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Guest Lecture

The insulin-like growth factors in critical illness: pathophysiology and therapeutic potential

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THE INSULIN-LIKE GROWTH FACTORS

The insulin-like growth factors (IGF) are two polypeptides which are highly homologous to proinsulin and have all the biological properties of insulin. They have rapid insulin-like metabolic actions and slower growth-promoting actions; in addition they can be potent cell survival factors, they can promote the differentiation of undifferentiated cells and they can stimulate the differentiated function of many tissues throughout the body. Distinct from other tissue growth factors, the IGF have been heavily implicated also in the regulation of childhood somatic growth. They are, therefore, pluripotential peptides which can have important effects in most tissues in the body. The IGF are the most prevalent growth factors present in the body, at least in the soluble form.

INSULIN-LIKE GROWTH FACTOR RECEPTORS

The actions of the IGF are elicited by interaction with cell surface receptors. There are three distinct receptor types which bind the IGF; the type I or IGF-I receptor, the IGF-II receptor and the insulin receptor. The mitogenic and anabolic effects of the IGF are believed to be mediated by signalling through the IGF-I receptor. Although it was originally thought that the metabolic effects were mediated by cross-reactivity with insulin receptors, differences between IGF-I and insulin actions in animal studies (Giacca *et al.* 1990) and *in vitro* studies with chimaeric receptors (Lammers *et al.* 1989) have indicated that the metabolic actions of the IGF are mediated by the IGF-I receptor. Whilst physiologically it appears that metabolic actions of IGF are through the IGF-I receptor, it is likely that pharmacologically high doses of IGF can trigger metabolic actions by interacting with insulin receptors. The IGF-I receptor shares a high degree of homology with the insulin receptor, both receptors having the same heterotetrameric structure formed from two α- and two β-subunits; the homology extends to 85% in the cytoplasmic tyrosine kinase domain which is thought to initiate intracellular events involved in receptor signalling. In cells with equivalent populations of the IGF-I and insulin receptors both ligands appear to have the same metabolic actions. The integration of the two systems is compounded by the presence of hybrid receptors formed by an α–β dimer of the insulin receptor dimerizing with an α–β dimer of the IGF-I receptor (Moxham *et al.* 1989); the significance of these hybrid IGF-I–insulin receptors is at

present unclear. Physiologically the differences in the metabolic actions of the IGF and insulin at the whole-body level appear to be due to the differences in the relative distribution of their respective receptors in the main metabolic target tissues. Whilst insulin has important metabolic effects on the liver, adipose tissue and skeletal muscle, the metabolic effects of IGF are predominantly in skeletal muscle in adults; this is because hepatocytes and mature adipocytes appear to have very few IGF-I receptors.

REGULATION OF INSULIN-LIKE GROWTH FACTORS

The regulation of IGF production and circulating levels is complex. Unlike insulin, the IGF are produced in many tissues throughout the body (Han *et al.* 1988). The factors regulating production of IGF vary between different tissues and with development. This is facilitated by different promoters which are present on the IGF genes (Sussenbach *et al.* 1991). Although the IGF are produced in many tissues, it appears that the liver is the main source of the IGF which is found at high levels in the circulation. In human subjects, IGF-II is generally present in the circulation at concentrations fourfold higher than those of IGF-I; despite this very little is known regarding the regulation or role of this prevalent circulating growth factor, although there are good animal data implying a role in fetal growth (DeChiara *et al.* 1990). After early childhood, IGF-II levels vary very little throughout life and are not altered by hormonal manipulations or in many pathological conditions. Although IGF-I levels are much lower, they vary much more both physiologically and pathologically and, hence, much more has been deduced regarding the regulation and role of IGF-I. Circulating IGF-I levels rise throughout childhood especially throughout the pubertal growth spurt; they then fall back to prepubertal levels and remain fairly constant throughout adult life and then fall gradually with old age (Smith *et al.* 1989). These changes parallel those of growth hormone (GH), and IGF-I administered to children lacking the effects of GH produces marked increases in longitudinal growth, implying that IGF-I has a major influence on childhood growth (Clemmons & Underwood, 1994).

