

Endocrine Function of the Human Placenta : Lessons from the Lifeline

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The placenta, which is an association of maternal and fetal tissues which develops during pregnancy in most mammals, has been developed during the transition from oviparity to viviparity in order to facilitate the intrauterine development of the fetus. It functions as an extremely efficient endocrine gland; an equivalent of the hypothalamo-pituitary-gonadal axis, though transient in nature. This is well exemplified by the human placenta which elaborates protein, peptide and steroid hormones which regulate the development of the fetus and also maintain the pregnancy. Results obtained in our studies reveal that the synthesis and secretion of the protein and steroid hormones by the human placenta is subject to regulatory mechanisms that are similar, though not identical, to those operative in the hypothalamo-pituitary-gonadal axis.

Key Words : Placenta, Chorionic Gonadotropin, Placental lactogen, GnRH, Progesterone, Estrogen

Introduction

Gestation is a composite of integrated processes, resulting in the successful development and birth of a new member of the species. During the course of evolution, mammals have adapted themselves to viviparity in order to protect the developing embryo from adverse environmental conditions. Successful viviparity requires that the mother provide the fetus with the substances essential for its growth. In addition, an intrauterine environment suitable for the development of the fetus must be created and maintained. To satisfy these requirements, an omnipotent, ephemeral organ called the "placenta" has evolved, which protects and nourishes the fetus until such time that it is capable of an independent existence. Placenta is an apposition of maternal and fetal tissues which serves a variety of functions like transport of nutrients and oxygen from maternal compartment to the fetus and removal of waste products from the fetus. It physically anchors the fetus to the uterus and provides protection from the

attack of the maternal immune system. In addition, it serves as an extremely efficient endocrine organ elaborating protein, peptide and steroid hormones (Strauss et al. 1996).

The Placenta: An Omnipotent Organ

The placenta is a unique organ in that, it differentiates and grows from an embryonic tissue to reach maturity in a period of only weeks or months depending on the species and ceases to exist after gestation. In short, the placenta exhibits a biological primacy that reflects its indispensable role in the perpetuation of species. Both, the structure and the function of the placenta are dictated by the complementary necessities of promoting growth on one hand and maintaining an equilibrium between the fetal and maternal systems on the other.

Throughout pregnancy, placenta retains the primary role of membranes, that is, a selective permeability of materials. It serves the function of transport of many essential nutrients which is

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accelerated by a variety of transport mechanisms. However, the transfer of particulate matter, such as blood cells, is severely restricted, a function aptly described as the "placental barrier" (Dancis & Schneider 1975). Nutrients like free fatty acids are very efficiently transported by simple diffusion (Morriss et al. 1994, Booth et al. 1981), while amino acids are transported from maternal circulation to the fetal circulation by active transport (Yudilevich & Sweiry 1985) and fat soluble vitamins are passively diffused. During the transfer of Vitamin A, it is known to complex with retinol binding protein and pre-albumin in fetal plasma thus restricting its transfer back into maternal blood (Ismadi & Olson 1975). Placenta has low density lipoprotein receptors which facilitate the uptake of LDL, circulating in the maternal blood which is used for the synthesis of steroids in the placenta (Biezanski 1969, Winkel et al. 1980 a,b).

It is very well known that placenta also serves as a barrier for maternal proteins. However, it is interesting to note that immunoglobulin G (IgG) and retinol binding protein can cross the placental barrier from the maternal to the fetal side freely (Vahlquist & Nilsson 1984, Bromble 1970, Ockleford & Clint 1980). The mechanism by which IgG escapes intracellular degradation and is transported into fetal circulation is still an unanswered question (Morriss et al. 1994). It is also well established that placenta is an immunologically privileged tissue, protecting fetus from the maternal immunological attack until parturition (Lanman 1975). The placenta produces several immunosuppressive factors which aid in performing this function.

An Endocrine System in Itself

In addition to the above functions, one of the most important functions of the placenta is to serve as a transient endocrine gland which is well exemplified by the human placenta. The human placenta serves as a special endocrine organ by its ability to elaborate a variety of peptides and proteins, some of which function as hormones. In addition, placenta also produces large quantities of progesterone (P_4), cortisol, estradiol 17 β (E_2), estrone and estriol. From the rich variety of its hormonal products, it appears to combine the biosynthetic ability of the hypothalamus, the pituitary, the gonads and the adrenal cortex, hence behaving like a miniature endocrine system.

Almost all of the placental peptide and protein hormones synthesized at different stages of pregnancy that have been studied, are structurally and functionally very similar, or in some cases identical, to those produced by the hypothalamus and the pituitary. To cite a few examples, chorionic gonadotropin (CG), which is synthesized in the placenta, is similar to luteinizing hormone (LH) (Fiddes & Talmadge 1984) and placental lactogen (PL) exhibits structural and functional homology with prolactin (PRL) and growth hormone (GH) (Bewley et al. 1972, Seeburg 1982, Selby et al. 1984, Cooke et al. 1981). Placental GnRH is identical to the hypothalamic GnRH (Tan & Rousseau 1982) and a group of peptides derived from a pro-opiomelanocortin (Liotta & Krieger 1980, 1986) like molecule have also been isolated from human placenta. There have also been reports of MSH, insulin and CRH being synthesized by the placenta (Frim et al. 1988).

