Regulation of insulin-like growth factor I binding in the rat uterus by growth hormone and thyroxine

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Summary. The insulin-like growth factor-I (IGF-I)-binding sites in the rat uterus were characterized further and the effects of growth hormone and thyroxine examined. The ^{125}I -labelled IGF-I binding sites on uterine membranes demonstrated relative binding affinity of less than 20% for IGF-II, less than 1% for insulin and no affinity for an unrelated peptide, epidermal growth factor, compared with 100% for IGF-I confirming the specificity of these binding sites. Scatchard analysis of the specific binding data revealed the presence of a single class of high-affinity binding sites ($K_d = 2.50 \pm 0.68 \text{ nmol l}^{-1}$, with a binding capacity of $1.02 \pm 0.13 \text{ pmol mg}^{-1}$ membrane protein in the uterus of the pituitary-intact ovariectomized rat. After hypophysectomy, the uteri from these rats had significantly ($P < 0.05$) increased IGF-I binding sites, without significant changes in their affinity. Administration of growth hormone with or without L-thyroxine reversed this increase in IGF-I binding. Injection of thyroxine alone to the hypophysectomized ovariectomized rats had no significant effects on the uterine IGF-I binding sites. These data show that growth hormone, but not thyroxine, can regulate IGF-I binding sites in the rat uterus, possibly through regulating IGF-I production.

Keywords: growth hormone; IGF-I binding; thyroxine; uterus; rat

Introduction

Insulin-like growth factors (IGFs) are a family of polypeptides with insulin-like and growth-promoting activity and they play major roles in mammalian development and growth (Froesch *et al.*, 1985; Underwood *et al.*, 1986). There are two molecular forms of IGF, IGF-I and IGF-II. IGF-I is found in plasma in concentrations of up to $1 \mu\text{g ml}^{-1}$ and is circulated bound to a large carrier protein. Although plasma IGF-I is traditionally considered to originate in the liver (Froesch *et al.*, 1985), recent reports indicated that it is synthesized at multiple sites in the body (D'Ercole *et al.*, 1984; Lund *et al.*, 1986; Han *et al.*, 1987), including the uterus (Murphy *et al.*, 1987; Norstedt *et al.*, 1989). Both oestradiol and growth hormone were demonstrated to increase IGF-I messenger ribonucleic acid (mRNA) in the uterus (Murphy & Friesen, 1988).

Several lines of evidence also indicate that IGF-I receptors are distributed widely in various tissues (Ewton & Florini, 1981; Bhaumick & Bala, 1985; Adashi *et al.*, 1988). Recently, IGF-I receptors have been demonstrated in the rat uterus (Chandrasekhar *et al.*, 1990; Ghahary & Murphy, 1989). The studies examining the endocrine requirement for optimal uterine decidualization indicated that both growth hormone and thyroxine were required in addition to oestradiol and progesterone (Kennedy & Doktorcik, 1988). Thus, it is possible that growth hormone and thyroxine may regulate uterine IGF-I or IGF-I receptors in the uterus. Here, we examined the regulation of IGF-I receptors in the uterus by growth hormone and thyroxine.

Materials and Methods

Materials

L-Thyroxine, oestradiol and progesterone were obtained from Sigma Chemical Co. (St Louis, MO). Ovine growth hormone (oGH, NIADDK-oGH-15) was a generous gift from the National Hormone and Pituitary Programs, National Institute of Diabetes, Digestive and Kidney diseases. Recombinant human IGF-I was purchased from Amgen Biologicals (Thousand Oaks, CA) and radioiodinated recombinant human IGF-I $2000 \text{ Ci mmol}^{-1}$ was purchased from Amersham Co. (Arlington Heights, IL). All other chemicals used were of analytical reagent grade and were obtained from local sources.

Animals and treatments

Female Sprague-Dawley rats, hypophysectomized at 200–225 g body weight, were obtained from Harlan-Sprague-Dawley (Indianapolis, IN). Pituitary-intact female rats of the same strain were also obtained from the same supplier at 150–160 g body weight. The rats were housed under temperature- and light-controlled conditions (lights on from 05:00–19:00 h) with free access to food and water. The hypophysectomized rats were also given 2% glucose in addition to water.

