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Secretion of pituitary-like peptides by the human placenta

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Evidence for the secretion of protein hormones by the placenta is conclusive only for human chorionic gonadotrophin (hCG) and human placental lactogen (hPL). Nevertheless, there is some evidence that material resembling pituitary thyroid stimulating hormone (TSH) and a peptide resembling adrenocorticotrophin (human chorionic corticotrophin) may also be secreted.

Human chorionic gonadotrophin

STRUCTURE

Ascheim and Zondek (1927) first demonstrated the presence of gonadotrophic substances in the urine of pregnant women. Such urine injected into infantile mice induced premature oestrus with follicle ripening and luteinisation. hCG is a glycoprotein structurally related to the pituitary hormones TSH, FSH, and LH and comprises two subunits (Reichert *et al.*, 1970; Pierce and Liao, 1970). The α-subunit (92 amino-acids) shows extensive structural sequence homology with FSH, LH, and TSH, and the β-subunit (145 amino-acids) is similar to that of LH apart from an extra 30 C-terminal amino-acids (Swaminathan and Bahl, 1970). The carbohydrate content comprises over 20% of the molecule (Diczfalusy and Troen, 1961) and is essential for biological activity (Whitten, 1948).

Isolated α- and β-subunits possess very little intrinsic biological activity but, because of the close structural similarities, an α-subunit of hCG can be combined with the β-subunit of LH or TSH with restoration of a high degree of biological activity appropriate to the β-subunit (Pierce, 1971). Interestingly, the β-subunits of all the glycoprotein hormones show a significant degree of homology, suggesting that they evolve from a common ancestral gene (Pierce, 1971; Acher, 1976) (Fig. 1).

SITE OF SYNTHESIS

In the early placenta maternal blood circulates in the intervillous space and bathes the chorionic villi. The chorionic villus comprises a core of fetal mesenchyme and fetal capillaries outside which is the trophoblast. The latter consists of an outer syncytial layer, the syncytiotrophoblast, and an inner layer, the cytotrophoblast, which disappears late in pregnancy. The syncytiotrophoblast is differentiated into thin areas overlying fetal capillaries and thicker areas not adjacent to capillaries which are probably responsible for the synthetic functions of the placenta. Studies using fluorescent antibodies have shown that hCG is probably synthesised and secreted by the syncytiotrophoblast (Midgley and Pierce, 1962). Thiede and Choate (1963) suggested that hCG synthesis may begin in the cells of the cytotrophoblast before their transition into the syncytium, but in a more recent study of placentas obtained between 6 and 41 weeks gestation the hormone was demonstrated only in the syncytial layer and a few cells of the amniotic epithelium (de Ikonicoff and Cedard, 1973).

The concentration of hCG in maternal serum reaches a peak around the 8th to the 10th week of gestation and the normal trophoblast secretes around

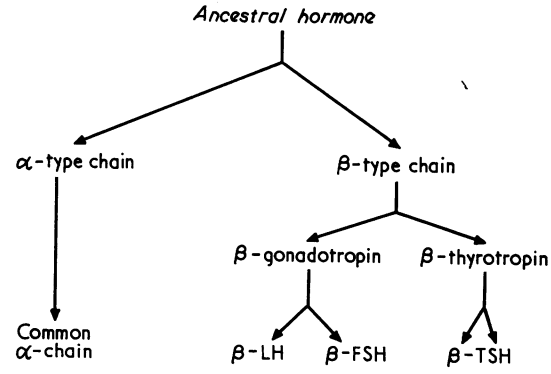

Fig. 1 *Hypothetical evolution of the dimeric hormones luteinising hormone (LH), follicle stimulating hormone (FSH), and thyrotrophin (TSH). Duplication of an ancestral structural gene and subsequent differentiation led to an α-type chain and a β-type chain. Successive duplications of the β-gene and selective evolution gave the specific β-chains of three hormones (Acher, 1976).*

$1{\cdot}4 \times 10^{-2}$ IU hCG/day. The trophoblast neoplasm, choriocarcinoma, has much reduced hCG synthetic capability both *in vivo* and *in vitro*.

