Endogenous mediators of growth

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Growth comprises three separate components; whole-body growth, tissue turnover and repair, and last, the response to external stimuli. Whole-body growth can be divided into changes associated with muscle, skin, viscera and skeleton. Because of our interest in bone metabolism the present review will concentrate on the processes associated with skeletal growth, turnover, and response to external stimuli. Although some of the factors which will be discussed are specific to bone the majority affect other body tissues.

There are too many endogenous mediators of growth to list in a review such as this. Basically such mediators can be divided into two groups (Table 1), those which have a systemic action such as growth hormone (GH) and those which act locally such as the transforming growth factors (TGF). Of those systemic factors, some such as GH have a general effect on a number of tissues. However, there are also systemic factors such as gonadotropins and ACTH which have trophic actions on particular target organs. Endogenous mediators of growth are varied as well as numerous. For instance, calcium can be considered a growth mediator for the parathyroid gland as prolonged hypocalcaemia results in a hypertrophy of the parathyroid gland.

Bone growth and metabolism rely on the action of a number of factors affecting the proliferation and differentiation of the major bone cell types and modulating their activity. The skeleton serves a structural role, but it is also responsible for maintaining normocalcaemia. These two functions may occasionally be contradictory but the primary role is the maintenance of serum Ca and at times of Ca stress (e.g. pregnancy and lactation) this occurs at the expense of skeletal integrity. Therefore, skeletal growth and metabolism are governed by two series of factors; those which control skeletal growth (e.g. GH and insulin-like growth factor-1 (IGF-1)) and the calciotropic hormones (parathyroid hormone (PTH), calcitonin and 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$)). It is becoming apparent that there is some overlap between these two systems as PTH, which at low levels is considered to be anabolic to the skeleton (Parsons *et al.* 1975), has recently been shown to increase the production of IGF-1 in bone cells (Canalis *et al.* 1989; Linkhart & Mohan, 1989).

The control of bone growth and metabolism is dependent on the activity and interactions of particular cell types. These include the growth plate chondrocytes which are primarily responsible for longitudinal growth. The two cell types which are responsible for the turnover and repair of the skeleton are the osteoblasts which form bone and osteoclasts which resorb bone. This remodelling process is also important in intramembranous (skull growth) and endochondral bone formation during bone development and growth. The osteocyte, which is an osteoblast embedded in the matrix, is probably responsible for sensing the changes in mechanical demand (Pead *et al.* 1988).

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Table 1. *Some examples of the various types of endogenous mediators of growth*

Systemic	
(a) General	Growth hormone (GH)
	Insulin-like growth factors (IGF)
	Epidermal growth factor
	Thyroid hormones
	Insulin
(b) Organ specific	Gonadotropins
	Thyroid-stimulating hormone
	Adrenocorticotrophin
	Calcium
Local	
	Insulin-like growth factors (IGF)
	Transforming growth factors (TGF)
	Platelet-derived growth factor
	Epidermal growth factor
	Osteoclast growth factor (OGF)
	Interleukins

LONGITUDINAL BONE GROWTH

The characteristic histological features of the long bones are developmentally regulated. At birth the columnar structure of the epiphyseal growth plate is incompletely formed. As a laminar organization of chondrocytes develops in the distal direction (Sissons, 1961) there is a uniformly distributed cell layer consisting of cells which rarely divide (Kember, 1978), which is termed the germinal layer. During longitudinal growth these cells differentiate and enter the proliferative layer where the cells multiply before maturing into hypertrophic chondrocytes. These cells produce the cartilaginous matrix which subsequently becomes calcified before being resorbed and replaced by osteoblast-mediated bone formation (Isaksson *et al.* 1987). Thus, longitudinal growth results from the increased production of cells and their subsequent expansion.

Post-natal longitudinal bone growth is primarily dependent on GH, but while effects of GH are easily recognized, the site and cellular mechanisms behind these effects are unclear. Salmon & Daughaday (1957) postulated that response to GH was mediated through the hepatic production of somatomedins, now termed insulin-like growth factors (IGF). This is based on the fact that addition of GH to explanted cartilage fragments produces little effect on metabolism. However, addition of serum stimulated a number of cellular functions associated with proliferation and differentiation. Serum from hypophysectomized animals had a lesser effect but subsequent GH therapy resulted in a serum with normal growth-promoting activities. The structure of both IGF has been elucidated (Rinderknecht & Humbel, 1978*a*,*b*) and the genes encoding these factors characterized (Jansen *et al.* 1983, 1985).

However, Schoenle *et al.* (1982) infused high doses of purified IGF-1 into hypophysectomized rats but could only induce a slight increase in bone growth which was of a similar magnitude to that induced by GH alone. Similarly, in a study using recombinant IGF-1, high doses had to be used to induce bone growth, while GH was more effective at a fiftyfold lower dose (Skottner *et al.* 1987). It is now apparent that while administration of

large doses of IGF-1 significantly increases body-weight, it produces only small effects on longitudinal bone growth.

