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Newly Discovered Activities for Calcitriol (1,25-Dihydroxyvitamin D_3): Implications for Future Pharmacological Use

Robert U. Simpson and Ronald Weishaar

Summary

Recent studies have yielded new insights into the critical importance of adequate vitamin D_3 intake and metabolism. Investigations of the actions of 1,25-dihydroxyvitamin D_3 (calcitriol) on novel target tissues has revealed that this hormone has functions other than its recognized action in regulating blood calcium and phosphate levels. Reports have characterized calcitriol receptors and activities in organs and tissues as diverse as pancreas, skeletal and heart muscle, blood cells, brain, skin, pituitary, parathyroid, kidney, bone and intestine. These studies suggest functions for calcitriol as varied as the regulation of insulin and prolactin secretion, muscle contractility, immune cell metabolism, melanin synthesis and differentiation of blood cells. This information may ultimately help us to understand the etiologies of several kinds of organ dysfunction and lead to the development of tissue-specific agents for new therapies.

Introduction

Studies of the function and endocrinology of vitamin D_3 over the last 80 years have elucidated the role of this prohormone in regulating calcium and phosphate concentrations in blood. During this period, great increases in our understanding of the significance of vitamin D have taken place. First the identification of the antirachitic factor and its importance in controlling blood calcium and phosphate levels was a major insight in basic physiology and had important therapeutic application. Secondly, studies on the metabolism of vitamin D led to the identification of the hormone, 1,25-dihydroxyvitamin D_3 or calcitriol. This discovery resulted in the rationale for clinical use of calcitriol in renal disease and increased our understanding of the basic mechanisms and action of this steroid hormone. The expression of vitamin D action is now known to involve the functioning of intracellular receptors, the regulation of gene transcription and the translation of mRNA. Initially, investigators extensively characterized a specific receptor for 1,25-dihydroxyvitamin D_3 in intestine and bone. Recently, calcitriol receptors have been characterized in tissues not originally recognized as calcitriol responsive.[1–3] Subsequent *in vivo* and *in vitro* studies have to some extent corroborated receptor studies and demonstrated previously unappreciated functions for calcitriol. Current data now suggest that 1,25-dihydroxyvitamin D_3 affects the functioning of organ systems that are not directly responsible for regulation of blood calcium and phosphate. We refer to excellent reviews on the vitamin D system as sources of reference for much of the discussion in this text.[4–6] It is the intention of this report to review the recent studies and summarize the information that pertains to our expanded view of calcitriol for health. Our intention is also to suggest future possible pharmacologically important uses for the vitamin D hormone.

REVIEW ARTICLES

Regulation of Vitamin D_3 Metabolism

The observation that a time lag exists between vitamin D_3 administration *in vivo* and changes in bone and intestinal metabolism, together with the finding that vitamin D_3 lacks activity *in vitro*, suggested that vitamin D_3 required bioactivation to produce its effects. Subsequent studies established that a metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3 (calcitriol), is the functionally active form of the vitamin, and that its metabolism is tightly controlled.

The bioactivation of vitamin D_3 can be influenced by a number of factors, including serum calcium and phosphate levels, calcitriol itself, parathyroid hormone, estradiol and prolactin. Circulating levels of calcitriol are increased in response to reductions in serum levels of calcium and phosphate. This response is apparently mediated in part by parathyroid hormone, since it has been shown that parathyroid hormone exerts a direct stimulatory effect on 1-α-hydroxylase activity.[7] In addition, parathyroidectomy has been shown to blunt the increase in circulating calcitriol levels observed during lactation in rats.[4-6] Estradiol and prolactin also stimulate the bioactivation of calcitriol, and the dopamine agonist bromocryptine – which decreases prolactin secretion – has been shown to lower plasma levels of calcitriol in rats.[4-6] Organs involved in regulating 1,25-dihydroxyvitamin D_3 metabolism *in vivo* are illustrated in Fig. 1.