The introduction of highly sensitive and specific radioimmunoassays and radioreceptor assays for hCG (Vaitukaitis *et al.*, 1972) have clearly demonstrated secretion of the hormone within a few days of conception (the basis of an early pregnancy test) and *in-vitro* studies using placental slices have shown that isolated α- and β-subunits are secreted as well as intact hCG (Franchimont *et al.*, 1972).

BIOLOGICAL FUNCTIONS OF hCG

The biological role of hCG is unknown. Various hypotheses put forward include stimulation of the corpus luteum to maintain progesterone output, a possible direct effect on the fetal testes causing sexual differentiation, and depression of maternal immunity preventing rejection of the fetus.

Early progesterone secretion by the maternal ovary is believed to prevent expulsion of the ovum by 'damping down' spontaneous uterine contractility through a direct membrane effect rendering the uterine muscle insensitive to circulating endogenous oxytocin (progesterone block) (Bengtsson and Schofield, 1963). Thus in some species such as the sheep progesterone levels fall precipitously before the onset of labour. This fall may facilitate an increase in concentration of uterus-stimulating prostaglandins, particularly $PGF_2\alpha$, into the utero-ovarian vein blood (Liggins *et al.*, 1973). Such a clear cut relationship does not exist in man, however, and an inhibiting effect of progesterone has not been observed (Fuchs and Stakemann, 1960). Nevertheless, Csapo *et al.* (1971) found significantly lower progesterone levels in women whose labour was short. The evidence suggests that progesterone, like maternal oestradiol, is more likely to play a facilitatory role in labour than to be the initiating agent (Turnbull *et al.*, 1977). This whole field has been the subject of a recent Ciba Foundation Symposium (No. 47, New Series). hCG has been implicated in the sexual differentiation of the male fetus but the peak of fetal plasma testosterone does not coincide with the hCG peak or with the fetal pituitary gonadotrophin peak, the latter occurring between the 16th and the 24th week of gestation (Fig. 2).

hCG also has TSH-like activity and high concentrations can be shown to interfere with the binding of labelled TSH to human and animal thyroid membranes and thus, by inference, to bind to the TSH receptor or a closely allied site (Hall, 1977). It has not so far been shown to activate adenylate cyclase in the human thyroid and it has not been possible to administer it in sufficiently high doses to examine the other aspects of TSH activity. Clinical

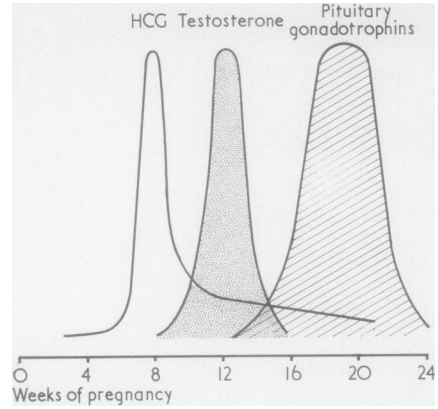

Fig. 2 *Immunoreactive HCG, testosterone, and LH in the fetal circulation in relationship to gestational age.*

hyperthyroidism, however, does occur in women with trophoblastic neoplasia (Odell *et al.*, 1963), thus providing indirect evidence for a thyroid stimulating role of hCG. Kenimer *et al.* (1975) have shown that on a molecular basis hCG contains about 1/4000 of the thyrotrophic activity of human pituitary TSH.

CLINICAL USES OF hCG

Over the years hCG has had important therapeutic and diagnostic roles in reproductive endocrinology. Thus injection of purified hCG has been used to induce ovulation in anovulatory women, and the specific measurement of serum hCG by radioimmunoassay can now be used to diagnose pregnancy within a few days of fertilisation. Sensitive specific hCG measurements can also be used to monitor the treatment of choriocarcinoma (Bagshawe, 1969), and this, in combination with advances in chemotherapeutic regimes, has dramatically reduced mortality of this otherwise uniformly lethal disease. Finally, the possibility remains, albeit remote, that immunisation against hCG could be used to control human fertility (Stevens, 1975).