Due to the ability of the human placenta to synthesize a wide variety of molecules and perform diverse range of functions, it is a highly complex system to study. Further, the lack of compartmentalization of its various activities (a single cell, the syncytiotrophoblast, in placenta has the capacity to synthesize protein, peptide and steroid hormones) has resulted in the non-availability of suitable model systems to study its functions in greater detail. Only a few of the hormones like hCG, hPL, GnRH, progesterone and estrogen have been well characterized and hence, this review will focus principally on these hormones. Also, the human placenta produces many of these hormones in large quantities and as a result, the effects of a given hormone are understood better by employing inhibitors of hormone synthesis or action rather than by employing the hormone *per se*. The roles of several placental hormones have been understood in our laboratory by the use of inhibitors rather than the hormones themselves.

Human Chorionic Gonadotropin

Chorionic gonadotropin (CG) belongs to a family of glycoprotein hormones and is synthesized and secreted in large quantities from the syncytiotrophoblast cells of human placenta during the first trimester of pregnancy. CG can be detected in maternal blood approximately 8-9 days following preovulatory surge of LH coinciding with the time

of implantation (Lenton et al. 1982). Its concentration in the blood rises steeply in early pregnancy, reaching a peak around 8 to 10th week of gestation and the serum levels are approximately 100 IU/ml during this period. It then declines fairly rapidly and by the 24th week, is at a level of about 10% of the peak value and continues to remain at that level for the rest of the pregnancy (Ashitaka et al. 1974, Kletzky et al. 1985). CG is metab. lized by the liver and kidneys and only a small fraction of the total is excreted in the urine.

Structure of CG

CG consists of two dissimilar subunits, α and β , held together by non-covalent interactions. The α -subunit is common to the other glycoprotein hormones, namely FSH, LH and TSH. However, the β -subunit, which confers biological specificity, is different among all the glycoprotein hormones. CG has a molecular weight of 36.7 kDa, with carbohydrates constituting 30% of the total molecular weight. The α and β subunits have molecular weights of 14.5 and 22.2 kDa, respectively. The α subunit, which is identical in its amino acid sequence with the α subunit of hLH, hFSH and hTSH is comprised of 92 amino acid residues. It consists of a single polypeptide chain with N-linked complex carbohydrate units at positions 52 and 58 (Endo et al. 1979 and Kessler et al. 1979a) and five disulfide bonds (Mise & Bahl 1980). The β -subunit comprises of 145 amino acid residues and differs in its amino acid sequence with β -subunit of hFSH and hTSH. It is a single polypeptide chain with six oligosaccharide units (Kessler et al. 1979b) and six disulfide bonds (Mise & Bahl 1981). A major function of the carbohydrate residues of CG is to prolong its half-life in circulation (Rosa et al. 1984). Binding studies with CG/LH receptors have shown that the dissociated subunits of CG are virtually devoid of biological activity (Strickland & Puett 1981, Morgan et al. 1974 and Rayford et al. 1972) and the biological specificity of each hormone is determined by the structure of the β -subunit (Strickland & Puett 1981).

The elucidation of the crystal structure of hCG (Lapthorn et al. 1994) has contributed greatly to the understanding of the molecule. The folding topology of hCG is most unusual in that it has unexpected folding motifs, interlocking subunit-subunit interactions and symmetry relationships, as well as

an unusually high ratio of protein surface to hydrophobic core. The heterodimer adopts an elongated shape consisting primarily of β sheets with the two subunits intertwined into each other. The core structure of each subunit contains a cystine knot motif, where a disulfide bridge connecting two strands penetrates into an eight-residue circle generated by a pair of disulfide bridges connecting two other strands. Extending out from the cysteine knot in the α - and β -subunits are a pair of highly twisted hairpin loops, in almost parallel planes, which project in one direction, and a long, flat hairpin loop which projects in the opposite direction. The subunits are intimately associated over their interacting surfaces, and in the process bury a surface area of approximately 4000 \AA^2 . The intermolecular interactions are of the β sheet type at the heart of the interface, as well as in a segment where the disulfide tethered carboxy terminal tail of the β -subunit embraces a long loop of the α -subunit. This "seat-belt" arrangement has implications for possible *in vivo* folding pathways for hCG $\alpha\beta$ heterodimer formation, because this process will be governed by the precise order of disulfide bridge formation.

Several histochemical studies using specific antibodies have suggested that CG is synthesized in the syncytiotrophoblast (Gartner et al. 1975 and Look et al. 1972). Studies using placental explants suggest that first trimester human placenta (FTHP) secretes more CG than the term placenta (Flurry et al. 1977 & Boothby et al. 1983). Thus, more mRNA for α - and β -CG subunits have been observed in FTHP than in the term placenta (Daniels-McQueen et al. 1978). The β -CG mRNA level in the FTHP is high since transcription of the gene is very active, although the levels of α -CG mRNA are always one or two folds higher than the β -CG. Use of immunochemical methods and *in situ* hybridization studies have shown that α -subunit is synthesized not only in the syncytiotrophoblast layer but also in the cytotrophoblast layer. As syncytial formation may proceed through cellular intermediates of cytotrophoblastic origin, it has been suggested that transcription of the α -CG gene is initiated during this process but prior to completion of syncytial formation (Gartner et al. 1975, Hoshina et al. 1979, 1982). However, β -CG subunit is secreted only by intermediate and syncytiotrophoblast cells (Hoshina et al. 1985).