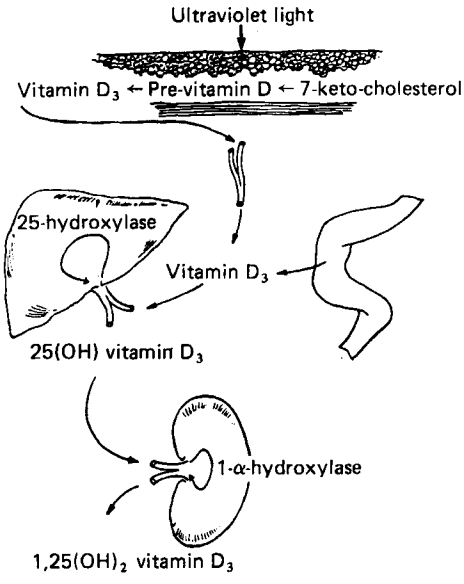

Fig. 1. *Organs involved in metabolism of vitamin D.*

Pharmacokinetics, Structure-Activity Relationships and 'Vitamin D Receptor Agonists and Antagonist'

Vitamin D_3 can enter the circulation in two ways. In the skin, ultraviolet light catalyzes the conversion of 7-ketocholesterol to pre-vitamin D_3, which then thermally equilibrates to vitamin D_3. Vitamin D_3 is also readily absorbed from the small intestine. Vitamin D_3 and its metabolites are transported in the circulation bound to a 52,000 molecular weight protein which has been termed the serum vitamin D binding protein[3-6].

Like vitamin D_3, calcitriol is also orally active, and absorption occurs in the proximal portion of the small intestine. The absorption of calcitriol appears to require complex formation with bile salts, and such absorption can be impaired by biliary cirrhosis. Studies have demonstrated that when given chronically via oral administration, 25 hydroxyvitamin D_3 is more effective than calcitriol for maintaining serum calcium and in promoting bone mineralization. These observations likely indicate that calcitriol is subject to rapid inactivation or elimination; an important consideration when attempting to develop useful therapeutic agents which act as 'vitamin D agonists'. In this regard, it has been shown that fluorination of calcitriol to produce 24,24-difluoro-calcitriol increased the biopotency and serum half-life of this analogue.[8] It has also been reported that 1-fluoro-vitamin D_3 possesses a selective stimulatory effect on bone calcium mobilization, compared to intestinal calcium transport.[9]

A number of analogs of calcitriol have been evaluated for biological activity and/or therapeutic utility. Several recent studies have employed competitive binding experiments to identify structural features which enhance the activity of various vitamin D_3 congeners as calcitriol receptor agonists. For such studies the ability of these congeners to compete with [^{3}H]calcitriol for binding sites on the intracellular receptor binding protein is evaluated. Such experiments have demonstrated that the 1-α-OH group contributes substantially to the potency of vitamin D_3. Activity is also enhanced considerably by the presence of a 25-OH group, with calcitriol being 100 to 1,000-fold more potent than 1α-hydroxyvitamin D_3. The presence of two adjacent hydroxyl groups, e.g. 1,24R-25-trihydroxyvitamin D_3, impairs interaction with the vitamin D receptor, and lowers potency. Structural considerations for vitamin D_3 metabolites and synthetic vitamin D analogues have been described in detail by Stern.[6]

To date, no compound has been identified which exerts a classic antagonist effect (high-affinity binding, no intrinsic activity) on the intracellular vitamin D receptor. However, a number of agents have been shown to impair calcitriol synthesis and may, therefore, be considered to 'antagonize' the response to vitamin D_3. Such agents include inhibitors of 25-hydroxylase such as 24-nor-25-hydroxy vitamin D_3, which also lacks intrinsic vitamin D receptor 'agonist' activity.[4-6] Several metabolites of vitamin D_3 have been shown to inhibit 1-α-hydroxylase activity; however, these metabolites also possessed intrinsic receptor 'agonist' activity.[4-6]

Intestine

Our greatest understanding of the mechanism of action of calcitriol on subcellular processes comes from studies of its effects on the intestine. Vitamin D, acting via its metabolite, calcitriol, stimulates Ca^{2+} transport from the lumen of the intestine through the cell and into the blood. Along with the intestinal transport of Ca^{2+}, calcitriol stimulates the transport of PO_4^{2-} from intestinal lumen to blood.[4-6] It is now recognized that calcitriol induces the synthesis of a cytosolic CaBP.[10] This action most likely does not account for all the processes activated by calcitriol and required for calcium transport across the intestinal epithelia. Therefore, many studies have concerned themselves with identifying other proteins or factors that are synthesized in intestine and under the control of calcitriol. For example, a membrane-bound calcium-binding protein complex possessing phosphatase activity has been reported to be under the direct control of vitamin D.[11] A different mechanism of action – alteration of membrane phospholipid composition – for calcitriol has been supported by Rasmussen and coworkers.[12] These data suggest that the activity of calcitriol might not be explained entirely by the activation of nuclear transcription of specific genes. Clearly, much work remains to be done before a clear understanding of how calcitriol affects intestinal cell processes is reached.

