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General Review

Role of Insulin-like Growth Factors in Steroid Modulated Proliferation

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The mechanism by which steroids influence cell proliferation is poorly understood although an understanding of this process might facilitate the development of strategies to modulate the tissue-specific activity of steroid hormones. In this article, the evidence that steroid hormones interact with the insulin-like growth factor (IGF) signal transduction pathway is reviewed for three different tissues. In osteoblasts, oestradiol stimulates the production of IGF-I which appears to act as an autocrine growth factor. In uterine tissue, oestradiol increases the synthesis of IGF-I in the stroma which then modulates the proliferation of epithelial cells although there is also evidence that oestradiol can modulate the sensitivity of uterine epithelial cells to IGFs. In breast cancer, oestrogens may increase IGF-II synthesis in epithelial cells, increase the sensitivity of breast cancer cells to IGFs (possibly by modulating type I IGF receptor levels) as well as resulting components of the IGF signal transduction pathway resulting in induction of immediate early genes. There therefore appears to be a variety of ways in which oestradiol interact with the IGF signal transduction pathway and these may be applicable to other malignant and normal tissues and other groups of steroid hormones.

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INTRODUCTION

Steroids have a wide variety of effects on target organs including the regulation of specific genes, cell proliferation and differentiation. The proliferative response to steroids is complex and the mechanisms involved are poorly understood. Because the effects of steroids are thought to be mediated through the regulation of the expression of repertoires of responsive genes, however, it is generally considered that the proliferative response is initiated by the induction of genes involved in controlling cell division or the progression of cells through the cell cycle.

Knowledge of the mechanism of action of steroid hormones at the molecular level has increased dramatically in recent years and has been the subject of extensive reviews [1, 2]. The genes encoding the major families of steroid receptors have been cloned. In addition, sequences responsible for the specific binding of steroids to the promoter regions of steroid responsive genes have been identified and characterized and the structure of DNA receptor complexes elucidated [3, 4].

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Growth factors, by definition, regulate the proliferation of cells and multiple families of growth factors, growth factor receptors and growth factor signal transduction pathways have been identified. Although it is possible that steroids exert their proliferative effects by regulating the expression of genes involved in the direct regulation of the cell cycle or enzymes involved in DNA synthesis, one hypothesis which had received much attention is that steroids modulate cell proliferation by interacting with and modulating the activity of growth factor signal transduction pathways.

Although several families of growth factors have been implicated [including the transforming growth factors and epidermal growth factor (EGF)], much attention has been focused on the insulin-like growth factor (IGF) family and the purpose of this article is to review the evidence that IGFs are involved in steroid induced cell proliferation.

THE INSULIN-LIKE GROWTH FACTORS

The IGFs are so called because of their structural homology to proinsulin. They were discovered as a result of three separate lines of research and were

eventually purified and sequenced in 1976. Whereas insulin is composed of two chains (B and A) linked by two disulphide bonds, IGF-I and -II are both single chain molecules. Mature IGF-I is a 70 amino acid peptide and contains a 12 amino acid C domain which lies between the B and A domains and is equivalent to the C domain of insulin. It also has 8 additional amino acids at the C terminus which form the D domain. Mature IGF-II has 67 amino acids with 8 amino acids in the C domain and 6 amino acids in the D domain.

IGFs are important molecules controlling growth and development. IGF-I is synthesized principally in the liver under the control of growth hormone. It mediates the effects of growth hormone and is a key molecule regulating postnatal growth [5]. The function of IGF-II has been more difficult to elucidate. It was originally presumed that it played a role in foetal development and that IGF-I assumed the role of IGF-II postnatally. There are now convincing genetic studies in mice which have shown that both IGF-II and -I play important roles in foetal development [6, 7].

IGF-I is present at relatively constant levels in the circulation which peak during periods of more rapid growth such as puberty. It is present in the circulation as a complex with IGFBP-3 which is itself associated with an 83 kDa protein to give a complex of approx. 150 kDa. IGFBP-3 has been reported to modulate the biological activity of IGF-I with some reports suggesting an enhancement and some an attenuation of biological activity depending on the experimental system used. Six IGF binding proteins have now been identified and their possible function in steroid mediated proliferation is discussed later in this review.

