are numbered from the first amino acid of the equine CG-β subunit mature protein as 1. Amino acids corresponding to deleted sequences are dashed and the portions have not cloned in a donkey CGβ shown by hyphens. Potential cysteine amino acids are indicated by asterisks. Donkey CGβ is initiated from 85 Ala.

et al., 1994). The disulfide Cys 26~110 is completed after the α /β association has occurred (Huth et al., 1992), indicating that the seat-belt region is important in maintaining the integrity of the heterodimer (Lapthorn et al., 1994).

Table 2. Structural similarity of glycoprotein α -subunit sequences (%)

	Equine	Bovine	Human	Rat	Mouse	Sheep	Marmoset
Equine		82.5	74.2	81.5	80.0	79.7	83.9
Bovine	76.8		79.0	82.8	82.9	96.7	83.7
Human	69.3	70.8		71.7	72.9	79.7	85.2
Rat	76.0	88.3	71.7		98.9	81.5	77.6
Mouse	77.7	90.0	73.3	98.3		81.5	78.7
Sheep	77.7	97.5	71.7	88.3	90.0		83.8
Marmoset	80.0	80.2	72.7	81.0	82.7	81.0	

*The similarity of amino acid and nucleotide sequences of glycoprotein α -subunit is shown. Values above the diagonal indicate nucleotide sequence similarity. Values under the diagonal indicate coding amino acid sequence similarity.

Table 3. Structural similarity of glycoprotein β -subunit sequences (%)

	eCG	hCGβ	maCGβ	baCGβ	hLHβ	bLHβ	pLHβ
eCG		76.5	73.7	77.6	81.0	84.2	86.8
hCGβ	65.0		79.8	91.2	92.7	80.4	80.6
maCGβ	65.3	69.7		82.9	80.9	78.5	78.5
baCGβ	71.4	80.9	72.9		92.1	81.7	84.6
hLHβ	63.9	87.0	77.7	87.5		82.7	84.6
bLHβ	76.7	66.2	60.9	71.3	70.2		88.9
pLHβ	86.6	60.9	73.6	78.3	74.5	85.8	

*The similarity of amino acid and nucleotide sequences of glycoprotein α -subunit is shown. Values above the diagonal indicate nucleotide sequence similarity. Values under the diagonal indicate coding amino acid sequence similarity. The names over each column represent as; CG: chorionic gonadotropin; LH: luteinizing hormone; e: equine; h: human; ma: marmoset; ba: baboon; b: bovine; p: porcine.

3. Glycosylation

The oligosaccharides on these glycoprotein hormones have been implicated in several actions, such as intracellular stability, correct protein structure folding and intracellular compartmentalization, modulation of the half-life in the peripheral circuation, subunit assembly and hormone-receptor interactions (Pierce & Parsons, 1981; Sairam & Bhargavi, 1985; Hartree & Renwick, 1992). The carbohydrate content of eCG, over 40% (w/w), is the highest in the glycoprotein family, including LH, FSH, TSH and hCG (Christakos & Bahl, 1979). The glycosylation sites on LH, FSH, and CG are illustrated in Fig. 9.

There have two N-glycosylation sites on the α-subunits of all these glycoproteins, whereas the numbers of such sites on their β-subunits differ depending on the hormone: LH β- and FSH β-subunits bear one and two sites, respectively, there are two on hCG, whereas eCG has only one. In horse, a single gene encodes both eCG and eLH β-subunits (Min et al., 1994). The difference between eCG and eLH lies in the structure of their glycoresidues, which are both sialylated and sulfated in LH and sialylated in CG (Fig. 10).

The α-subunit of eCG has two N-glycosylation sites (Asn 56 and Asn 82) and its β-subunit has