Osteoporosis

Vitamin D-dependent rickets was cured with the therapeutic application of the anti-ricket factor vitamin D. Recently, it

was also shown that calcitriol is of great therapeutic use in treatment of such diseases as renal osteodystrophy, vitamin D-resistant rickets due to an inborn error of vitamin D metabolism, and certain hypoparathyroid disorders.[4-6] Osteoporosis is an age-related disorder that affects over 15 million persons in the United States. The bone wasting associated with this disease suggests the possible therapeutic usefulness of calcitriol for the treatment of osteoporosis. Initial clinical studies focused on the action of the prohormone, vitamin D, on postmenopausal osteoporosis.[13] These studies did not demonstrate a therapeutic use of vitamin D for treatment of osteoporosis. It has been argued that use of vitamin D for the treatment of postmenopausal osteoporosis has not been effective due to decreased metabolism of vitamin D to the active metabolite calcitriol in the aged.[14] Analysis of serum calcitriol supports this contention, showing that postmenopausal women have a significant decrease in serum calcitriol levels. Studies using 0·25 μg calcitriol showed that calcitriol was ineffective in treating this disease.[15] Others, however, using higher concentrations of calcitriol, have demonstrated significant improvement of bone parameters in osteoporetic patients.[16] Further clinical testing of calcitriol for the treatment of postmenopausal osteoporosis is on-going and essential for establishing the therapeutic usefulness of calcitriol.

Kidney

A major action of calcitriol is to regulate renal 25-hydroxyvitamin D_3-1-α-hydroxylase (1α(OH)ase). It has been shown that calcitriol brings about a suppression of 1α(OH)ase concomitant with an increase in 25-hydroxyvitamin D_3-24-hydroxylase (24(OH)ase). These observations have been supported by *in vivo* and *in vitro* studies.[4-6] This action of calcitriol is analogous to the action of other steroid hormones to effect a feedback inhibition of their synthesis. The feedback inhibition of 1α(OH)ase is dependent on blood calcium concentrations since it has been observed that calcitriol will, in fact, stimulate its own synthesis in hypocalcemic animals.

Possible actions of calcitriol on renal calcium and phosphate handling are not at present fully characterized. Studies have shown that vitamin D increases renal retention of calcium. Also, it has been shown that vitamin D increases renal absorption of phosphate. However, these *in vivo* studies could not eliminate the possible secondary action of other renal active agents to elicit these effects. In spite of this, calcitriol receptors have been demonstrated in distal renal tubule cells, cultured kidney cells and kidney homogenates.[3-6] Further evidence that calcitriol has a direct effect on kidney functions comes in the data showing that a specific vitamin D-dependent calcium-binding protein is present in kidney tubule cells.

Vitamin D and Skeletal Muscle

Several authors have suggested a potential role for vitamin D in the regulation of skeletal muscle contractile function. Such involvement is based upon the observation that vitamin D deficiency is associated with skeletal muscle myopathy and muscle fibre atrophy, conditions which are reversible upon administration of vitamin D or its metabolites.[17] In addition, Curry *et al.* have shown that the rate of calcium uptake by skeletal muscle sarcoplasmic reticulum isolated from vitamin D-deficient rabbits is significantly reduced compared with that of sarcoplasmic reticulum isolated from normal rabbits.[18] This reduction is apparently not related to any change in the ATPase activity of the sarcoplasmic reticulum. We reported that receptors for calcitriol exist in skeletal muscle myoblasts and at significantly lower concentrations in excised muscle tissue.[19] Others have demonstrated that calcitriol stimulated calcium transport in cultured muscle myoblasts.[20] A possible dihydroxyvitamin 1,25D_3 specific effect on muscle myoblasts is currently being studied in our laboratory.

In a recent study, Wassner and co-workers reported that weight gain and skeletal muscle mass were reduced in vitamin D-deficient rats, and that myofibrillar protein degradation was increased.[21] According to the authors, these changes were associated with the onset of hypocalcemia and could be reversed by the addition of vitamin D to the diet or by feeding the vitamin D-deficient rats diets that contained sufficient quantities of calcium to restore circulating calcium to normal levels. Thus, although vitamin D repletion leads to improved muscle protein anabolism and an increase in skeletal muscle mass and weight gain, these effects have yet to be proved to be a result of the direct action of vitamin D or a vitamin D-derived metabolite on skeletal muscle.