To examine the possibility that GH might have a direct effect on the growth plate, Isaksson and colleagues (Isaksson *et al.* 1982; Isgaard *et al.* 1986; Nilsson *et al.* 1987) infused low concentrations of GH (50 ng/d) directly into the growth plate or into the knee joint and showed that it stimulated longitudinal growth compared with the contralateral limb. IGF-1 was also capable of stimulating epiphyseal cartilage width but a combination of the two growth promoters produced no additional increase in bone growth. Other groups have confirmed much of this work (Russell & Spencer, 1985; Schlecter *et al.* 1986).

These studies have been supported by the findings that many tissues produce IGF-1 (D'Ercole *et al.* 1984) and has led to the development of an alternative concept of GH action whereby GH has a direct effect on peripheral tissues which in turn mediate the effect by the local production of IGF-1 (Underwood *et al.* 1986). Many of the organ-specific growth mediators outlined in Table 1 have been shown to stimulate the local production of IGF-1 and IGF-1 may in turn potentiate the response to those growth mediators (for review, see Holly & Wass, 1989). One of the important questions for future research is whether the direct or indirect actions of GH on peripheral tissues are physiological and what is the relative contribution of each pathway to the final effect.

BINDING PROTEINS

Both GH and IGF are either found free or complexed to binding proteins within the circulation. Human plasma is known to contain at least two binding proteins (>100 K and 80–85 K) for growth hormone each with varying degrees of affinity for human GH (Baumann *et al.* 1986; Herington *et al.* 1986). The high affinity peak (80–85 K) is responsible for 80–90% of the complexed GH (Baumann *et al.* 1989). Under physiological conditions approximately 50% and 29% of the circulating 22 K and 20 K human GH respectively are in a complexed form (Baumann *et al.* 1988). At GH concentrations greater than about 20 ng/ml the complexed fraction declines in a concentration dependent manner due to partial saturation of the binding protein (Baumann *et al.* 1988).

The concentrations of both binding proteins vary considerably from subject to subject in health and disease but are grossly maintained within the normal range in a number of diverse conditions which are known to result in abnormal GH levels such as acute infection, liver cirrhosis, acromegaly, uraemia (Baumann *et al.* 1989). The levels of binding protein were, however, significantly lower in neonates but reached the adult levels by 1 year of age (Baumann *et al.* 1989). Limited evidence exists that the two binding proteins are differentially regulated with the larger binding protein (>100 K) having sex hormone dependency but not the high-affinity binding protein (80–85 K).

More than 98% of plasma IGF-1 and IGF-2 is bound to a 150 K complex whose concentration is GH dependent (Baxter & Martin, 1986). Acidification results in an acid-stable subunit of 50 K with a binding site with equal high affinity for either IGF-1 or IGF-2 (Martin & Baxter, 1986).

A group of smaller binding proteins (25–43 K) are also present in serum and one of these (26 K) is found in amniotic fluid (Povoa *et al.* 1984). The serum levels of this protein are low in acromegaly and raised in hypopituitarism so it is believed to be

inversely related to GH (Drop *et al.* 1984). Injection of GH to GH-deficient patients resulted in a fall in the level of binding protein (Busby *et al.* 1988), although it has been reported that the levels rise during the nocturnal increase in GH secretion (Baxter & Colwell, 1987). Studies on nutritional influences on this protein have shown that its concentration falls after a meal (Baxter & Colwell, 1987; Busby *et al.* 1988) and rises during overnight fasting (Busby *et al.* 1988) and during the infusion of glucose and insulin (Sukkari *et al.* 1988).

As the IGF- and GH-binding proteins have only recently been characterized the role of such binding proteins in the expression of the biological activity of these growth promoters is as yet unclear. One function that has been attributed to them is to protect GH (Baumann *et al.* 1987) and IGF (Cohen & Nissley, 1976) from degradation and prolonging their biological half-life by restricting their access to degradation sites. A number of other functions have speculatively been attributed to the GH-binding protein. These include inhibition of GH action by interference with GH-receptor binding, enhancement of GH action by acting as a cofactor for receptor binding or acting as a circulating hormone reservoir during periods of fluctuations in GH secretion (Baumann *et al.* 1987). Similarly, inhibition (Drop *et al.* 1979; Zapf *et al.* 1979; Knauer & Smith, 1980) or enhancement (Clemmons *et al.* 1986; Elgin *et al.* 1987) of the activity of IGF-1 has been attributed to the low-molecular-weight IGF-binding proteins. The 150 K IGF-binding protein is considered due to its very long half-life (Hodgkinson *et al.* 1987) to constitute an IGF storage compartment.