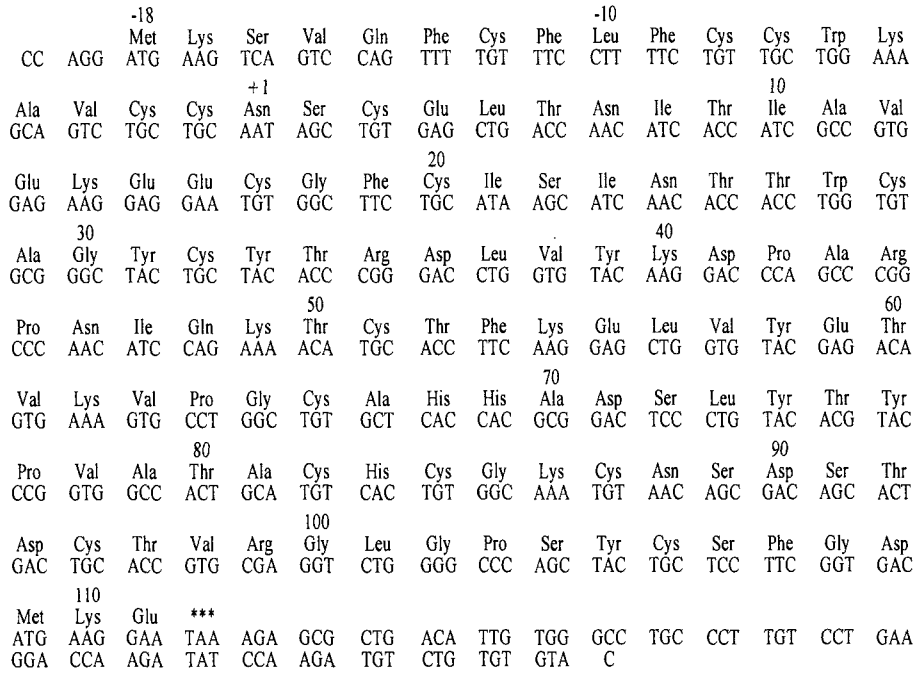

Fig. 8. Nucleotide sequences of eFSH β -subunit cDNA and its deduced amino acids. The eCG β -subunit contains an open reading frame encoding 111 amino acids including a signal peptide of 18 amino acids.

one (Asn 13). In addition to these N-glycosylation sites, the β-subunits of human and baboon CGs are heavily glycosylated C-terminal extensions, which increase the average lengths of these β-subnuits from 120 to 145 amino acids (Pierce & Parsons, 1981; Crawford et al, 1986). Analysis of a purified preparation of eCG revealed that its β-subunit consists of 149 amino acids (Sugino et al., 1987), which was confirmed by the molecular cloning of its cDNA described previously (Min et al., 1994, 1996). There seem to be at least four to six, or even as many as 11, O-glycosylation sites on the extended C-terminal region of the eCG β-subunit (Bousfield et al., 1992; Min et al., 1996). In that point, the role of oligosaccharides in the function of eCG is distinct from that in the case of hLH, hFSH and hCG. Removal of the N-linked oligosaccharides from gonadotropins by enzymatic or chemical methods has been shown to reduce their adenylate cyclase stimulating activities, suggesting that, in general, these oligosaccharides are required for efficient signal transduction (Sairam & Bhargavi, 1985; Calvo et al., 1986; Min et al., 1996). Recombinant eCGs including the mutants which lack oligosaccharides will be useful tools for analyzing the structure-function relationships of gonadotropins in the horse as well as other species.

4. Pattern of α and CG β Messenger RNA Expression

The control of α-subunit gene expression has been examined in considerable detail (Delegeane et al., 1987; Jameson et al., 1988; Bokar et al., 1989). A practical matter responsible for much of the focus on a gene expression has been derived human choriocarcinoma cell lines that express the endogenous

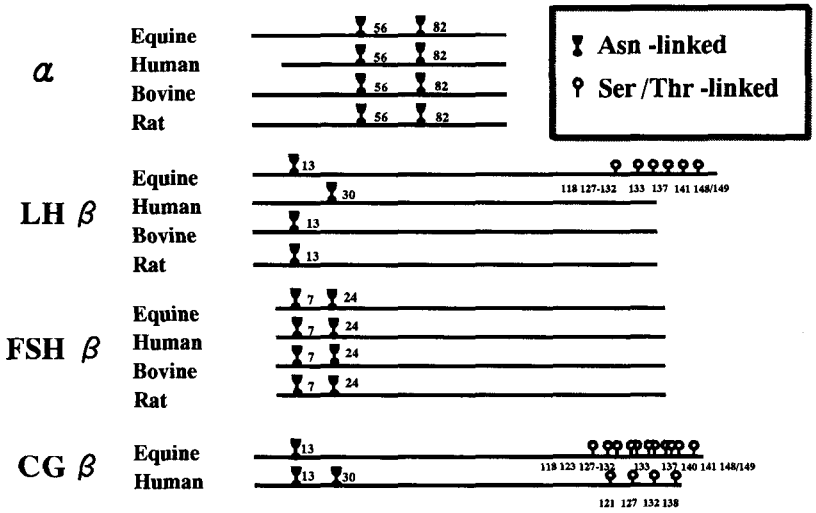

Fig. 9. Glycosylation sites on the α - and β -subunits of glycoprotein hormone. The α - and β -subunits of equine, human, bovine and rat glycoprotein hormones are shwon, and their homologies with respect to the Asn glycosylation site location and numbers of amino acids are indicated. The α -subunits of all these hormones from four species have two Asn glycosylation sites located at homologous positions (Asn^{56} and Asn^{82} in equine α). LHβ has only a single Asn glycosylation site and two analogous sites are present on FSHβ from all four species. Unlike LHβ and FSH β , CGβ has a 25~29 amino acid residue extension peptide at its carboxyl terminus, and eCG β possesses several O-glycosylation sites in its C-terminal extension, at least 11 of which are glycosylated.