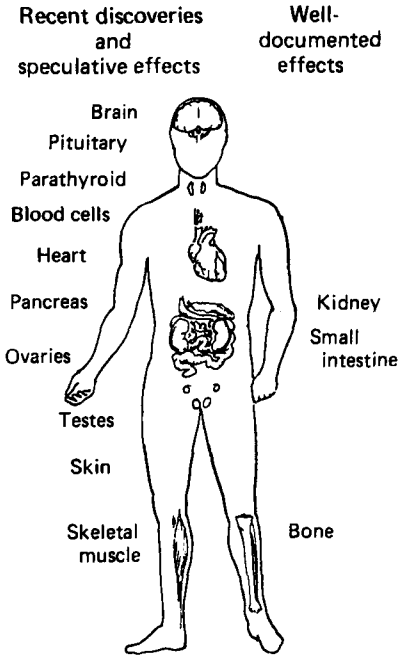

Fig. 2. *Target tissues for calcitriol.*

Vitamin D and Cardiac Muscle

It has been known for many years that administration of pharmacological quantities of vitamin D can produce myocardial failure, presumably due to hypercalcemia and calcification of cardiac muscle. Such hypercalcemia can persist for months following discontinuation of vitamin D administration. Furthermore, vitamin D has been reported to alter the ultra-structure of cardiac muscle endoplasmic reticulum.[22] A physiological role for vitamin D in influencing myocardial calcium homeostasis has been suggested by the recent observation of Simpson *et al.* that cardiac cells grown in culture contain a specific receptor for calcitriol.[23] This receptor has also been identified in whole left ventricular muscle from adult rats. In addition, Thomassett and co-workers have shown that the 10K vitamin D-dependent calcium-binding protein, but not the 28K calcium binding protein, is present in rat heart muscle.[24] The relevance of this latter observation is not known at the present time.

Receptors for calcitriol have also been identified in uterine muscle.[25]

Cancer Cells

The initial observation that cancer cells possess calcitriol receptors was that of Eisman and collaborators.[26] Since this report, a number of malignant tumors and cell lines have been characterized as

possessing calcitriol receptors. Furthermore, calcitriol has been shown to inhibit proliferation of breast and melanoma cell lines and to initiate terminal differentiation of myeloid leukemia cells.[27]

Possible clinical usefulness of calcitriol for control of certain malignant cancers was supported by studies that correlated 1,25-dihydroxyvitamin D_3 receptor concentrations and potency of supra-physiological levels of 1,25-dihydroxyvitamin D_3 action to inhibit cancer cell proliferation.[28] Furthermore, it has been shown that survival time of mice injected with leukemia cells was greater in animals dosed with calcitriol than control mice.[29] Obvious disadvantages are present in the use of calcitriol for control of endocrine-associated malignancies. Hypercalcemia and bone wasting induced by this agent would be prevalent side-effects. However, the use of calcitriol in concert with hypocalcemic agents (e.g. diphosphonates, glucocorticoids) may prove useful. Future development of vitamin D analogs that possess cancer-inhibitory activities but are devoid of calcium-mobilizing actions may permit therapeutic application of calcitriol.

The observations that calcitriol receptors exist in leukemia cells and that calcitriol stimulates differentiation of these cells into mature monocytes suggests that the hormone may be involved in the regulation of monocyte differentiation. Recently, this work has been extended to show that calcitriol stimulates the aggregation of precursor cells into poly-nuclear osteoclastic-like cells. This process was suggested as a possible mechanism for calcitriol-induced osteoclastic bone reabsorption. Receptors for calcitriol have also been characterized in activated T cells.[30] Therefore, a function for calcitriol in the immune response may exist.

Skin, Brain and Sex Tissue

Calcitriol receptors have been demonstrated in mammary and skin tissue.[1-3] A possible role of calcitriol in calcium handling by skin, sweat glands and mammary glands is suggested. However, little data on hormone action have been presented. An increase in 7-dehydrocholesterol levels in skin due to calcitriol has been identified. This suggests that calcitriol may have a feedback-inhibitory action on the synthesis of vitamin D_3 in skin (see ref. 3 for review). Recently, Hosoi *et al.* demonstrated that calcitriol exerted a time- and dose-dependent stimulatory effect on melanin synthesis in B16 mouse melanoma cells.[31] This stimulatory effect was apparently the result of an increase in the activity of tyrosinase, a key enzyme involved in regulating melanin synthesis. According to the authors, since pigmentation of the skin prevents the penetration of sunlight to the dermis, stimulation of melanin synthesis by calcitriol may represent a negative feedback mechanism for suppressing the conversion of pre-vitamin D_3 to vitamin D_3.