Whilst traditionally GH has been considered the prime regulator of hepatic IGF-I production and, hence, circulating IGF-I levels, there is also convincing evidence that insulin has a prime role in the regulation of IGF-I. This appears to be due to insulin regulating hepatic GH receptor levels (Baxter *et al.* 1980) and to a direct effect of insulin on IGF-I production (Maes *et al.* 1986). There also appears to be a direct effect of nutrition on IGF-I production and circulating levels. Fasting appears to reduce hepatic GH receptors (Strauss & Takemoto, 1990) leading to an uncoupling of GH regulation; after 3 d of fasting, GH injections in normal subjects fail to increase IGF-I (Merimee *et al.* 1982). In addition protein restriction causes post-receptor resistance to the action of GH (Maiter *et al.* 1988) leading to decreased IGF-I production and also to resistance to IGF-I actions (Snyder *et al.* 1988). Indeed a number of studies have suggested that the circulating concentration of IGF-I is a very good index of nutritional status (Unterman *et al.* 1985; Burgess, 1992).

INSULIN-LIKE-GROWTH-FACTOR-BINDING PROTEINS

The very high levels of IGF found in the body are maintained by the presence of specific binding proteins (IGFBP). Alone the IGF are about 7·5 kDa in size, but they are found

in the body almost exclusively in complexes of about 50 kDa and 150 kDa with these IGFBP. Binding to the IGFBP greatly reduces the clearance of the IGF enabling the relatively high levels to be maintained; alone the IGF have a circulating half-life of about 8 min (similar to that of insulin) but complexed with the IGFBP this is extended to many hours, particularly when in the 150 kDa complex (Guler *et al.* 1989*b*). In association with IGFBP the total IGF concentration in the circulation is about 100 nmol/l in adult man which is about 1000-fold higher than that of insulin and it is evident that most of this IGF cannot be active (Baxter, 1991). Six IGFBP have now been identified, they are all products of distinct genes and appear to form a family of closely-related proteins (Drop *et al.* 1991). Whilst they have a relatively high amino acid homology and share a number of structural features, they all appear to have distinct functional properties. The reason for six IGFBP and the precise role of each is at present unclear, although there is accumulating evidence to support certain roles for some of the IGFBP.

Most of the IGF in the circulation is held in the 150 kDa complex which is formed with IGFBP-3 and a further acid-labile subunit (ALS), which alone does not bind IGF (Baxter & Martin, 1989). The large complex appears to be retained relatively within the circulation and, thus, forms a large latent store of IGF unavailable to tissue receptors (Binoux & Hossenlopp, 1988). Since there are no intracellular stores of IGF maintained within the tissues, this provides an alternative readily-available source of IGF for the tissues. The affinity with which the IGF are bound to IGFBP-3, and the other IGFBP, is considerably higher than that with which they bind to the cell IGF-I receptors, implying that the equilibrium would not favour receptor interactions (Kiefer *et al.* 1992). This raised the question: 'If the binding proteins maintain extracellular stores of IGF, then how is this IGF made available to receptors in target tissues?' An apparent solution to this question came with the observation that IGFBP-3, which holds most of the IGF, is subjected to specific proteolytic modification which does not destroy the binding protein but which lowers the affinity with which it binds IGF. This was demonstrated first in pregnant women (Giudice *et al.* 1990; Hossenlopp *et al.* 1990), but has subsequently been shown to be a general occurrence (Holly *et al.* 1993). The proteolytic modification of IGFBP-3 increases the biological availability of the IGF that it carries (Holly *et al.* 1993). Subsequently, proteases specific for a number of other IGFBP have been reported, suggesting that this may represent a general mechanism whereby IGF held on IGFBP may be made available for cell receptors. The other IGFBP for which there is sufficient data to indicate a specific role is IGFBP-1; this is produced predominantly in the liver under insulin regulation. Increases in insulin suppress IGFBP-1 production and levels fall, with a half-life of about 50 min. The acute insulin-dependent variations in IGFBP-1 are associated with variations in IGF activity (Taylor *et al.* 1990). Thus, when insulin levels fall, IGFBP-1 levels rise and restrict IGF activity. This suggests that IGFBP-1 may have a 'counter-regulatory' role restricting the insulin-like actions of the IGF and coordinating IGF action with that of insulin itself. Administration of IGFBP-1 to rats leads to an immediate significant rise in blood glucose (Lewitt *et al.* 1991), implying that the IGF may play a role in normal carbohydrate homeostasis which is modulated by IGFBP-1.