Gene Organization of α - and β -CG Subunits

Within a mammalian species, the amino acid sequence of α -subunit is the same for all glycoprotein hormones. The α -subunit in humans is coded by a single gene (Boothby et al. 1981) and consists of four exons separated by three introns. The α -subunit can be divided into three domains based on the gene structure : N-terminal domain comprising of 1-10 residues, the middle domain with residues 11- 71, and residues 72 - 92 forming C-terminal the domain. The first domain shows the greatest degree of difference suggesting that this domain is not important for biological activity or dimerization. However, the second and third domains show high degree of homology between the human and bovine sequences and these domains are linked by disulfide bridges. The β subunit, however has diverged considerably resulting in manifestation of different type of biological activity. The amino acid sequence of the β subunit of CG has 80% homology with human pituitary LH (Fiddes & Goodman 1980), although CG β subunit is unique in that it contains an extension of 31 amino acids at its carboxy-terminal (Jameson et al. 1986). This extension has been shown to be due to the loss of an extreme stop codon in the β -CG gene. Placenta-specific expression of the human α -CG subunit gene requires the presence of at least two distinct cis-acting elements. Among these, cAMP response element (CRE) is one which functions by binding the ubiquitous protein CREB. The other region is the upstream regulatory element (URE) which has binding sites for at least two trans-acting factors. The CRE and the URE together form a tissue-specific enhancer and are critical for placenta-specific expression of α -CG subunit.

In contrast to a single gene which codes for α subunit, eight separate β -CG subunit genes have been isolated and out of these six of them code for β -CG, one codes for β -LH and the other is a pseudogene. Interestingly, these genes have similar structures and of these, four (viz.1,2 and 5,6) have been shown to exist as inverted pairs. In both the cases the genes are arranged in a convergent transcriptional order. The other four β -CG genes, 3 & 4 and 7 & 8 are arranged in tandem pairs. (Boorstein et al. 1982). The β -CG and β -LH genes contain 3 exons and 2 introns (Policastro et al. 1983). The CG- β genes and the LH- β gene have been mapped and found to be in a single cluster in human

genomic DNA (Policastro et al. 1986). All these genes show conservation of 5'-flanking sequences that seem only weakly homologous to canonical CAAT and TATAA promoter elements. Using RFLP and transient expression in COS cells, it has been shown that only three of the seven CG β genes are expressed in the placenta, viz. 3,5 and 6. Presumably, these genes have additional transcription-control sequences that are not shared by the other CG- β genes or by LH β gene. However, the transcripts of CG- β genes 2, 6, 7 and 8 are shown to represent less than 10% of the total β -CG gene expression. Two regions which are required for efficient transcription, the enhancer consensus sequence at - 49 and an upstream region from the CCAAT box around -100, have been identified in the genes 5 and 6 (Talmadge et al. 1984). A sequence responsible for cAMP induction of the CG- β gene was identified in the region of -292 to + 357, which is a promoter-regulatory region. Since this region contains no close homology to the α -CG subunit CRE, cAMP responses of these two genes must be mediated via different cis-acting elements and transacting factors.

Role of CG During Pregnancy

The precise physiological role of CG in human pregnancy is not fully understood. During the initial 6-8 weeks of pregnancy, CG act as a luteotropin, by rescuing the *corpus luteum* in a fertile cycle. The rescue results in the maintenance of progesterone production well beyond the time when luteal regression normally occurs in a non-fertile cycle, thereby maintaining pregnancy until placental progesterone production is established. As mentioned above, CG appears in the maternal blood by about 10th day after ovulation (Leuton et al. 1982). This period coincides with the stage of luteal rescue, when the *corpus luteum* has been reported to show enhanced sensitivity to CG treatment both *in vivo* (Tu 1978) and *in vitro* (Dennefors et al. 1982). Several experimental studies using non-human primates and clinical studies have shown that after 25th day of pregnancy, ovariectomy has no effect on the course of pregnancy. However, during the period when *corpus luteum* is sensitive to CG its levels are extremely low and CG is detectable only by the most sensitive RIA. In contrast, CG is at its peak levels subsequent to the stage at which removal of *corpus luteum*, on which it is supposed to act, has no effect on the course of pregnancy. It has also been reported

that placental progesterone production is not stimulated by addition of CG (Menon & Jaffe 1973). These observations, thus, do not support the suggestion that the main function of CG is only to maintain progesterone production during pregnancy. In an attempt to explain these parameters several other functions have been attributed to CG. A very important finding in understanding the function of CG has been the finding of Shi et al. (1993) who demonstrated that hCG can regulate the differentiation of cytotrophoblasts into syncytiotrophoblasts. CG is known to stimulate testosterone production by the fetal testes and the concentrations of testosterone peaks between 11 and 17 weeks post-conception (Reyes et al. 1973, 1976) which coincides with the maximum concentrations of CG in the fetal serum and amniotic fluid (Reyes et al. 1976) in the male fetuses. In addition, it has been shown that CG stimulates thymidine incorporation into Leydig cells and it has been suggested that it may regulate Leydig cell proliferation as well as testosterone biosynthesis in the fetal testis. Based on these observations, it has been suggested that CG has a role in fetal sex differentiation. In support of this is the observation that, even in the bonnet monkey, serum testosterone levels reach a peak around a period when CG levels are maximal (Rao & Kotagi 1982).