IGFs can act through three receptors the type I and II IGF receptors and the insulin receptor. The most important receptor for transmitting mitogenic signals is the type I IGF receptor and IGF-I has the highest affinity for this receptor. IGF-II has a slightly lower affinity wheres insulin has a 100-fold lower affinity than IGF-I for the type I IGF receptor. The signal transduction pathway for IGFs has been partially elucidated but the parts of the pathway which discriminate between signals from the insulin receptor and type I IGF receptor are not known.

ROLE OF IGFs IN STEROID INDUCED PROLIFERATION OF NORMAL CELLS

The stimulatory effects of IGF-I on normal growth and development clearly occur in a highly regulated and controlled way. Indeed, a variety of syndromes have been identified which result from abnormal circulating IGF levels e.g. Laron type Dwarfism [11, 12].

Although many normal tissues are regulated by steroids, there are relatively few in which significant progress has been made in understanding the mechanisms by which steroids regulate cell proliferation. In the two examples discussed below, the IGF signal transduction pathway has been implicated.

Effects of oestrogens on bone cells

Oestrogens act directly on bone cells and oestrogen deficiency is responsible for the bone loss observed following the menopause [13]. Exogenous oestrogens in the form of oestrogen replacement therapy effectively prevent bone loss [14], and several studies have suggested that oestrogens act directly on bone cells [15, 16]. Osteoblasts contain oestrogen receptors [17] and experiments in culture have demonstrated that oestrogens stimulate the proliferation of osteoblasts [16] and increase the synthesis of procollagen mRNA [16, 18].

The original discovery that IGF-I mediates the effects of growth hormone on cartilage emphasizes the importance of IGF-I as a growth factor for bone. It is not, therefore, surprising that a connection between oestradiol and the IGFs has been sought. Centrella *et al.* [19] demonstrated that osteoblasts contain type I IGF receptor and Ernst *et al.* [16] showed that the oestrogen-induced increase in cell proliferation can be blocked by antibodies against IGF-I and that oestradiol increases IGF-I mRNA levels in osteoblast cells [16]. More recently oestradiol has been shown to increase IGF-I mRNA levels by increasing transcription of the IGF-I gene [20]. Thus in bone cells it is suggested that steroids increase cell proliferation by increasing the production of IGF-I which then acts as an autocrine growth factor (Fig. 1).

Effects of oestrogens on the uterus

The rat uterus has long been used as an experimental system for studying the mechanism of steroid hormone action [21]; although in recent years its popularity has waned with the use of more easily manipulated

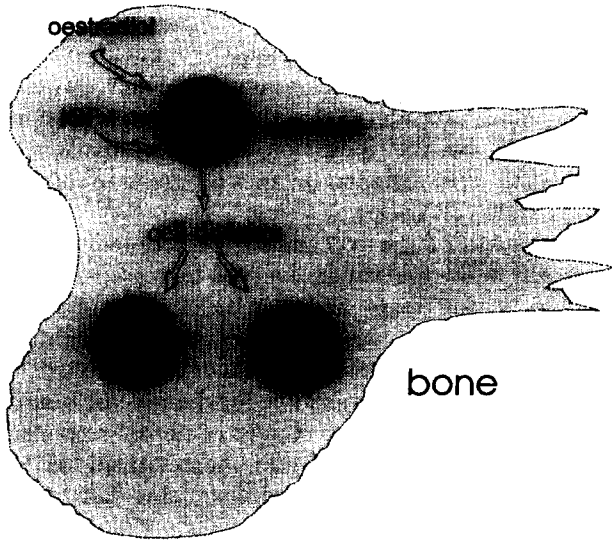

Fig. 1. Involvement of IGF-I in the stimulation of osteoblast cell division by oestrogens.