The role of the other IGFBP is still far from clear. In addition to storing, transporting and targeting IGF to specific tissues, there is accumulating evidence indicating that a number of IGFBP may interact with cells, modulating their response to stimulation (Clemmons *et al.* 1991). With very high levels of the pluripotential IGF present

throughout the body it seems that the numerous independently-regulated binding proteins may confer specificity, enabling the actions of the IGF to be elaborated in the most appropriate manner at any particular time within any tissue.

PATHOPHYSIOLOGY OF INSULIN-LIKE GROWTH FACTORS IN CRITICAL ILLNESS

Until recently the effects of critical illness and stress on the IGF seemed relatively straightforward. In critically-ill and nutritionally-depleted subjects circulating IGF-I levels decrease (Frayn et al. 1984; Phillips & Unterman, 1984; Ross et al. 1991a), despite a rise in GH, indicating a relative resistance to this action of GH. These changes have been considered to be adaptive, mobilizing metabolic fuels in the compromised subject. Thus, there is an increase in GH levels and direct GH actions, increasing lipolysis and causing insulin resistance, decreasing peripheral glucose utilization, but also a decrease in indirect anabolic GH actions mediated by IGF-I; the decrease in IGF-I resulting in a fall in protein synthesis. The net effect of these changes is mobilization of metabolic fuels for essential functions but at the expense of peripheral metabolic stores and catabolism ensues. Such catabolism is associated with many conditions of critical illness and results in increased morbidity and mortality. However, these deductions regarding the changes in the IGF system were made before the intricacies of the system for storing, transporting and modulating IGF became apparent.