The existence of a brain–pituitary axis for calcitriol effects was suggested by autoradiographic studies of Stumpf *et al.*[32] Specific localization of [^{3}H]calcitriol in neurons of rat forebrain, hindbrain and spinal cord was demonstrated. Receptors have also been identified for calcitriol in ovaries and testes; however no clear functional role for calcitriol in these tissues has as yet been identified.[1-3, 33]

Exocrine Function of Calcitriol

The presence of calcitriol receptors in parathyroid, pancreas, testes, ovaries and pituitary has raised the possibility of a direct action of this hormone in the regulation of hormone synthesis and secretion. We will review the data presented to support a functional role of calcitriol in exocrine cell processes.

Pancreas

Recent studies have provided evidence that vitamin D plays a role in regulating pancreatic metabolism. Such an involvement is supported by several observations, including the presence of a receptor for calcitriol in the pancreas, and of a vitamin D-dependent calcium-binding protein (CaBP).[34-35] Kadowaki and Norman have shown that the pancreatic CaBP is homologous with the intestinal CaBP.[5] In addition, changes in dietary calcium and phosphorus, as well as changes in circulating levels of calcitriol, produce comparable changes in pancreatic and intestinal CaBP levels (for review see ref. 5).

In 1980, Norman and co-workers demonstrated that vitamin D deficiency inhibited insulin secretion from the perfused pancreas, whereas vitamin D repletion improved insulin secretion.[36] An involvement of vitamin D with insulin secretion is also implicated by the observation that although pancreatic levels of CaBP are low compared with levels in the intestine or the kidney, pancreatic CaBP is present exclusively in the B-cells.[5] Together these observations imply a physiological role for vitamin D in modulating calcium metabolism in the pancreas, and indicate that vitamin D may exert an influence on insulin secretion as well. However, vitamin D deficiency also leads to decreased food intake and hypocalcemia. The direct action of calcitriol on insulin secretion therefore remains to be firmly established.

Parathyroid

There is *in vivo* and *in vitro* evidence to support a direct action of calcitriol in regulating secretion of parathyroid hormone (PTH) from the parathyroid gland. Evidence has been presented showing that a calcitriol receptor exists in excised parathyroid tissue from avian and mammalian species.[34] Furthermore, immunochemical vitamin D-dependent CaBP have been localized to the parathyroid.[24] Functional studies have suggested that calcitriol in normal calcemia can suppress the secretion of PTH. However, *in vitro* studies designed to demonstrate a direct action of calcitriol on parathyroid tissue have yielded ambiguous data. It has been shown that calcitriol inhibited, stimulated or had no effect on release of PTH from parathyroid cells dosed *in vitro* (for review see ref. 3). These discrepancies in results might be explained by the nutritional status of tested animals or in terms of the *in vitro* incubation conditions for PTH release. Recent studies, however, do support an *in vivo* role for calcitriol in regulation of PTH secretions.[37]

Pituitary

Pituitary cells have been shown to possess specific calcitriol receptors and the vitamin D-dependent calcium-binding protein.[1, 34] It has recently been shown that calcitriol has a specific and selective action to increase prolactin synthesis and secretion from pituitary cells.[38] This has been shown measuring the peptide and specific mRNAs for prolactin. The effect of 1,25-dihydroxyvitamin D_3 on prolactin mRNA synthesis was diminished in low-calcium-containing media. These findings demonstrate that calcitriol stimulates prolactin gene expression and that calcium is involved in this expression. In addition, autoradiography and immunohistochemistry techniques have provided evidence that calcitriol specifically localizes in those cells that secrete thyroid-stimulating hormone.[39] These data indirectly suggest that calci-

triol may modulate thyroid hormone secretion.

The presence of receptors for calcitriol in exocrine tissues suggests a functional role for calcitriol in endocrine-controlled processes. However, with the exception of the action of calcitriol on PTH secretion, a clear picture of the regulation of hormone secretion by calcitriol remains to be established, and further studies are required to translate *in vitro* observations of calcitriol secretory control to *in vivo* relevance.

Conclusions

During the last two decades the important role of calcitriol in regulating bone mineralization and intestinal calcium transport has been well documented. Moreover, the ability of calcitriol to treat renal osteodystrophy has been firmly established. In addition, clinical studies are currently under way that are aimed at demonstrating the ability of calcitriol to prevent the onset or retard the progression of osteoporosis.