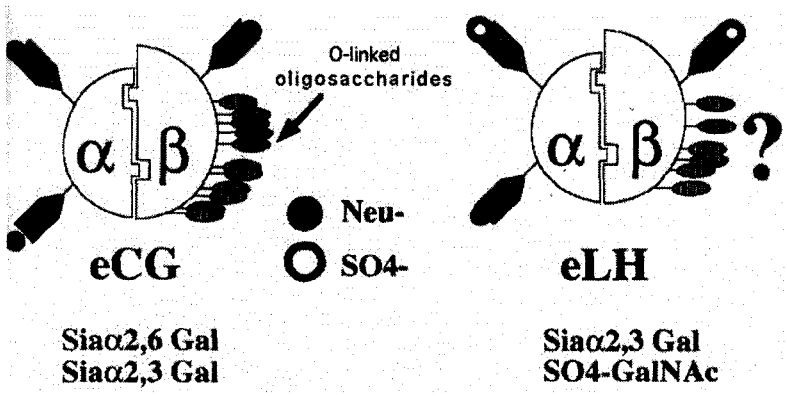

Fig. 10. Oligosaccharide structure of equine chorionic gonadotropin.

gene and the absence of established pituitary cell lines in the gonadotrope lineage. These observations have raised several important questions including what is responsible for the cell-type-specific expression of a gene and why the gene is expressed to placenta in primates and equids but not in other mammals.

Northern blot analysis of mRNA prepared from

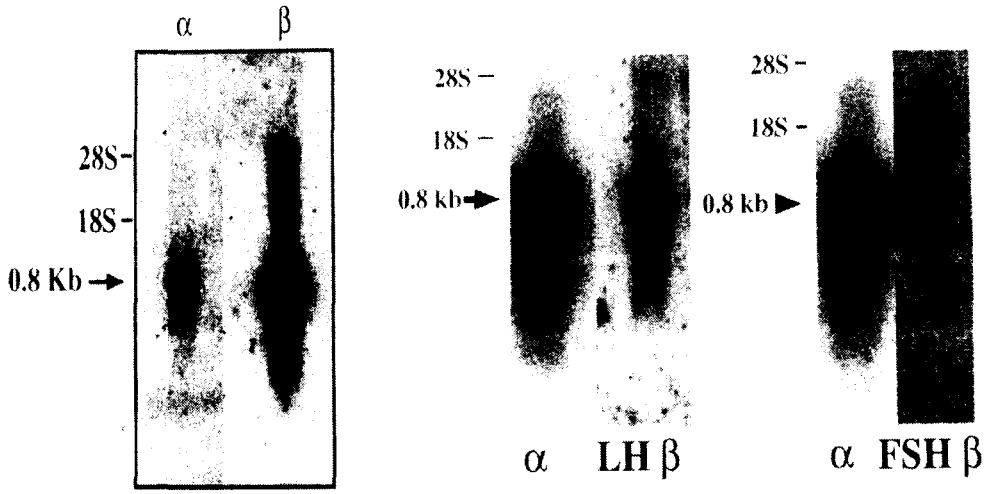

Fig. 11. Northern blot analyses of equine chorionic gonadotropins. Total RNA preparation (20㎍) extracted equine placenta (day 70 of gestation) and pituitary were electrophoresed on 1.0% agarose/formamide gel, transferred to a nylon membrane, and hybridized with [^{32}P]-labeled each subunits cDNAs. The mobilities of 28S and 18S are shown on the left side and the arrow indicate 0.8 Kb eCG α -subunit mRNAs.

equine placenta revealed a transcript of the predicted size. An intense mRNA ratio of α to β at first trimester placenta was about 1: 5 (Min et al., 1994) (Fig. 11).