Another important function ascribed to CG concerns with the suppression of maternal immune system so that the fetus is not rejected. In fact, the early appearance of CG in the embryo and its subsequent synthesis and secretion during human pregnancy in the placenta make it a potential candidate to be an immunoregulatory agent. Several workers using crude CG preparations have demonstrated its ability to block PHA induced lymphocyte transformation, mixed lymphocyte reactions and antigen-induced blastogenesis. However, these immuno-suppressive actions of CG were questioned later by various workers using purified CG (Nisula & Bartocci 1984). It is now clear that CG is not a systemic immunosuppressant, although it may serve an important immunoregulatory function locally in the placenta. It has also been reported that CG is responsible for fetal adrenal growth prior to mid-gestation, but has a decreasing role in late pregnancy as fetal ACTH begins to play a greater role. During the second trimester, CG causes ultrastructural changes in the

fetal adrenals which are consistent with increased steroidogenic activity (Johanisson 1979). It is also pertinent to note that in the increase in production of DHAS by fetal zone *in vitro* is more in response to CG than that with ACTH (Seron-Ferre et al. 1978). In postnatal life, CG administration to human neonates causes definite elevations in plasma DHA and DHAS levels (Dell'Acqua et al. 1978) and thus CG has long been an attractive hormone to be implicated in fetal-zone maintenance. Also, it is interesting to note that the fetal zone disappears rapidly and coinciding with the fall in the level of CG, it regresses completely at term and this perhaps helps to explain fetal-zone involution after birth. The demonstration of CG-receptors in the human myometrium (Korneyi et al. 1993) suggests that hCG may play a role in the growth of myometrium during pregnancy. It may also have other regulatory effects on the myometrium which might be crucial in the maintenance of pregnancy.

Regulation of CG Secretion

As mentioned earlier, placenta synthesizes more CG in the first trimester than in mid and term pregnancy and the synthesis of β -CG subunit is rate limiting in the synthesis of CG. Accordingly, more mRNA for the β -CG subunit is seen in the first trimester human placenta. This phenomenon of rapid increase in the CG level between 8-12 weeks of pregnancy followed by a sudden decline after this period has attracted the attention of several investigators. While, it was obvious that some sort of regulatory mechanism must be operating, until recently, nothing was known about the possible factors involved in this regulatory process. Although, earlier it was suggested that the secretion of CG is autonomous, recent studies have clearly established that the synthesis and secretion of CG is subject to regulation by a variety of factors. In studies using placental explants, Belleville et al. (1978) reported that addition of P_4 and E_2 had no effect on CG secretion. However, Wilson et al. (1980, 1984) have demonstrated that addition of P_4 to placental explants resulted in an inhibition of CG secretion. Maruo et al. (1986) reported that while E_2 has no role, P_4 exerted an inhibitory control over CG secretion. In contrast to these observations, studies of Ahmed et al. (1988) revealed that addition of P_4 to placental explants resulted in an increase in CG secretion. More recently, studies from our laboratory have demonstrated that

the synthesis of CG is negatively modulated by E_2 and positively modulated by P_4 as judged by the change in the levels of immunoreactive CG and α - and β -CG mRNA (Rao et al. 1995). Inhibition of aromatase by the addition of 1,4,6-androstatrien-3,17-dione resulted in a significant increase in CG levels; this increase was partially reversed by the simultaneous addition of E_2 (Sharma et al. 1993). Addition of P_4 resulted in a significant increase in the levels of α - and β -CG mRNA as well as immunoreactive CG (Rao et al. 1995).

Studies from our laboratory and elsewhere have also indicated that, GnRH may be a possible regulator of CG secretion since physiological concentrations of GnRH stimulate CG secretion from placenta in a dose-dependent manner, whereas GnRH antagonist inhibits CG secretion (Siler-Khodr & Khodr 1981, Siler-Khodr et al. 1986, Mathialagan & Rao 1986a). In support of the involvement of GnRH in the regulation of CG synthesis and secretion by human placenta are the reports describing the presence of GnRH synthesizing system, independent of hypothalamus, in the placenta (Mathialagan & Rao, 1986b). Several other investigators have also reported the isolation and characterization of GnRH from human placenta (Khodr & Siler-Khodr, 1980). The presence of specific receptors for GnRH in the human placenta has also been reported though there also appear to be low affinity receptors in addition to the classical high affinity receptors (Belisle et al. 1984, 1987). These lines of evidence indicate that GnRH may definitely be one of the factors in the regulation of CG synthesis by human placenta.

In contrast to the *in vitro* studies on the role of GnRH in the regulation of CG synthesis and secretion by human placenta, very little information is available using *in vivo* models. One of the main problems is the lack of a suitable animal model. For obvious ethical concerns, human female cannot be used. Of the available non-human primate models, though the great apes synthesize CG, studies using them are quite expensive and time consuming, in addition to the problem of their non-availability. In contrast, both the Rhesus and the Macaque are easily available. However, the CG profile in them is quite different from that of the humans. CG levels are low and are detectable over a narrow period, from day 25 to day 45 of a fertile cycle. We initiated studies towards using the bonnet monkey, *Macaca radiata* as an *in vivo* model, by developing an RIA for monitoring CG

levels during early pregnancy (Rao et al. 1984) which was subsequently modified into a sensitive ELISA (Chakrabarti & Rao 1987). Using these, CG levels in the bonnet monkey were determined. It was also observed that administration of GnRH during the ascending phase of CG resulted in a significant increase in serum CG levels but not during the descending phase (Rao & Moudgal, 1984). The increase observed was not due to interference with LH as the ELISA was very specific to CG and LH did not crossreact in the assay. Subsequently, by delivering the GnRH agonist, Buserelin by Alzet mini-osmotic pump to pregnant bonnet monkeys, it was observed that the sharp fall in CG, which occurs after 33rd day of fertile cycle, is prevented (Rao & Chakrabarti 1990). This was the first evidence *in vivo* for the regulation of CG by GnRH.