Recent studies looking in more detail at the components of the system have revealed further changes which may suggest an alternative interpretation of the role of IGF in the metabolic adaptations occurring in critical illness. In intensive-care patients, fasting IGFBP-1 levels are high but the inverse relationship between IGFBP-1 and insulin appears to be maintained despite subjects exhibiting insulin resistance with respect to carbohydrate metabolism (Ross et al. 1991b). However, the most marked alteration in the IGF system seems to be a dramatic increase in the activity of the circulating IGFBP-3 protease (Davies et al. 1991). This protease activity in the sick patients appears to be under some form of metabolic regulation; its activity increases during fasting and decreases when nutritional support is provided (Davies et al. 1991). Similarly in severely-malnourished subjects there is an increase in the protease activity which decreases with refeeding (Pucilowska et al. 1993). An acute insult such as surgical stress or trauma also appears to result in increased activity of the same protease (Cwyfan Hughes et al. 1992; Davenport et al. 1992; Timmins et al. 1993). The increase in activity occurred within 24 h of major surgery; this occurred even in a subject undergoing total hepatectomy, indicating that the protease was not of hepatic origin (Cwyfan Hughes et al. 1994). Following surgery there was also a shift in distribution of IGF-I from the 150 kDa complex into 50 kDa complexes; this appears to be a second effect independent of the IGFBP-3 protease (Cwyfan Hughes et al. 1992). This shift in distribution of IGF towards smaller complexes should also increase the availability of IGF to tissue receptors. Whilst the significance of the IGFBP-3 protease is yet to be determined the inference is that, as in pregnancy, there is a fall in affinity with which the IGF is bound to IGFBP-3 and a resultant increase in availability of IGF to the tissues. The circulating half-life of administered IGF-I appears considerably shorter in critically-ill patients (Miell et al. 1992), consistent with a reduction in the affinity of the main binding protein, resulting in increased IGF-I turnover and increased availability of the circulating IGF to its target tissue receptors.
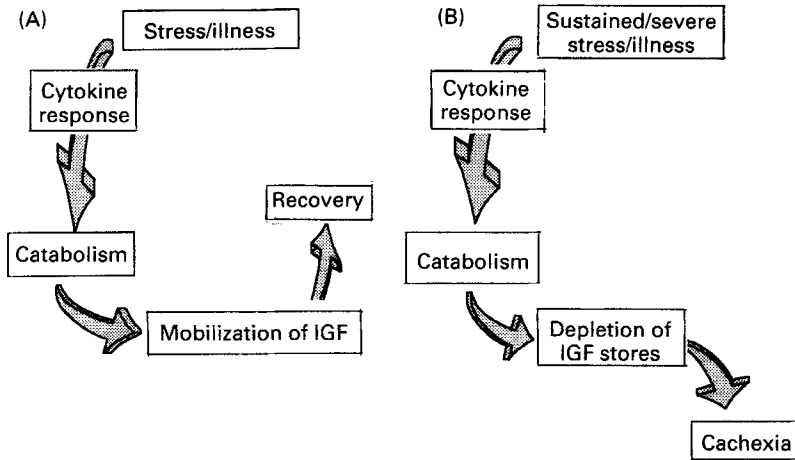
Fig. 1. (A) The role of insulin-like growth factors (IGF) in the catabolic response to critical illness or stress. (B) Changes in IGF with sustained or severe stress or illness which can lead to cachexia.

These new findings suggest that following stress and in severe illness the body is activating mechanisms for mobilizing the large latent reservoir of IGF which is maintained in the circulation. This implies that the changes in IGF are not permissive, allowing the catabolism, but are rather an adaptive response leading to increased anabolism to counter the catabolic condition induced by cytokines and other components of the stress response. This is supported by a study in which rats were made catabolic by partial resection of the bowel, with some animals receiving a constant infusion of IGF-I throughout the entire experiment to prevent any fall in IGF-I concentration (Lemmey et al. 1991). The catabolic response to the insult was exactly the same in the animals receiving IGF-I as that in the controls, indicating that this catabolic response was not due to a fall in IGF-I concentration. The only difference between the two groups of animals was that in those receiving IGF-I there was a more rapid improvement in N balance during the recovery period. This occurred at the time when in our studies we observed changes which would have resulted in mobilization of the endogenous IGF. Therefore, this study in rats is consistent with our new findings, indicating that the changes in the IGF system are involved in the recovery from catabolism (Fig. 1(A)) rather than in the induction of the catabolism. With a stress that is particularly severe and/or sustained, the mobilization of IGF may then lead to depletion of the circulating IGF reservoir since increases in IGF-I production are insufficient to maintain stores, presumably due to the GH and insulin resistance. Despite the mechanisms to increase the availability of IGF, if stores become depleted then availability and hence tissue activity would eventually decrease, and without the anabolic counterbalance of the IGF the catabolism can proceed to cachexia (Fig. 1(B)).

THERAPEUTIC POTENTIAL OF INSULIN-LIKE GROWTH FACTORS IN CATABOLIC CONDITIONS

Catabolism is a common problem in many critically-ill patients and is associated with increased morbidity and mortality. Nutritional support with either enteral or parenteral

feeding increases protein synthesis, but not sufficiently to counter the catabolism experienced by most critically-ill patients (Douglas & Shaw, 1989). This has led to many attempts to use anabolic factors to correct the catabolism. Earlier studies with insulin found a transient improvement in N balance (Woolfson et al. 1979). More recent studies with GH have been more successful, although relatively high doses of GH have been used since patients with catabolism develop GH resistance. The more severely ill the patient the more resistant they become to the anabolic effect of GH (Douglas et al. 1990). In addition GH can have adverse effects, increasing the glucose intolerance associated with glucocorticoid administration (Horber & Hammond, 1990) and traumas such as extensive burns (Belcher et al. 1989).