Human placental lactogen

STRUCTURE

Josimovich and MacLaren (1962) found a substance in human placenta and in fetal and maternal serum which had lactogenic activity *in vivo* and *in vitro* and they showed its partial immunological identity with human growth hormone (hGH) of pituitary origin. This substance was therefore called human placental lactogen (hPL). However, both its somatotrophic and lactogenic actions are very weak (Florini *et al.*, 1966) although about 80% of the amino-acid residues of

hPL and hGH are identical, with close similarities in their sequences. These two hormones also show considerable homology with human prolactin, which suggests that all three hormones evolve from a common ancestral polypeptide.

SITES OF SYNTHESIS AND SECRETION

Josimovich and Atwood (1964) showed that hPL could be extracted from placental tissue but not from pituitary tissue of pregnant and non-pregnant women or from the fetus. hPL was later localised by immunofluorescent techniques to the syncytiotrophoblast (Sciarra *et al.*, 1963), though some doubt has been cast on these findings (Gau and Chard, 1976). Studies using cultured fragments of placentas taken at various stages of gestation show that hPL is present in the supernatant medium (Grumbach and Kaplan, 1964), and specific incorporation of ^{14}C-labelled amino-acids into hPL by placental fragments taken at term has been clearly shown by radioimmunoelectrophoresis (Gusdon and Yen, 1967).

In the last trimester of pregnancy the amount of hPL synthesised by the placenta is of the order of 1 g per day (Kaplan *et al.*, 1968), a rate of production greatly in excess of that of any other peptide hormone. Thus a woman in the last month of pregnancy secretes more hPL than she does insulin in her entire life. The high production rate, despite a short half life of 10-20 minutes, explains the very high circulating hPL levels at term—about 6 mg/l of plasma.

hPL secretion seems to be autonomous. Thus manipulation of blood glucose and insulin levels, known to alter both hGH and human prolactin secretion, have only minor effects on hPL (Spellacy *et al.*, 1971) and hPL levels rise progressively in the maternal circulation throughout gestation (Fig. 3). The measurement of hPL levels by immunoassay is a well established test for abnormal pregnancy (Letchworth *et al.*, 1971), and serial measurements in a particular 'at-risk' patient may be of great value as an indicator of failing placental function (Letchworth and Chard, 1972). This test, however, reflects only placental function whereas the measurement of urinary oestrogens, especially oestriol, gives an indication of both fetal and placental wellbeing.

BIOLOGICAL FUNCTIONS

As with hCG, the biological role of hPL remains unclear. Although hPL levels are not related to blood glucose levels it has been suggested that hPL may be responsible for some of the metabolic changes that occur in pregnancy, including reduced glucose tolerance and the rise in free fatty acids (Grumbach *et al.*, 1966). hPL does possess weak anti-insulin activity—an action which may involve

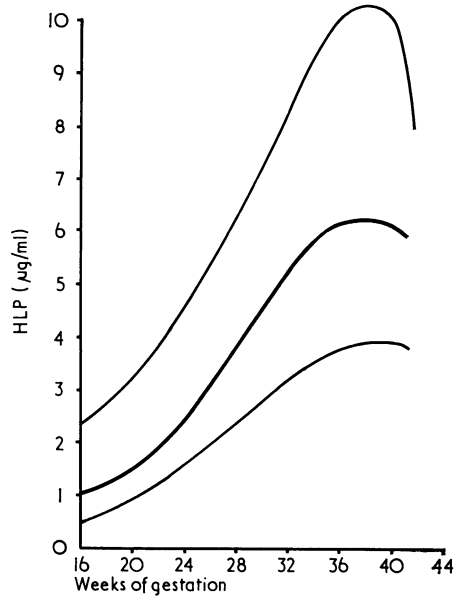

Fig. 3 *Immunoreactive human placental lactogen levels throughout normal pregnancy showing the mean and two standard deviations. Note that the distribution is skewed, being greater above the mean than below it (Courtesy Professor T. Chard).*

synergism with placental steroid hormones such as oestrogens and progesterone.