Dibutyryl cAMP, theophylline and other phosphodiesterase inhibitors stimulated CG secretion from trophoblast as well as from cell-lines suggesting that cAMP could be a second messenger in this pathway (Wilson et al. 1980, Ringler et al. 1989). Studies carried out using choriocarcinoma cells by Anderson et al. (1990), have shown that addition of 8-Br-cAMP increased α - and β -CG subunit mRNA levels in a dose-dependent manner. The changes in subunit mRNA levels correlated with changes in secretion of their respective protein subunits, suggesting that cAMP regulates CG biosynthesis primarily at a pre-translational level. However, there is a difference in the transcriptional regulation of the two genes by cAMP. The increase in β -CG expression followed by cAMP addition was slow compared to the increase in the case of α -CG subunit expression. It was also reported that addition of cycloheximide had no effect on cAMP induced transcription of the α -CG gene, whereas it almost completely blocked the effect of cAMP on β -CG gene transcription, suggesting that the effect of cAMP on α -CG gene transcription involves pre-existing factors, whereas β -CG transcription requires *de novo* protein synthesis. As mentioned above, the sequence in β -CG that confers cAMP responsiveness in transfection studies lacks homology to the α -CG subunit CRE suggesting that cAMP exerts a direct effect on α -CG gene expression and indirectly on the β -CG gene by probably inducing synthesis of intermediary protein. Pestell et al. (1994) have demonstrated that c-jun represses transcription from α - and β -CG promoters

and that this effect is mediated via the CRE in both the promoters.

It has been reported that treatment of choriocarcinoma cells with phorbol ester, phorbol myristate acetate leads to a time dependent increase in the α - and β -CG mRNA's (Anderson et al. 1990) suggesting that protein kinase C (PKC) may also be involved as a second messenger in the regulation of synthesis of CG subunits. Studies from our laboratory have shown the involvement of Ca^{2+} , PKC, and calmodulin, in the GnRH-induced CG secretion (Mathialagan & Rao 1989, Sharma and Rao 1991, 1992). Addition of Ca^{2+} chelators, EDTA and EGTA caused a significant decline in GnRH-induced CG secretion. Addition of GnRH caused an increase in the uptake of Ca^{2+} which correlated with an increase in CG secretion (Mathialagan & Rao 1989). Also, addition of A 23187, a calcium ionophore, resulted in an increase in CG secretion which was inhibited by lanthanum, a calcium antagonist (Sharma & Rao 1992). In addition, experiments monitoring the effect of calcium using the incorporation of radioactive amino acid precursors into hCG revealed that newly synthesized hCG is preferentially secreted over the stored hormone in the absence of calcium (Sharma & Rao 1993). Addition of activators of PKC like PMA and DiC8 resulted in a significant dose-dependent increase in the synthesis and secretion of CG. Similarly, addition of inhibitors of calmodulin resulted in an inhibition of secretion of CG (Sharma & Rao 1991). In addition, studies from our lab have revealed an interesting fact that secretion is a stimulus for the synthesis of hCG by the first trimester human placenta (Sharma & Rao 1995).

Recently, Myat-Thanda et al. (1996) have demonstrated that hCG release is inhibited by nitric oxide in choriocarcinoma cells, JEG-3 and BeWo. It has also been suggested that CG is regulated by growth factors like EGF, PDGF and thyroid hormone (Siler-Khodr & Khodr 1981, Khodr & Siler-Khodr 1980 and Iwashita et al. 1986). Addition of triiodothyronine (T3) and EGF resulted in an increase in CG levels. Boime et al (1982) have suggested that regulation of CG synthesis may occur at the level of translation through changes in the concentration of electrolytes like Mg^{+2} and spermidine. The results of their studies suggest that the α : β subunit ratio is dependent on the concentration of Mg^{+2} and spermidine in the cell-free extracts.

In addition to P_4 and E_2 , placenta is also known to synthesize significant amount of glucocorticoids during pregnancy. The levels of glucocorticoids increase during gestation and rapidly increase at the time of labor. Wilson and Jawad (1982) have shown that glucocorticoids stimulate CG synthesis in the term placental culture system. This was further confirmed by Ringler et al. (1989) who demonstrated that dexamethasone, a synthetic glucocorticoid, stimulated CG production in cultured cytotrophoblasts by increasing the abundance of α - and β -CG mRNA levels. Studies in our laboratory have indicated that glucocorticoids may differentially regulate CG mRNA levels in FTHP and term placenta. While addition of dexamethasone stimulated both α - and β -CG mRNA levels in FTHP, in term placenta an increase was observed only in the levels of α -CG mRNA (Prasad 1993). With the development of methods to isolate cytotrophoblasts and syncytiotrophoblasts it has been suggested that the change in the synthesis of CG may be due to change in the number of cytotrophoblasts as ratio of cytotrophoblasts to syncytiotrophoblasts decreased with progress in pregnancy. Thus, the synthesis and secretion of CG appear to be under the control of more than one factor and perhaps also varies at different stages of gestation.