Many of the anabolic effects of GH are believed to be mediated by IGF-I and the availability of this peptide from recombinant DNA technology has led to preliminary studies investigating its use in catabolic conditions. Initial studies with normal volunteers in whom catabolism was induced by severe dietary restriction for 2 weeks have compared infusions of IGF-I with daily subcutaneous injections of GH (Clemmons et al. 1992). At the doses chosen, IGF-I and GH both attenuated the negative N balance to similar extents. However, whilst GH raised serum glucose and insulin levels, IGF-I had the opposite effect, lowering serum glucose and dramatically suppressing insulin secretion. In an identical study the same group went on to compare IGF-I infusion alone with IGF-I infusion in combination with daily subcutaneous GH (Kupfer et al. 1993). The combination caused significantly greater N retention than IGF-I used alone. Furthermore, the addition of GH considerably attenuated the IGF-I-induced hypoglycaemia and the suppression of insulin secretion.

There are now many studies under way investigating the use of IGF-I in a variety of catabolic conditions and within the next couple of years a clearer picture of its usefulness should emerge. It appears that adequate supplies of substrate are essential for an anabolic response to IGF-I (Clemmons & Underwood, 1994). Some preliminary findings indicate that an initial anabolic effect subsequently declines with continued administration in catabolic patients with acquired immune deficiency syndrome (Lieberman et al. 1993) and head injury (Chen et al. 1993). This may result from reduced GH levels due to IGF-I feedback; the reduced GH levels then lead in turn to decreases in the levels of IGFBP-3 and the ALS which together maintain the major proportion of circulating IGF in the large 150 kDa complex. Continuous IGF-I administration results in decreases in IGFBP-3 and ALS and the initial rises in serum IGF-I concentrations are not sustained (Kupfer et al. 1993). The falls in levels of IGFBP-3 and ALS are prevented by co-administration of GH and the rise in serum IGF-I concentration is then sustained (Kupfer et al. 1993).

The in vivo studies with IGF-I have indicated that in addition to an anabolic response primarily in skeletal muscle, IGF-I can also have particular effects on specific organs which may prove also to be therapeutically useful. Administration of IGF-I significantly improves renal function in patients with chronic renal failure (O'Shea et al. 1993) and this appears to be due to effects on both glomerular filtration rate and renal blood flow (Guler et al. 1989a). Marked increases in cardiac output (Elahi et al. 1991) without an increase in blood pressure have been described also; these appear to be due to increased peripheral blood flow (Guler et al. 1989a; Copeland & Nair, 1994). This, in turn, may be due to vasodilation secondary to induction of endothelial cell NO production (Tsukahara et al. 1994). Animal studies have indicated that the intestines are particularly responsive to the growth-promoting actions of IGF-I (Lemmey et al. 1991) and treatment of patients

following bowel resection is an obvious application which could prove particularly beneficial, speeding up their recovery and their independence of parenteral nutrition. Stimulatory effects of IGF-I on the immune system and the immune response to challenge have also been reported (Kudsk *et al.* 1994).

The clinical studies with IGF-I are only just beginning. Initial studies have indicated that adequate nutrition may be essential to gain full effects and that, under some conditions, IGF-I may be better used in combination with GH. Administration by intravenous infusion has been associated with a number of adverse side effects, mainly related to its hypoglycaemic actions, and the pharmacokinetics following subcutaneous injections indicate that the latter is the preferable mode of administration and leads to sustained elevations of serum IGF-I levels (Takano *et al.* 1991). However, the best mode of delivery, the most effective dosage regimen, the best applications and the potential drawbacks are still to be determined.

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