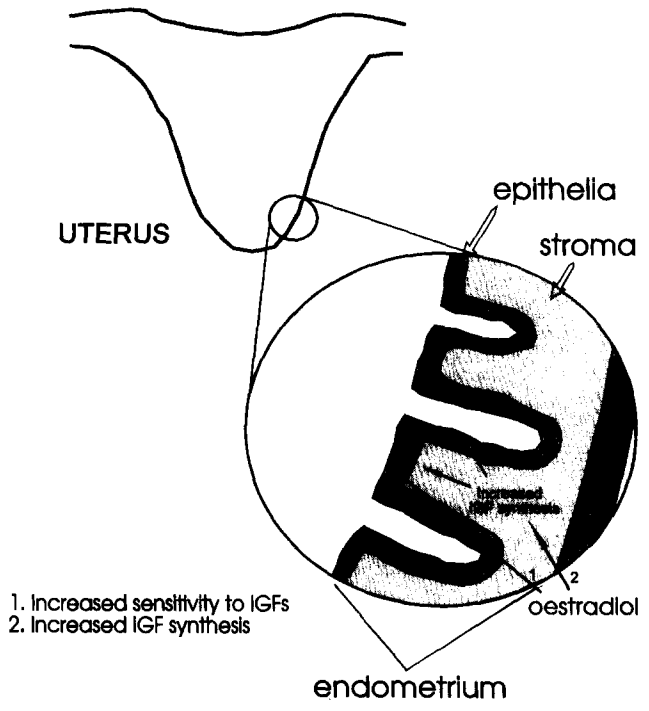

Fig. 2. Involvement of IGFs in the stimulation of uterine cell division by oestrogens.

oestrogen-responsive cell lines established from other sources.

In immature or ovariectomized rats, oestrogen administration results in a marked cellular hypertrophy and hyperplasia. Following administration of oestradiol to the immature rat, the most marked changes are in the stroma and epithelial cells. In the mature rat, there are marked changes in the luminal and glandular epithelium of the uterus during the oestrus cycle with little change in the stroma and myometrial layers.

The mechanisms involved in the proliferative response have been elusive partly because cultures of uterine cells lose oestrogen responsiveness [20]. This observation in itself led to the concept that factors (estromedins) were synthesized in other tissues under the control of oestrogens and were then transported to the uterus via the circulation where they were responsible for stimulating DNA synthesis [23]. Evidence against this concept was provided by the elegant experiments of Stack and Gorski [24] who demonstrated that intraluminal injection of oestradiol stimulated DNA synthesis in the injected but not the contralateral horn. While effectively ruling out the estromedin hypothesis, these experiments were consistent with oestrogens increasing the responsiveness of uterine cells to a circulating growth factor. It was subsequently shown that stromal cells are important to maintaining the oestrogen responsiveness of transplanted and cultured epithelial cells [25] again emphasizing the involvement of paracrine growth factors in oestrogen responsiveness (Fig. 2).

Recent research has focussed on the role of EGF and IGFs although the relative contribution of the two groups of factors is not known. Oestradiol increases the amount of immunoreactive IGF-I in rat uteri [26] and also increases the levels of IGF-I and -II mRNA quite dramatically [27]. *In situ* hybridization suggests that IGF-I mRNA is more abundant in the smooth muscle and outer stroma although it can be found in the periglandular and periepithelial stroma [28]. Type I IGF receptors have been demonstrated in the uterus and their level increases following oestrogen treatment [29]. Most importantly, it has been shown that IGF-I stimulates DNA synthesis in uterine tissue from immature rats in organ culture [30] and interestingly this stimulation is only observed in the presence of oestradiol or in tissue obtained from oestrogen primed animals suggesting that oestrogen increases the responsiveness of uterine cells to IGF-I [30].

Overall these data suggest that IGF-I and -II and possibly the type I IGF receptors are under oestrogen control in the uterus (Fig. 2). Current knowledge of the cells producing these growth factors and the cells in which the proliferative response is observed suggests that IGFs act as oestrogen-induced paracrine growth factors in this tissue. Oestrogen also appears to increase the responsiveness of the uterine epithelium to IGFs although the mechanism by which this occurs is obscure.

The concepts discussed above may well be applicable to the effects of oestrogens and other steroids on other normal tissues (e.g. the effects of androgens on the prostate). In organs such as the prostate, it is known that components of the IGF signal transduction pathway are present [31, 32] but the way in which this pathway and steroid signalling pathways interact is not known. These concepts are also highly relevant to the effects of steroids on hormone-responsive tumour cells and this is discussed in the following section.