Human Placental Lactogen

Human placental lactogen (hPL) is a single chain polypeptide hormone with a molecular weight of 22 kDa. It is composed of 191 amino acids and contains two intramolecular disulfide bridges (Josimovich & Levitt 1976) but no carbohydrate residues. hPL shows 96% homology with human growth hormone (hGH) and only 67% homology in amino acid sequence with human prolactin (hPRL), although it is functionally similar to PRL. Due to their structural similarities hGH, hPRL and hPL are thought to have originated from a common ancestral polypeptide by repeated gene duplication (Barsch et al. 1983 and Selby et al. 1984). hPL is synthesized as a pro-hormone having a 25 amino acid residue signal peptide. Immunoreactive hPL appears in the maternal circulation by the third week of pregnancy and later the maternal serum levels steadily increases until about 34 weeks and remain constant till the end of the pregnancy. The concentration of hPL at term gestation ranges between 5 and 15 mg/ml

(Braunstein et al. 1980). hPL mRNA is localized to the syncytial cells of the trophoblast, which suggests that hPL is synthesized by these cells (Boime et al. 1982, Hoshina et al. 1985). In term placental tissue, the steady state level of hPL mRNA is five times greater than in FTHP (Boime et al. 1982).

Regulation of hPL secretion

The regulation of synthesis and release of hPL is markedly different from that of the pituitary hormones, viz., growth hormone and prolactin, in spite of the striking similarities in its chemical and biological properties. Recent studies strongly suggest that the synthesis and release of hPL may be regulated, at least in part, by several novel factors including high density lipoprotein (HDL) and hPL releasing protein found in serum from pregnant women as well as butyryl cAMP which stimulated release of hPL (Welsch 1979). It has been reported that addition of calcium to term placental minces also increased hPL secretion. In contrast, estradiol and progesterone have been reported either to stimulate hPL secretion from placental explants (Belleville et al. 1978) or to have no effect.

Functions of hPL

The major role of hPL is to bring about changes in the maternal intermediary metabolism and to increase availability of glucose for fetus. hPL principally acts as an insulin antagonist mobilizing fatty acids from maternal depots, which allows the mother to spare glucose to accommodate increasing fetal needs in late pregnancy (Grumbach et al. 1966). However, several clinical studies do not show an absolute requirement of hPL in the maintenance of pregnancy (Sideri et al. 1983).

Gonadotropin Releasing Hormone

In placenta, synthesis of gonadotropin releasing hormone (GnRH) has been demonstrated (Khodr & Siler-Khodr 1980) and subsequent purification of placental GnRH has revealed that the primary structure is identical to that of hypothalamic GnRH (Tan & Rousseau 1982). Using immunohistochemical methods (Miyake et al. 1982). GnRH has been localized to cytotrophoblasts and the outer surface of the cell membrane of syncytiotrophoblasts indicating that syncytiotrophoblast cells are targets of GnRH. The placental GnRH levels are relatively

high between 12 to 23 weeks of pregnancy and then decline to a very low level. The important function of GnRH is to stimulate the secretion of CG and it has also been observed that it regulates the production of placental steroids and prostaglandins. GnRH has been reported both to stimulate (Siler-Khodr et al. 1986) and to inhibit (Wilson & Jawad 1980) the release of estradiol and progesterone by placental explants and cells *in vitro*. Stimulation of release of PGE-2a and PGE-2b *in vitro* by GnRH in the placenta has been demonstrated (Siler-Khodr 1983). Placenta is also known to have substances with TRH-like activity (Gibbons et al. 1975), somatostatin-like activity which are localized in cytotrophoblasts (Etzrodt et al. 1980). GH-releasing factor like activity (Baird et al. 1985) has also been demonstrated in extracts obtained from human term placenta.

Placental Steroid Hormones

The human placenta has the capacity to elaborate two major types of steroid hormones, namely progesterone and estrogens. The estrogens include primarily estriol (E_3) and also a significant quantity of estradiol-17B (E_2) and estrone (E_1). Unlike the peptides formed in the mother and fetus, the steroids readily cross the placenta. The synthesis of these steroids are known to occur even at the blastocyst stage of development prior to implantation (Meyer 1955). Preimplantation blastocysts can inter-convert pregnenolone to progesterone and estrone to estradiol. The early gestation placenta can convert cholesterol to pregnenolone (Gunasegaram et al. 1978, 1979) and dehydroisoandrosterone to estrogen (Smith & Axelrod 1969). The human placenta does not become the dominant source of steroid hormones until the 8th week of gestation. Until the placenta acquires this capacity, the primary source of P_4 and E_2 is the *corpus luteum* (Csapo et al. 1972, 1973). In animals with short gestation periods (20-60 days), this prolongation of the function of the *corpus luteum* is sufficient to provide all the extra steroid hormones needed for the successful completion of pregnancy. In humans, the placenta assumes varying degree of steroid producing activity and replaces ovarian function completely. The human trophoblast produces CG which maintains and stimulates the *corpus luteum* to produce P_4 until at least 8 weeks of gestation (Moudgal et al. 1972), after which the placenta becomes the dominant source of P_4 . In