It is well documented that the human placenta secretes free α-subunits (Ozturk et al., 1988a,b). This can be explained by the imbalanced synthesis of α- and β-subunits. Since accumulation and secretion of a net excess α-subunit have been observed in the pituitary gland as well as in the placenta (Edmonds et al., 1975), sharing of α-subunits in the glycoprotein hormone family may require the excess expression of α-subunit rather than of each individual β-subunit. However, secretion of free α-subunit from equine placenta has not been clearly demonstrated. Interestingly, Couture et al. (1993) showed that the free eCG β -subunit exists in serum and urine, whereas the free eCG α-subunit was undetectable. In our results, the expression of α-subunit in the equine placenta was lower than that of the eCG β-subunit, suggesting that the biosynthesis of eCG β is dominant and secretion of free eCG α-subunit is reduced in the equine placenta at day 70 of gestation. We also analysed mRNA expressions of α-, LH β- and FSH β-subunits from equine pituitary. The intensity of the α-subunit RNA was greater than that of the β-subunit, suggesting that the expression of α-subunit was dominant in the equine anterior pituitary (Fig. 11).

Boothby et al. (1983) reported the presence of twice as much hCG α mRNA as hCG β mRNA. In contrast, the amount of eCG β mRNA was higher than that of eCG α mRNA in the equine placenta. Thus, the subunit mRNA levels seem to be independently regulated and their imbalance may account for differences in the quantities of α- and β-subunits in the placenta. Animals, including horses, have a single copy of the cAMP response element (CRE)-like element in the 5'-flanking region of the α-subunit gene (Fenstermaker et al, 1990), while the human α-subunit gene has the

repeated copies of the CRE. Inspection of the genomic DNA sequence in the 5'-flanking region that contains the α-gene' CRE revealed by Steger et al, (1991).

Ⅳ. 요 약

임신초기 말의 태반으로부터 분비되는 융모성 성선자극 호르몬 (eCG)은 황체형성 (LH), 난포자극 (FSH) 및 갑상선자극 (TSH)호르몬과 같이 알파 및 베타 단체의 비공유결합으로 구성되어져 있는 당단백질 호르몬이다. 알파단체의 아미노산 배열은 동물종내에서 동일하지만, 베타단체는 호르몬에 따라 작용의 특이성을 가지고 있다고 알려져 있다. 말의 융모성 성선자극 호르몬은 모체의 자궁내막에 침입한 태아 유래의 융모조직 (자궁내막배)에서 합성 · 분비되어진다.

eCG는 당단백질 호르몬중 탄수화물 함량이 40%이상으로 가장 많이 함유되어 있으며, 알파단체는 두 개의 N-linked 당쇄첨가 부위 (아미노산 56 및 82번), 베타단체는 13번에 1개의 N-linked 당쇄첨가 부위와 카르복실기 말단부위에 적어도 11개의 O-linked 당쇄첨가 부위를 가지고 있는 것이 특징이다. 또한, eCG는 다른 동물에 있어서 강력한 난포자극 및 황체형성 호르몬의 기능을 가지고 있는 아주 특이한 호르몬이다. 말의 태반과 뇌하수체 조직으로부터 eCG α 및 β단체와 eFSH β단체의 cDNA를 cloning하였으며, 각 단체의 mRNA 발현은 태반과 뇌하수체에서 독립적으로 조절되어진다. 따라서, eCG의 기능 및 수용체에 대한 호르몬의 특이한 작용을 분자생물학, 생화학적인 측면에서 연구하는데 아주 흥미로운 호르몬이다. 왜 eCG가 이러한 이중활성을 가지고 있는지에 대해서는 아직까지 구체적으로 연구된 바가 없지만, 지금까지의 eCG연구를 종합하면, eCG의 알파 및 베타 단체의 cDNA의 유전자 구조 (알파단체는 96개 아미노산; 베타단체는 149개 아미노산)가 밝혀짐으로서 각각의 당쇄첨가 부위에 대한 기능연구에 박차를 가하게 될 것으로 보인다. 따라서 Site-directed mutagenesis를 활용 어느 특정부위의 당쇄 수식이 없는 유전자 재조합 eCG에 대한 연구로 이들 당단백질 호르몬에 대한 생물학적 특성에 대해서 확실하게 밝혀질 것으로 기대하고, 이러한 연구가 계속 진행되고 있으며, 가까운 미래에 eCG에 있어서 지금까지 의문으로 남아있는 난포자극 및 황체형성의 이중활성에 대한 당쇄의 기능이 완전히 해결될 것으로 기대한다. eCG의 황체형성에 대한 당쇄의 기능은 본 연구팀에 의해 알파단체의 56번 N-linked 당쇄첨가 부위가 필수불가결하다는 결과를 얻었지만, 앞으로 난포자극 활성에 미치는 당쇄의 중요성에 관해서는 현재 연구 중에 있다.

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