From previous observations (Ghahary & Murphy, 1989; Chandrasekhar *et al.*, 1990) it was noted that ovarian steroids altered the uterine IGF-I receptor content and therefore all the rats used were ovariectomized. The rats were ovariectomized under ketamine anaesthesia (Ketalar: Parke-Davis, Morris Plains, NJ) and allowed at least 5 days to recover from surgery. At the start of the experiments, hypophysectomized ovariectomized rats had been hypophysectomized for 10–14 days. The rats then received s.c. injection of oGH ($120 \mu\text{g}$, twice in $0.03 \text{ mol NaHCO}_3 \text{ l}^{-1}$ in $0.15 \text{ mol NaCl l}^{-1}$, daily) and thyroxine ($1 \mu\text{g}$, in $0.15 \text{ mol NaCl l}^{-1}$, daily). As controls, a group of pituitary-intact ovariectomized rats from the same colony received vehicle only. The treatments were continued for 3 days and four animals per group were killed by decapitation and exsanguination.

Membrane preparation

The uteri were rapidly removed, cleaned, weighed and placed in ice-cold buffer for at least 1 min. The details of preparation of the membranes from the uterus have been described elsewhere (Chandrasekhar *et al.*, 1990). Briefly, the uteri were cleaned and homogenized with three 15 s bursts at a setting of 60 in Tekmar Tissuemizer (Tekmar Co., Cincinnati, OH) in $10 \text{ mmol Tris-HCl l}^{-1}$; containing $1 \text{ mmol CaCl}_2 \text{ l}^{-1}$ and $250 \text{ mmol sucrose l}^{-1}$. The homogenate was then centrifuged at $700 g$ for 10 min and the supernatants were recentrifuged at $35\,000 g$ for 40 min. The pellet was washed twice and resuspended in $10 \text{ mmol Tris-HCl l}^{-1}$ containing $1 \text{ mmol CaCl}_2 \text{ l}^{-1}$. All the above procedures were carried out at 4°C . The concentration of protein in the membrane preparations was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

IGF-I binding assay

Specific binding sites for IGF-I were measured on the uterine membrane preparations as described previously (Chandrasekhar *et al.*, 1990). Briefly, two replicates of uterine membranes from each membrane preparation containing $50 \mu\text{g}$ protein per tube were incubated with $0.02 \text{ nmol } ^{125}\text{I}$ -labelled IGF-I with or without various concentrations (0.42 – 53 nM) of radioinert IGF-I in $200 \mu\text{l Tris-HCl}$ with 0.1% gelatin. The optimal binding assay conditions in terms of time, temperature and pH were described previously (Chandrasekhar *et al.*, 1990). The binding assays were performed by incubating the uterine membranes at 4°C for 20 h at pH 7.8. At the end of the incubation, the membrane-bound ^{125}I -labelled IGF-I was separated from free ^{125}I -labelled IGF-I by centrifugation. The pelleted membrane-bound ^{125}I -labelled IGF-I fraction was washed twice with 1 ml of fresh ice-cold Tris-HCl buffer with 0.1% gelatin and the bottom tips of the microfuge tubes were cut and counted for ^{125}I in a gamma counter (Packard Auto-gama, Downers Grove, IL, USA). Approximately 9.0% of the total counts added was specifically bound in the absence of radioinert IGF-I and 1.8% of the total counts added was nonspecifically bound in the presence of 53 nM radioinert IGF-I. The binding affinity and the total binding capacity of IGF-I for binding the uterine membranes were determined from a Scatchard plot of the specific binding data (Scatchard, 1949). The specificity of the binding of IGF-I to the uterine membranes was determined from a log-dose inhibition of specific binding of ^{125}I -labelled IGF-I by radioinert IGF-I, IGF-II, insulin and epidermal growth factor (EGF), in terms of relative binding affinity as described for other receptors (Singh *et al.*, 1985).

Completeness of hypophysectomy

At autopsy, the sella turcica of all hypophysectomized ovariectomized rats was examined for pituitary remnants which, if present, resulted in the exclusion of that animal from the study.

Data analysis

Data from the binding assays were plotted according to the graphical representation of Scatchard (1949). Regression analysis was used to calculate line slopes and intercepts (Snedecor & Cochran, 1967). The apparent dissociation constant (K_d) was calculated as the negative reciprocal of the slope and binding site concentration was determined from the x -axis intercept. The significance of treatment effects was determined by analysis of variance and when significant interactions were found, Duncan's new multiple range test (Steel & Torrie, 1960) was used for comparisons between animal groups.