Thyrotrophic hormone

The evidence for the existence of a placental thyroid stimulating hormone is sparse. However, two thyroid stimulating substances have been reported—human chorionic thyrotrophin (hCT) (Hershman and Starnes, 1969) and human 'molar'* thyrotrophin (hMT) (Hershman and Starnes, 1971). hCT is the smaller molecule and shows immunological cross-reactivity with some anti-human TSH antisera whereas hMT does not. hMT is the longer acting *in vivo*. At present this subject remains rather confused and the existence of a true placental thyroid stimulator separate from hCG remains to be established.

Adrenocorticotrophic hormone

That pregnant women have plasma and urinary free cortisol levels which are higher than normal has long been known, but in man there is not the clear association between rising corticosteroid levels in the fetus and the onset of parturition that is seen in other species such as the sheep. The high cortisol levels

*Derived from a hydatidiform mole.

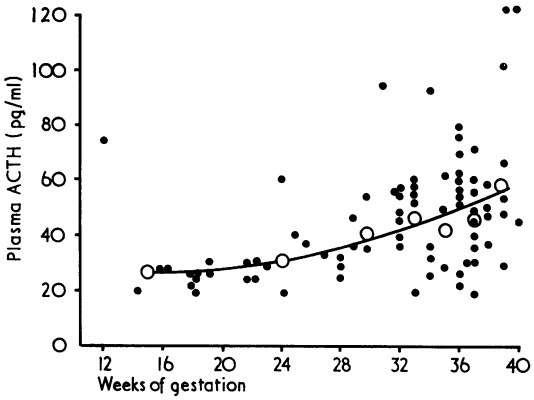

Fig. 4 *Maternal plasma ACTH levels in normal pregnancy. Individual levels are shown (●) together with the logarithmic mean (○) estimated for 10-20 weeks, 21-27 weeks, 28-31 weeks, 32-33 weeks, 34-35 weeks, 36-37 weeks, and 38-40 weeks. Note a few high levels which may have been caused by unrecognised stress during sample collection.*

show resistance to suppression by exogenous steroids (Rees *et al.*, 1975), especially in late pregnancy. There is also a progressive though not dramatic increase in maternal circulating ACTH levels throughout pregnancy (Fig. 4). These facts accord with an extrapituitary source of ACTH not subject to normal feedback mechanisms. The levels of ACTH in extracts of placenta considerably exceed those that can be attributed to the contained blood (Genazzani *et al.*, 1975; Rees *et al.*, 1975); the material extracted is immunologically identical with pituitary ACTH and is also biologically active in the cytochemical ACTH bioassay (Rees *et al.*, 1975).

If a placental source of ACTH is accepted its part in the control of the fetal adrenal must still remain in doubt. As with the other placental hormones already discussed, maternal levels are two to three orders of magnitude greater than those in the fetus, and there is no evidence to suggest that placental ACTH behaves differently from the pituitary hormone, although its relatively low molecular weight (4500) might enable it to diffuse more freely in both directions. The possibility that placental ACTH provides a drive to the fetal adrenal cannot be excluded (Chard *et al.*, 1977).

Placental hormones and the immunology of pregnancy

Why the fetus is not rejected by the mother as a foreign antigen is a subject that has recently been extensively reviewed (Beer and Billingham, 1976). Several facts are clear. Firstly, the fetus is antigenic and possesses a full complement of both paternal and maternal antigens. Secondly, the mother is capable of reacting immunologically to these antigens. There must therefore exist some barrier between the two which could depend on special properties of the syncytiotrophoblast or in the hormones synthesised therein. Both hPL and hCG can inhibit the lymphocyte transformation induced by phytohaemaglutinin (a T-cell mitogen) '*in vitro*'—generally held to be an indication of immunocompetence. Also the carbohydrate moiety of hCG may represent a surface antigen on trophoblastic cells, by analogy with the carriage of ABO blood group antigens by some glycoproteins of saliva (Adcock *et al.*, 1973). Thus a high concentration of hCG surrounding the trophoblast at implantation could block the rejection of the trophoblast by maternal lymphocytes. However, hormones analogous to hCG and hPL have not been demonstrated in the trophoblast of other species, such as the rat. Consequently, unless other hormones with similar effects on lymphocytes can be shown to be synthesised by trophoblastic cells in such species any major role for hCG and hPL in the immunology of pregnancy must remain suspect. Further discussion of this fascinating subject is outside the scope of this paper.

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