IGFs AND CANCER

The ability of tumour cells from a wide variety of sources to respond to IGFs is an increasingly recurrent theme in the literature and has been reviewed by us and others [33–35]. This has led to a realization that IGFs may be important mitogens for cancer cells *in vivo*. There are several mechanisms by which this might occur. In the first, circulating IGFs may provide a reservoir of mitogen which could stimulate the proliferation of tumour cells by an endocrine mechanism. In the second, IGFs could be synthesized by stromal cells within the tumour and increase tumour cell proliferation by a paracrine mechanism. In both these cases, tumour cells may have altered receptor or signal transduction systems compared to normal cells which increase their responsiveness to IGFs. In the third, cells may acquire the ability to synthesize IGFs which could then act as autocrine or intracrine growth factors.

REGULATION OF SIGNAL TRANSDUCTION BY STEROIDS

Some types of tumours can respond to steroid hormones and the classic example is the responsiveness of breast cancer to oestrogens. There is considerable interest in elucidating the mechanisms by which oestradiol stimulates breast cancer cell proliferation as components of the mitogenic pathway could provide novel therapeutic targets for the treatment of hormone responsive and unresponsive breast cancer. The following sections discuss the evidence that the IGF signal transduction pathway is involved in mediating the effects of oestrogens on breast cancer cells.

Effects of oestrogens on the synthesis of IGFs in breast cancer cells

The responsiveness of breast cancer cells in tissue culture to IGFs was demonstrated in 1984 by Myal *et al.* [36] and Furlanetto *et al.* [37] and has been confirmed in a large number of laboratories under a variety of experimental conditions. Subsequently, several groups have focussed on the possibility that IGFs could be synthesized by breast cancer cells and act as autocrine growth factors. As part of a general strategy to identify growth factors synthesized by breast cancer cells, Huff *et al.* [38] identified IGF-I in tissue culture medium conditioned by various breast cancer cell lines using a radioimmunoassay. They suggested that highly tumourigenic oestrogen unresponsive cell lines produce most IGF-I, and provided evidence that the secreted IGF-I was of similar size to authentic IGF-I and that cells produced IGF-I mRNAs of 4.7, 1.4 and 0.3 kb. A subsequent study [39] suggested that IGF-I secretion was under hormonal control as it was inhibited by the antioestrogens tamoxifen, 4-hydroxytamoxifen and LY117018 and was increased by physiological concentrations of oestradiol in the absence of phenol red. The effect of oestradiol was thought to be post-transcriptional as the RNA hybridizing to the IGF-I probe was not regulated by oestrogens.

These studies suggested an elegant model of oestrogen regulated proliferation in which IGF-I acts as an oestrogen regulated autocrine growth factor, however, subsequent studies did not confirm this simple view. Although one other study claimed to have detected low levels of IGF-I secretion by the MCF-7 cell line [40], van der Burg *et al.* [41] failed to detect IGF-I secretion using a bioassay rather than an immunoassay and Yee *et al.* [42] using an RNase protection assay failed to detect IGF-I mRNA in five human breast cancer cell lines. In contrast to the findings with cell lines, IGF-I expression was detected in most breast tumours, fibradenoma and normal breast and *in situ* hybridization demonstrated the present of IGF-I RNA in stromal but not normal or malignant breast epithelial cells [42] suggesting that if IGF-I plays a role in breast cancer it acts as a paracrine growth factor (Fig. 3).

IGF-II is thought to stimulate cell proliferation by acting through the type I IGF receptor and, like IGF-I, has been shown to be mitogenic for breast cancer cells [43–45]. It is somewhat less potent than IGF-I and several studies have examined the expression of this growth factor in breast cancer cells. In contrast to IGF-I there is convincing evidence that it is synthesized in a limited number of cell lines. Yee *et al.* [44] detected IGF-II mRNA in T47D and late passage MCF-7 cells whereas Osborne *et al.* [45] detected IGF-II secretion from all lines lines examined but detected IGF-II mRNA in MCF-7 and T47D cells only by Northern hybridization and RNase protection. Both of the above studies showed that IGF-II mRNA levels are highest in T47D cells and that oestradiol increased IGF-II mRNA levels in this oestrogen-responsive cell line. This data suggests that IGF-II could act as an autocrine mitogen and its regulation by oestradiol suggests that it could mediate the effects of oestradiol by acting as an oestrogen-regulated autocrine growth factor (Fig. 3). This view has received support from experiments with T61 human breast cancer xenografts [46]. In this model, tumour growth is inhibited by oestradiol and tamoxifen and the inhibition of