Results

Specificity of ^{125}I -labelled IGF-I binding

To examine the specificity of the ^{125}I -labelled IGF-I binding, we incubated uterine membranes obtained from pituitary-intact ovariectomized rats with $0.02 \text{ nmol } ^{125}\text{I}$ -labelled IGF-I l^{-1} , with or without various concentrations of radioinert ligands. ^{125}I -labelled IGF-I binding to uterine membranes was displaced in a dose-dependent fashion by IGF-I, IGF-II and insulin (Fig. 1), but not by EGF. From the log-dose inhibition curves, the relative binding affinity of IGF-II and insulin was calculated to be $<20\%$ and $<1\%$ compared with that of IGF-I for displacing ^{125}I -labelled IGF-I bound to the uterine membranes, respectively.

Fig. 1. Effect of insulin-like growth factor (IGF)-I (○), IGF-II (□), insulin (●) and epidermal growth factor (■) on specific binding of ^{125}I -labelled IGF-I to rat uterine membranes. Each value is a mean of triplicate determinations using pooled uterine membranes.

Uterine weight

Uterine wet weights from individual animals were determined and expressed as mean $\pm$ SEM per group for each treatment. The weight of the uterus was 103.7 ± 9.5 , 82.3 ± 8.2 , 98.0 ± 5.5 , 97.7 ± 6.3 , $95.3 \pm 6.8 \text{ mg}$ in pituitary-intact ovariectomized and hypophysectomized ovariectomized rats receiving vehicle, growth hormone, growth hormone and thyroxine, or thyroxine, respectively, and was not significantly different from each other.

Uterine IGF-I receptors

Scatchard plots of the specific binding data confirmed the presence of a single class of high-affinity binding sites for IGF-I on uterine membranes. The binding affinity calculated in terms of equilibrium dissociation constant, K_d , and the number of specific binding sites for IGF-I on uterine membranes of pituitary-intact ovariectomized rats were $2.50 \pm 0.68 \text{ nmol l}^{-1}$ and $1.02 \pm 0.13 \text{ pmol mg}^{-1}$ protein, respectively (Table 1). The K_d values from Scatchard analysis of IGF-I specific binding data from hypophysectomized ovariectomized rats injected with vehicle, growth hormone, growth hormone + thyroxine or thyroxine are given in Table 1. There were no significant differences in the K_d values among the treatment groups indicating that the affinity of the IGF-I binding to the uterus is not regulated by growth hormone or thyroxine. However, the concentration of specific IGF-I binding sites in the uterus are significantly ($P < 0.05$) higher in hypophysectomized ovariectomized rats compared with pituitary-intact ovariectomized rats (Table 1). The increase in concentration of IGF-I binding sites in the uterus of hypophysectomized ovariectomized animals was reversed by growth hormone, but not by thyroxine injection. The IGF-I binding sites were significantly ($P < 0.05$) higher in hypophysectomized ovariectomized rats receiving vehicle or thyroxine, compared with hypophysectomized ovariectomized rats receiving growth hormone with or without thyroxine and pituitary-intact ovariectomized rats. The difference in binding site concentrations among the last three groups was not significant.

Table 1. Specific binding of ^{125}I -labelled IGF-I by uterine membrane preparations from hypophysectomized ovariectomized and pituitary-intact ovariectomized rats: summary of analysis of Scatchard plots

Source of preparation	Apparent dissociation constant (nmol l^{-1})	Binding site concentration (pmol mg^{-1} protein)
Hypophysectomized ovariectomized rats		
Vehicle-treated	4.20 ± 0.85	2.04 ± 0.08^a
oGH-treated	3.90 ± 0.95	1.45 ± 0.20^b
oGH + thyroxine-treated	4.40 ± 1.05	1.44 ± 0.15^b
Thyroxine-treated	3.90 ± 0.87	2.20 ± 0.06^a
Ovariectomized rats	2.50 ± 0.68	1.02 ± 0.13^b

Means $\pm$ s.e.m. with different superscripts differ significantly ($P < 0.05$). Hypophysectomized ovariectomized rats received daily s.c. injections of $2 \times 120 \mu\text{g}$ ovine growth hormone (oGH) with or without $1 \mu\text{g}$ thyroxine or vehicle for only 3 days before being killed; ovariectomized rats received vehicle only. Each value represents the combined data obtained from two separate experiments.