Several recent studies have shown that in addition to its effect on bone, intestine and kidney metabolism, calcitriol may play an important role in regulating key metabolic processes in a number of other organs and cells, including pancreas, skin, cancer cells, cardiac and skeletal muscle, brain, parathyroid gland, testes and ovaries. These processes include insulin secretion, muscle contractility, cell differentiation and melanin synthesis. Such discoveries have prompted interest in the potential utility of vitamin D receptor agonists and antagonists, and agents which may influence 1,25-dihydroxyvitamin D_3 action as potential therapeutic agents for the treatment of diabetes, cancer, muscle myopathy, and other pathological conditions. Although there exists a paucity of such compounds, the tissue-selective effects of an analog of vitamin D indicate that it may be possible to design agents which limit the potent hypercalcemic effect of 1,25-dihydroxyvitamin D_3. If made available, tissue-specific calcitriol agonists and antagonists may provide useful therapeutic agents for novel treatment of endocrine diseases.

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REVIEW ARTICLES

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Human Choriogonadotropin

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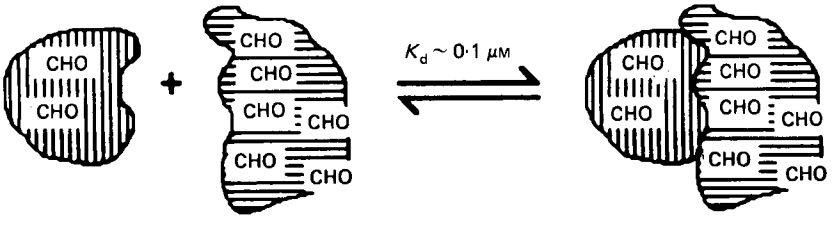
Summary

Human choriogonadotropin, a hormone derived from the syncytiotrophoblast cells of the placenta, is a member of the glycoprotein hormone family which also contains the pituitary hormones lutropin, follitropin and thyrotropin. These four hormones are comprised of two dissimilar subunits, one (α) being common to all four and the other (β) conferring hormonal specificity. Information is rapidly accumulating on the nature and regulation of the genes for these subunits, as well as the structural aspects, mechanism-of-action and physiological roles of these complex hormones. This mini review considers some of the recent advances in our understanding of human choriogonadotropin.

The Glycoprotein Hormones

There are four characterized members of the human (h) glycoprotein hormone family: choriogonadotropin (CG, generally designated as hCG), lutropin (LH, luteinizing hormone), follitropin (FSH, follicle-stimulating hormone) and thyrotropin (TSH, thyroid-stimulating hormone). hCG is a product of the syncytiotrophoblast cells of the placenta, and the other hormones are synthesized in and secreted by specific cell types of the adenohypophysis (i.e. the anterior lobe of the pituitary). There has also been a suggestion of an hCG-like material in human pituitary, and hCG and subunits are secreted by certain tumors. Each hormone contains a common α subunit and hormone-specific β subunit; only the αβ complex (Fig. 1) exhibits significant biological activity.

hCG exhibits LH-like effects and is responsible for maintenance of the corpus luteum and stimulation of progesterone production during the first 6–8



	α	β	αβ (hCG)
M_r (P)	10·2 K	15·5 K	25·7 K
M_r (C)	4·3 K	6·7 K	11·0 K
M_r (t)	14·5 K	22·2 K	36·7 K
No. of Res	92	145	237
No. of S-S	5	6	11
No. of N-l	2	2	4
No. of O-l	0	4	4

Fig. 1. *Schematic representation of the reversible formation of hCG from the constituent subunits; the K_d is about 0.1 μM. The α subunit is common to the four human glycoprotein hormones, and the β subunit confers hormonal specificity. Characteristics of the α and β subunits and the αβ dimer are provided. M_r(P), M_r(C) and M_r(t) denote the respective molecular weights (K = kilodaltons) of the polypeptide portion, the carbohydrate (CHO) portions (the subunits are heterogenous, and the values given are averages) and the total (i.e. polypeptide plus carbohydrate). The number of amino acid residues (Res), disulfides (S—S), N-linked oligosaccharides (N-l) and O-linked oligosaccharides (O-l) are also given. The disulfide pairings are controversial, but there is general agreement of disulfide bonds between half-cystines 7–31 and 10–32 in the α-subunit and between 23–72, 26–110 and 93–100 in the β subunit. Additional information is provided in refs. 4–6.*