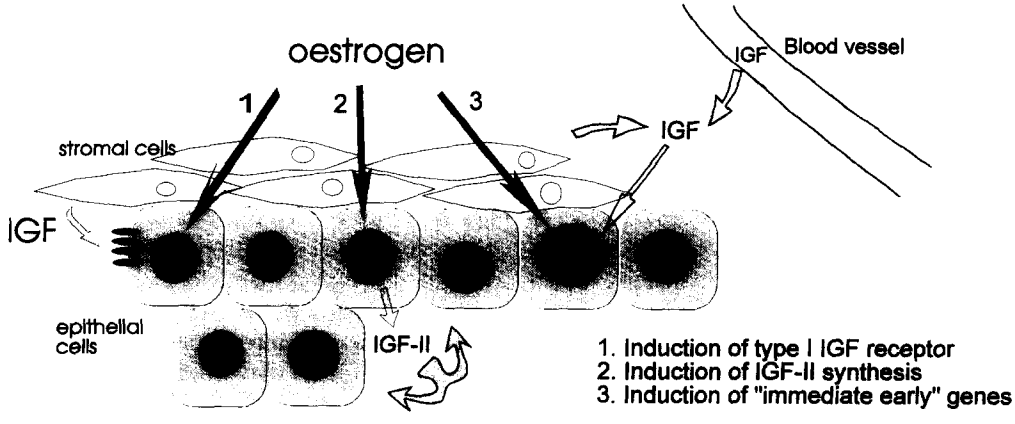

Fig. 3. Involvement of IGFs and the type I IGF receptor in the stimulation of cell division in oestrogen-responsive breast cancer cells by oestrogens.

growth is correlated with almost complete loss of IGF-II mRNA. It is not known why IGF-II is downregulated by oestradiol in T61 tumour cells but upregulated in MCF-7 and T47D cells and it is also unclear why tamoxifen should be almost as effective as oestradiol in downregulating IGF-II mRNA when tamoxifen generally acts as a partial oestrogen agonist.

The importance of IGF-II expression has also been emphasized in experiments in which IGF-II has been constitutively expressed in oestrogen-responsive breast cancer cell lines. Using a retroviral vector to overexpress IGF-II under the control of the cytomegalovirus promoter, Cullen *et al.* [47] demonstrated that MCF-7 cells show increased anchorage independent growth and reduced hormone responsiveness when they overexpress IGF-II. Daly *et al.* [48] showed a similar effect on hormone responsiveness when ZR-75 cells were transfected with a plasmid expressing IGF-II under the control of the metallothionin promoter.

Effects of oestrogens on type I IGF receptor and the IGF-I signal transduction pathway in breast cancer cells

The proliferative effects of both IGF-I and -II are mediated by the type I IGF receptor, a heterotetrameric protein which shows homology to the insulin receptor. Many studies have shown that blockade of this receptor by antireceptor antibodies (α-IR3) inhibits the proliferation of breast cancer cell lines and inhibits IGF-I and -II stimulated proliferation [45, 50–53]. Although the evidence presented in the above section has led to the suggestion that oestrogen-stimulated proliferation of breast cancer could involve induction of IGF-II, there are several lines of evidence that suggest that oestradiol acts to regulate the responsiveness of cells to IGFs and that this may involve an effect on the type I IGF receptor or downstream components of the IGF signal transduction pathway (Fig. 3).