Discussion

Specific binding sites for IGF-I have been demonstrated in a variety of tissues (Zapf *et al.*, 1978; Rechler *et al.*, 1980; Bhaumick *et al.*, 1981; Adashi *et al.*, 1988). The presence of IGF-I receptors in the rat uterus was previously reported (Chandrasekhar *et al.*, 1990). The studies reported here further confirm the specificity of the IGF-I binding sites in the rat uterus since the relative binding affinity of radioinert IGF-II was $<20\%$ compared with that of IGF-I for displacing ^{125}I -labelled IGF-I bound to the uterine membranes. The relative binding affinity of $<20\%$ and $<1\%$ for IGF-II and insulin compared with that of 100% for IGF-I for displacing ^{125}I -labelled IGF-I bound to the uterine membranes was similar to that reported for other tissues (Bhaumick & Bala, 1985; Tramontano *et al.*, 1987; Ritvos *et al.*, 1988).

The effects of growth hormone and thyroxine on IGF-I receptors in the uterus were investigated. It was observed, for the first time, that the concentration of IGF-I receptors was increased (two-fold)

in hypophysectomized ovariectomized rats compared with the pituitary-intact ovariectomized rats, suggesting that hormones from the pituitary gland are involved in the process. Hypophysectomized ovariectomized rats were, therefore, treated with growth hormone and thyroxine (a substitute for thyroid-stimulating hormone), since these hormones are required for normal uterine decidual cell reaction (Kennedy & Doktorcik, 1988). The results of the experiment indicated that treatment with growth hormone with, or without, thyroxine reversed the increases in IGF-I binding sites in the uterus of hypophysectomized ovariectomized rats, such that the uterine IGF-I binding sites in these animals were similar to pituitary-intact ovariectomized rats. Thyroxine alone, however, had no significant effect on the uterine IGF-I binding sites in hypophysectomized ovariectomized rats. The effects of growth hormone on IGF-I binding sites appeared to be specific since growth hormone had no significant effect on the uterine wet weight in these animals.

The mechanism(s) by which growth hormone induces IGF-I receptor regulation in the uterus is not clear. It is possible that these changes in IGF-I binding in hypophysectomized ovariectomized rats may be due to direct effects of growth hormone on the cells bearing IGF-I receptors, since the receptor/binding protein activity for growth hormone has recently been reported in the rat uterus (Lobie *et al.*, 1990). Alternatively, it is possible that the lack of growth hormone in hypophysectomized rats decreases IGF-I production (Reiter & Lovinger, 1981; Bucher *et al.*, 1983; Scott *et al.*, 1985; Hynes *et al.*, 1987) which, in turn, may result in increases in uterine IGF-I receptor concentration. Growth hormone administration to hypophysectomized animals has been demonstrated to induce elevations in immunoreactive-IGF-I concentrations (Roberts *et al.*, 1986; Maiter *et al.*, 1988; Daughaday & Rotwein, 1989). Presumably, the increased IGF-I concentrations might have induced downregulation of uterine IGF-I binding sites. Ligand-induced downregulation of the receptors has been reported for IGF-I in IM-9 lymphocytes (Rosenfeld *et al.*, 1982) and human skin fibroblasts (Rosenfeld & Dollar, 1982). Scatchard analysis of the binding data suggested that the decreases in IGF-I binding sites by growth hormone was accounted for by a decrease in the number of binding sites rather than a change in binding affinity. In the studies by Rosenfeld *et al.* (1982), the ability of IGF-I, IGF-II and insulin to decrease the number of IGF-I receptors was proportional to their relative potency in inhibiting binding of ^{125}I -labelled-IGF-I to IM-9 cells (IGF-I > IGF-II > insulin), suggesting that the downregulation depends upon receptor occupancy.

In summary, it has been confirmed that specific IGF-I receptors are present in the rat uterus and that these receptor concentrations are increased in hypophysectomized ovariectomized rats. Growth hormone, but not thyroxine alone, reversed this effect. The effects of growth hormone on IGF-I receptors of uterus may be mediated by IGF-I concentration and further studies are required to confirm this hypothesis.

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