The presence of these binding proteins has undoubtedly important implications in the metabolism, distribution and physiological activity of GH and the IGFs but these processes are as yet unclear and warrant further research.

LOCAL TURNOVER AND REPAIR

The activity of osteoblasts and osteoclasts is closely coupled (Rodan & Martin, 1981) which results in skeletal turnover without accretion or loss. Both the activity and the proliferation and differentiation of bone cells appear to be under the control of a number of growth factors. The bone matrix itself is a storehouse for a plethora of factors which can induce bone formation. In the 1960s, Marshall Urist (1965) showed that devitalized bone was capable of inducing endochondral bone formation. Several groups have pursued this line of research and have purified a number of growth factors (Reddi & Anderson, 1976).

In addition to these bone specific growth factors there are other growth factors which have a more general activity and are also found within the bone matrix. These include IGF-2 (Mohan *et al.* 1988), fibroblast growth factor (Hauschka *et al.* 1986) which is mitogenic for mesoderm- and neuroectoderm-derived cells (Gospodarowicz *et al.* 1987) and TGF-β (Sporn *et al.* 1987).

Work in our laboratory is concerned with two aspects of the control of bone cell activity. First the development of novel techniques which enable the *in situ* measurement of the biochemistry of bone cells (Bradbeer *et al.* 1988; Loveridge & Farquharson, 1989), and second the purification of a factor released from osteoblasts which stimulates the proliferation and differentiation of osteoclasts (Dickson & Scheven, 1989; Scheven *et al.* 1989). This factor affects the number of osteoclasts but not the activity of existing osteoclasts and has been termed osteoclast growth factor (OGF).

TGF-β is produced by osteoblasts in response to calciotropic hormones (Pfeilschifter & Mundy, 1987) and inhibits bone resorption induced by other factors (Pfeilschifter *et al.* 1988), although in some systems it stimulates bone resorption (Tashjian *et al.* 1985). TGF-β is produced as a latent precursor molecule which is then cleaved to release the biologically active TGF-β (Sporn *et al.* 1987), a process of which the osteoclast is capable (Oreffo *et al.* 1989). In some but not all in vitro assays TGF-β stimulates markers of bone formation such as osteoblastic alkaline phosphatase (*EC* 3.1.3.1) activity (Pfeilschifter *et al.* 1987). Thus, it has been suggested that TGF-β acts as an anabolic coupling factor by stimulating formation and inhibiting resorption. In our laboratory, however, while TGF-β inhibits osteoclastic activity it also inhibits osteoblastic activity (Loveridge & Farquharson, 1989), although the latter action is about 100 times less sensitive.

RESPONSE TO EXTERNAL STIMULI

The last aspect of growth which we will discuss is the response to external stimuli. In the case of bone it is obvious that external stimuli such as dietary deficiencies of vitamin D, changes in the availability of Ca or increased demand for Ca result in alterations in the Ca homeostatic mechanisms with a consequent loss of skeletal material. Similarly metabolic diseases such as diabetes can result in significant bone loss (Hui *et al.* 1985). What is less well characterized is the response to mechanical stress.

It is well known that the capacity of bone to remodel itself is influenced by the degree of mechanical loading. For instance, prolonged bed rest results in disuse osteoporosis (Asher, 1947; Krolner & Toft, 1983). Furthermore, short periods of mechanical stress in which the magnitude and distribution of the dynamic strains within the bone are different from those to which it is normally accustomed (Lanyon, 1984) result in profound changes in remodelling activity.

What is not clear at present is the mechanism of response to loading, although recent evidence suggests a possible role for the osteocyte (Pead *et al.* 1988).

SUMMARY

In the present review it is not possible to discuss the effects of the numerous endogenous mediators of growth. What we have attempted to do is to indicate the areas of controversy and the need for further research. In our view, four main questions arise. First, what are the relative contributions of the direct and indirect effects of GH? Indeed, if GH can produce all its effects by local production of IGF, is the original somatomedin hypothesis still tenable? Second, how is the biological activity of the IGF modified by the presence of binding proteins? Because of the role of binding proteins in modulating IGF bioactivity, care must be taken when interpreting results from immunoassays for IGF because this will only represent the concentration of IGF not the level of biological activity, a situation which is analogous to that which pertains with certain polypeptide hormones (for review, see Robertson *et al.* 1987). Third, how are the activities of the osteoblast and osteoclast coupled so that in the mature adult, bone formation and bone resorption are roughly equivalent? Understanding of this process will undoubtedly involve the elucidation of the roles and interactions between a number of locally acting growth factors and systemic hormones and will lead to the understanding of certain metabolic bone diseases such as osteoporosis. Last, how is the response to local stimuli

such as mechanical stress transduced? This is again probably dependent on the activity of local growth factors but may also involve changes in the interactions between bone cells and their underlying matrix components (Skerry *et al.* 1988).

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