The first observation is that regulation of IGF-II expression has only been observed in MCF-7 and T47D cell lines but not in other oestrogen-responsive cell lines such as ZR-75. The second observation is that addition of IGF-I, IGF-II or insulin to tissue culture medium should abrogate the requirement of oestrogen-responsive cells for oestrogen yet this has not been observed. Stewart *et al.* [43] found that in oestrogen-free culture conditions, insulin or IGFs had very little effect on cell proliferation and could not replace oestradiol. Three groups independently, although under rather different experimental conditions [43, 54, 55], have suggested that there is a synergistic effect between oestradiol and IGF-I. Stewart *et al.* [43] showed that in phenol red-free medium supplemented with charcoal-treated serum, oestradiol alone increased cell proliferation more than IGFs or insulin and that oestradiol appeared to dramatically increase the responsiveness of cells to the proliferative effects of IGFs. In addition, the observation that the IGF-I neutralizing antibody SM1.2 could partially inhibit the effect of oestradiol alone [43] was interpreted to suggest that oestradiol alone stimulates proliferation by sensitizing cells to IGFs which are present in charcoal-treated serum. Stewart *et al.* [43] also showed that oestradiol increases the level of the type I IGF receptor and the simple hypothesis was put forward that the increased responsiveness resulted from the increased levels of the type I IGF receptor (Fig. 3). That this model is an oversimplification is now apparent from experiments in which the type I IGF receptor has been overexpressed in MCF-7 cells using a retroviral expression vector [56].

Aakvaag *et al.* [57] measured the response of MCF-7 breast cancer cells to oestradiol, insulin and IGF-I in the presence of charcoal-treated serum. They observed an inhibitory effect of serum alone but the effect of oestrogen increased with the concentration of serum suggesting that oestradiol might be sensitizing the cells to the proliferative effect of a factor in the serum. In a subsequent study by this group [58] using serum-free medium a synergistic effect of oestradiol and IGF-I were observed and on the basis of additional experiments with αIR-3 (an antibody which blocks binding of IGFs to the type I IGF receptor) in which the antibody inhibited the stimulation of proliferation by oestradiol, concluded that oestrogens act by sensitizing cells to the growth stimulatory effect of IGF-I rather than by inducing the synthesis of an autocrine growth factor.

van der Burg *et al.* [55] have developed a form of charcoal treated serum in which growth factor activity is destroyed by treatment with dithiothreitol and iodoacetamide. Medium supplemented with this serum therefore lacks steroids and growth factors. Cells cultured in this serum become quiescent and oestradiol alone has very little mitogenic effect, again reinforcing the view that oestradiol does not act by inducing an autocrine growth factor. In agreement with the data of Stewart *et al.* [43] there was a large synergistic effect of oestradiol and insulin but van der Burg *et al.* [55] also observed that treatment of cells with insulin alone at a high concentration (10 μg/ml) resulted in the same level of proliferation as had been observed in the presence of lower concentrations of insulin together with oestradiol. In a more recent paper [58], van der Burg *et al.* have argued that oestradiol and insulin are responsible for inducing the expression of "immediate early" genes (fos and jun, respectively). In this model (Fig. 3), oestradiol and IGFs regulate distinct genes: the activity of both being required for maximal cell proliferation and this model is distinct from that of Stewart *et al.* [43] in which oestrogen increases the responsiveness to IGFs by upregulating the type I IGF receptor. Although the model of van der Burg *et al.* [58] is appealing, it does not explain how high concentrations of insulin alone stimulate cell proliferation to the same extent as low concentrations of insulin

together with oestradiol as high concentrations of insulin induce jun but not fos.

As more components of the IGF-I signal transduction pathway, such as the insulin receptor substrate IRS-I [59], are identified, it will become possible to examine the effects of oestradiol and IGFs on their activities. If oestrogens do control cell proliferation by acting through the IGF signal transduction pathway then this type of study should ultimately identify the number of components and the precise mechanisms involved.

Role of IGF binding proteins in oestrogen stimulated proliferation

Six IGF binding proteins (IGFBPs) have been identified which are encoded by distinct genes [60]. These proteins show limited homology overall but the number and positions of their cysteine residues are conserved. IGFBP-3 is the most abundant IGF binding protein in adults and most IGFs circulate as a complex of 150,000 M_W with IGFBP-3 (39,000–43,000 M_W) and an acid labile protein (100,000–110,000 M_W). A principle function of IGF binding proteins appears to be to stabilize IGFs. Free IGF-I has a half-life of <10 min in blood [62], a half-life of approx. 10 min when bound to IGFBP-1 and -2 and a half-life of >6 h when bound to IGFBP-3 as part of the large 150,000 M_W complex. IGFBP-1 and -2 are transported across intact endothelium and intact capillaries, whereas IGFBP-3, because of its large size, is not [63, 64]. IGFBP-1 and -2 may act to transport IGFs out of the vasculature. In experimental systems, addition of IGFBPs generally inhibit the activities of IGFs (reviewed in [61]) however under certain conditions, usually involving preincubation of cells with IGFBP, IGFBPs have been reported to accentuate the effects of IGFs [65].