placenta, P_4 is produced from cholesterol, and estrogen is produced from circulating C-19 steroid precursors derived either from fetal or maternal adrenals (Brinster 1973). Among steroidogenic tissues, the human placenta is unusual in that it cannot convert C21 steroids to C19 steroids. This is in contrast to the placenta of the sheep, in which the capacity to effect this transformation appears to be induced by cortisol. The early studies on the formation of pregnenolone by placental tissue indicated that cholesterol could be converted to pregnenolone and pregnenolone in turn, to progesterone. However, cholesterol was not formed from acetate (Levitz et al. 1962, Van Leusden & Vिलее 1965) by human placental tissue. Also, maternal cholesterol seemed to be the precursor of placental steroids (Hellig et al. 1970) with low-density lipoprotein (LDL) cholesterol being the form utilized for steroidogenesis (Winkel et al. 1980). Thus, the human placenta is not autonomous in terms of steroid hormone production since it relies on maternal cholesterol for production of progesterone and fetal and maternal adrenals as source of C19 steroids for estrogen biosynthesis.

Progesterone

The steroid hormone progesterone is so named after its supporting effects in pregnancy, and has been shown to be absolutely essential for the maintenance of pregnancy in all mammals studied so far (Csapo 1969). P_4 secreted by *corpus luteum* decreases after the 5-6th week of pregnancy due to the regression of *corpus luteum* and simultaneous increase of P_4 synthesis in placenta takes place. The placental synthesis of P_4 rapidly increases from 10-37th week of pregnancy and later it remains constant until parturition. In women, serum P_4 concentration rises relatively linearly with advancing gestation, attaining values of 150-200 ng/ml at term (Tulchinsky et al. 1972). The rate of P_4 production during late gestation approximates 250-600 mg/day (Solomon 1994) as against non-pregnant women in whom it is 15-20 mg/day. In some pregnancies involving multiple fetuses, 800 mg of P_4 is produced per day ! The MCR of P_4 in non-pregnant and pregnant women does not differ much. However, an increase in serum P_4 concentration with pregnancy in humans is observed (non-pregnant 6-10 ng/ml and pregnant 104 ng/ml) and it has been shown that this increase is the result of an

increase in the synthesis of P_4 and not due to a change in its metabolism (Lin et al. 1972).

The cholesterol side-chain cleavage reaction converts cholesterol to pregnenolone, which is then readily converted to progesterone. This step is rate-limiting for adrenocorticotrophic hormone (ACTH) in the adrenal; however, in the placenta the trophic factors controlling this conversion are not known. This cleavage requires molecular oxygen and NADPH. The cholesterol side-chain cleavage has a specific cytochrome P-450 (namely, P-450_{SCC}) in the adrenal, and there is evidence that the same P-450 is operative in the placenta (Simpson et al. 1978). An additional rate-limiting factor for placental steroidogenesis is the uptake of LDL-cholesterol from the maternal circulation.

Regulation of Progesterone Biosynthesis

Regulation of P_4 biosynthesis in human placenta seems to be strictly autonomous. Earlier suggestions, that CG regulates placental P_4 synthesis have not been substantiated (Vилее et al. 1966). It was found that CG and antibodies to CG were ineffective in altering the rate of conversion of pregnenolone to P_4 in placenta (Menon & Jaffe 1973 and Talwar 1979). Even though the levels of P-450_{SCC} in placental mitochondria are low, the specific activity of P-450_{SCC} is same as in ACTH stimulated rat adrenals (Simpson et al. 1978). In the human trophoblast, cholesterol from maternal compartment is the main substrate for P_4 synthesis. P_4 formation from cholesterol synthesized within the placenta is minimal since the *de novo* synthesis of placental cholesterol is limited as demonstrated in *in vitro* studies (Van Leusden & Vилее. 1965). Earlier work has shown that in primary culture, and in choriocarcinoma cell lines, human trophoblast cells have the capacity to take up and degrade LDL (Simpson et al. 1979) and to a lesser extent HDL. The presence of high affinity LDL receptor sites on trophoblast cells has been demonstrated (Winkel et al. 1980a&b). The receptors are known to have an apparent K_d of $1.03 \times 10^{-8}M$. At relatively high concentrations, HDL is also capable of increasing P_4 formation in human placental cells (Winkel et al 1980a). In view of the relative content of cholesterol in LDL, which is twice that of HDL, and plasma concentration of LDL, which is four fold greater than that of HDL, it has been suggested that under normal physiological conditions LDL is the primary source of cholesterol for the placental P_4

synthesis. It has also been demonstrated that radioactively labeled blood-borne cholesterol is converted to P_4 and the distribution of isotopic label indicates a preferential use of cholesterol over other possible precursors for P_4 biosynthesis (Hellig et al. 1969). Thus the supply of cholesterol to placental mitochondria appears to be rate limiting for P_4 biosynthesis. The concentration of LDL in maternal plasma is high and the rate of P_4 biosynthesis may be determined by the number of LDL receptors on the surface of trophoblasts (Simpson et al. 1979). Henson et al. (1988) have reported that E_2 increases progesterone production in Baboon placenta by increasing the uptake of LDL. Recent studies from our laboratory have indicated that E_2 upregulates the synthesis of P_4 (Shanker & Rao 1997) and increases the LDL-receptor mRNA levels in the human placenta (Shanker et al. 1997a). Interestingly, hCG was also able to stimulate LDL-receptor mRNA levels in the human placenta (Shanker et al. 1997a). We have also observed that P_4 might autoregulate its own synthesis in the human placenta (Shanker & Rao 1997), however, the mechanism of autoregulation is not yet clear.