Although early studies did not examine the synthesis of the six binding proteins, it is now established that all are synthesized and secreted by breast cancer cells [66]. The possibility that the synthesis of one or more of the IGFBPs may be under the control of steroid hormones and therefore modulate the responsiveness of breast cancer cells to oestradiol has been examined. Clemmons *et al.* [67] concluded that oestrogen receptor-negative cell lines secrete IGFBP-1, -3 and -4 whereas oestrogen receptor positive cell lines secrete IGFBP-2 and -4 but not -3 or -1. Subsequently, Shao *et al.* [68] and Pekonen *et al.* [69] have measured IGFBP expression in breast tumours. Shao *et al.* [68] showed by IGFBP-3 is expressed at higher levels in oestrogen receptor negative tumours. Pekonen *et al.* [69] detected expression of IGFBP-1, -2, -3, -4 and -5. They found elevated levels of IGFBPs in cancer compared to normal adjacent tissue and showed that IGFBP-3 was more likely to be expressed in oestrogen receptor negative tumours in agreement with the data of Shao *et al.* [68]. Recently Yee *et al.* [70] have shown that oestradiol decreases IGFBP-3 but increases IGFBP-2 and Sheikh *et al.* [71] have shown that oestradiol increases the expression of IGFBP-4 but not -5 in MCF-7 cells. Overall, these results show that expression of some of the IGFBPs is modulated by oestrogen. It would be expected that this would modulate the responsiveness of cells to IGFs but experimental evidence for this is still lacking.

Control of circulating IGF levels by steroids

Although the majority of this review has focused on the way in which autocrine or paracrine IGFs may mediate the effects of steroids on proliferation, there is a growing realization that steroids could affect the proliferation of IGF sensitive cells by controlling the circulating levels of IGFs. It has long been known that oestrogens cause an improvement in the clinical status of individuals with acromegaly [72], and this is associated with a reduction of IGF-I levels following oestrogen treatment [73]. This observation is particularly relevant to hormonally-responsive cancers such as breast cancer which are frequently treated with systemic hormonally-active agents. Peyrat *et al.* [74] have recently shown that IGF-I levels are increased in women with breast cancer compared to age matched controls. Colletti *et al.* [75] and subsequently Pollak *et al.* [76] and Lien *et al.* [77] showed that the anti-oestrogen tamoxifen significantly reduced circulating IGF-I levels. The magnitude of the decrease is surprisingly large (approx. 25%) and it has been suggested that the decrease in circulating IGF-I levels could contribute to the antitumour effects of tamoxifen.

Although the mechanism of this effect is not well characterized, it had been presumed that tamoxifen inhibits the release of growth hormone from the pituitary [78]. recent experiments, however, have suggested that tamoxifen can directly decrease IGF-I synthesis in the liver [79].

The true biological significance of these findings has yet to be assessed, but the clear implication is that biologically active IGFs derived from the circulation may be more significant than the IGFs produced locally. If this turns out to be the case, then the control of circulating IGF levels and the control of the responsiveness of cells to IGFs may be more important than the regulation of the synthesis of IGFs in steroid sensitive tissues in mediating the effects of steroids on proliferation.

CONCLUSIONS

Although IGFs are traditionally thought to be involved in mediating the effects of growth hormone in postnatal development, evidence is now accumulating which links IGFs with the control of cell proliferation in normal and neoplastic tissues. This article has reviewed some of the evidence that IGFs are involved in mediating the effects of steroids on cell proliferation

in normal tissues and in oestrogen responsive breast cancer cells. The extent to which the effects of oestrogens on other tissues are also mediated by IGFs and the extent to which IGFs mediate the effect of other steroids remains to be determined.

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