Apart from lipoproteins, other factors that have been reported to regulate P_4 synthesis include the cytokines IL-1a, IL-1b & TNF α (Feinberg et al. 1994), and Sn-1,2- diacylglycerols and phorbol esters (Kato et al. 1989). However, the mechanism of action of any of these is not known.

Function of Progesterone

Removal of the ovary during early pregnancy in the rat and human leads to abortion, but can be prevented by administration of P_4 alone in each case. Early secretion of CG ensures maintenance of *corpus luteum* function and thus P_4 production, until placental steroidogenesis is well established. P_4 inhibits the uterine secretion of prostaglandins, there by blocking the uterine contraction which would otherwise expel the embryo and thus P_4 plays an indispensable role in the maintenance of pregnancy (Csapo 1977). P_4 was found to have demonstrable immunosuppressive effects (Siiteri & Stites 1982) when administered locally in animals. It was found that P_4 at a concentration of 20 mg/ml suppressed mixed lymphocyte culture response (Clemens et al. 1979) such as thymidine incorporation and blast transformation. It is also possible that anti-inflammatory or immunosuppressive actions of

progesterone may be indirect and are mediated by regulation of synthesis of other specific uterine proteins (Siiteri & Stites 1982) such as uteroglobin or other substances such as prostaglandins which may have either stimulatory or inhibitory effects on the immune system. Recent studies from our laboratory have demonstrated the presence of progesterone receptors in the human placenta (Shanker et al. 1997b) by RT-PCR. This observation was confirmed by the demonstration of the absence of uterine contamination in the placental RNA samples used for the demonstration of progesterone receptor. This observation, in conjunction with the role of P_4 in the regulation of CG (Rao et al. 1995) suggests that P_4 plays a very important regulatory role in the placenta itself.

Estrogens

Placenta becomes the primary source of estrogens after the 9th week of gestation. The principle estrogens synthesized in the placenta are E_2 and E_3 and the physiologically active hormone is E_2 . Estrogens are also known to influence placental growth (Abdul-Karim et al. 1971). Earlier studies in our laboratory have shown that E_2 stimulates the synthesis of several proteins in the human placenta (Sharma et al. 1990). Also, as mentioned earlier, E_2 regulates the synthesis and secretion of hCG in the human placenta (Sharma et al. 1993).

During human pregnancy, large quantities of estrogen are produced. In pregnant women, at or near term, 15-20 mg E_2 and 50-100 mg E_3 are synthesized per day (Simpson et al. 1981). The precursor for estrogen synthesis in placenta is dehydroisoandrosterone sulfate which is synthesized by the maternal and the fetal adrenals and transported to the placenta. The sulfatase activity is very high in placenta (French & Warren 1966), which helps in the conversion of dehydroiso-androsterone sulfate to dehydroiso andro sterone and later to estrone by 3β HSD/ $D\Delta^{5-4}$ isomerase and the aromatising enzyme system. Estrone is eventually converted to E_2 . Human placenta lacks steroid 17α -hydroxylase activity and consequently cannot convert C-21 steroids to C-19 steroids. However, placenta has a remarkable capacity for aromatization of C-19 steroids (Siiteri & MacDonald 1963, Bolte et al 1964). Stimulation of the conversion of C-19 steroids to estrogen in the perfused human placenta by hCG has been reported (Cedard et al 1970).

Aromatase activity could be stimulated in choriocarcinoma cells by dibutyryl cAMP and theophylline (Bellino et al. 1978) suggesting a role for cAMP in hCG stimulated conversion of C19 steroids.

As mentioned earlier, estrogen plays a very important role during pregnancy. Its effects on the expression of hCG and LDL-receptor have been discussed. It has also been demonstrated in our laboratory that estrogen regulates actin in the human placenta (Sudha, 1997). The recent demonstration of estrogen receptors in the human placenta (Chibbar et al. 1995) suggests that estrogen may also play a crucial role in the regulation of placental function.

Concluding Remarks

It is evident from the literature reviewed, that we are dealing only with the tip of the iceberg in a highly complex and efficient system. As mentioned earlier, the lack of compartmentalization of the various activities of placenta has been one of the major problems in delineating the functions of placental hormones. As a consequence, very little is understood about their exact roles in the maintenance of pregnancy. However, from whatever we have been able to fathom, it is clear that viviparous species have been very successful in employing the development of placenta as a very efficient stratagem to counter the adverse environment of extra-uterine development. It is not merely a passive filter, but a very efficient endocrine organ, serving as a very

efficient hypothalamic-pituitary-gonadal axis, though transient in nature. It serves as an excellent model to study cell differentiation, regulation of gene expression, oncogenesis, mechanism of immuno-suppression and aging. It is no wonder that Beaconsfield et al. (1980), in their excellent, comprehensive review, remarked "It may turn out to be unfortunate that the apparent simplicity of the bacterium *Escherichia coli* seduced investigators into making it the basic organism for genetic research. If placental cells had been employed for the same purpose, the initial difficulties would have been greater but, today the universal model would be human in origin". Although a discarded tissue, one cannot but perhaps wonder that the placenta is nature's gift to the biologist. Moreover, its absolute necessity for the maintenance of pregnancy underlines its importance in the perpetuation of our species. It is, then, only apt to describe it as our "life line".

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