Specific binding of human growth hormone but not insulin-like growth factors by human adipocytes

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Binding of human GH (hGH) and insulin-like growth factors I and II (IGFI and II) to isolated human adipocytes from adult subjects was studied. Binding equilibrium for hGH at 24°C was reached at 120 min and half-maximal specific binding at 6–8 ng/ml. Apparent K_a was 2.1×10^9 M^{-1} and B_{max} 7.3×10^{-11} M/10^6 cells. The human fat cell growth hormone receptor recognized neither bovine, ovine or rat GH nor human prolactin or placental lactogen. No specific receptors for human IGFII could be demonstrated. Thus, human adipocytes do not possess IGF receptors but have specific GH receptors which recognize hGH but not GH from lower species.

Growth hormone *Insulin* *Insulin-like growth factor* *Adipocyte*

1. INTRODUCTION

The human organism is exclusively responsive to primate growth hormone (GH) whereas lower species respond to GH from human and many other animal sources [1]. An explanation for these findings is not readily available.

Isolated adipocytes or adipose tissue fragments from several species, including rat and human, respond to growth hormone stimulation [2–7]. We have also recently shown that isolated fat cells from normal rats possess specific binding sites for hGH [6,7]. In these studies, GH from species other than the human were recognized by the rat adipocyte GH receptors to variable degrees. Several previous studies have shown that rat fat cells also possess specific receptors for the insulin-like growth factors (IGF) [8], particularly IGFII [9]. The metabolic insulin-like effect of these growth factors seems to be mediated by their ability to bind to the insulin receptors [8]. However, a more complicated interaction between insulin and IGF is also evident as insulin increases IGFII binding in rat adipocytes by translocating receptors from an intracellular pool to the plasma membrane through an energy-requiring process very similar to the effect of insulin on glucose transport units [9].

The aim of the present study was to investigate whether human fat cells possess specific receptors for these growth factors.

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2. MATERIALS AND METHODS

2.1. *Tissue sources*

Biopsies of abdominal subcutaneous adipose tissue were obtained from 20 subjects, 34–75 years of age and with a relative body weight of 83–170%, undergoing elective surgery.

2.2. *Hormone binding*

Isolated fat cells were prepared by incubating small fragments with collagenase as described [10].

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The isolated cells ($\sim 3 \times 10^5$ cells/1.5 ml) were incubated with ^{125}I-labelled insulin, hGH, hIGFI or hIGFII in the presence of variable concentrations of unlabelled hormone (2–5000 ng/ml) in medium 199 containing 4% albumin for 2 h unless otherwise specified.

In the experiments aimed at elucidating receptor specificity, 3000 ng/ml of either hGH, rGH, bGH were added to the vial containing 4.4 ng/ml ^{125}I-hGH. Similarly, human thyroid stimulating hormone (hTSH), human adrenocorticotropic hormone (hACTH), human prolactin (hPRL) and human placental lactogenic hormone (hPRL) were tested for the degree of competition at concentrations of 500–5000 ng/ml.

At the end of the incubation period, samples of the cell suspension were centrifuged in microtubes through dinonyl phthalate as described by Gliemann et al. [11]. Total number of fat cells in each vial was calculated by dividing the triglyceride content by the mean cellular lipid weight as described [10]. Non-specific binding, estimated from the non-saturable binding [12], was subtracted. Specific hGH binding was expressed on a per cell basis after correcting for non-specific binding. Hormone degradation during the binding assay was monitored by measuring the precipitability of label with 10% trichloroacetic acid. The degradation was less than 15%.

2.3. *Glucose transport*

In some experiments glucose transport was measured essentially as described by Kashiwagi et al. [13]. Briefly, cells at a lipocrit of 3–5% were preincubated at 37°C with or without the addition of hormone in a glucose-free medium. After 15 min, [U-^{14}C]glucose was added at a final concentration of 50 nM (~ 0.2 μCi/tube). After 60 min, cells and medium were separated through dinonyl phthalate [11] and the uptake determined. Control experiments were also performed with 3-*O*-[^{14}C]methylglucose uptake using described incubation conditions [14].

2.4. *Hormones*

Purified hGH, with an estimated potency of 2 U/mg was kindly supplied by Kabi AB, Stockholm, Sweden. Human IGFI and IGFII were purified as described [15]. hACTH was provided by Kabi AB, Stockholm, Sweden, bGH (NIH-bovine GH-18), oGH (NIH-ovine GH-5-6) and rGH (NIH-rat GH-B-6) were provided by the Pituitary Agency of the National Institutes of Health.

Human IGFI and II were iodinated according to published procedures [15]. Purified hGH was iodinated with Na^{125}I as described by Thorell and Johansson [16]. ^{125}I-insulin was kindly supplied by Dr Sten Ivarsson, Malmö.

3. RESULTS

3.1. *hGH binding*

Initial experiments of the time-course for ^{125}I-hGH binding at 24°C showed that equilibrium had been established by 2 h and persisted throughout 3 h. All subsequent binding experiments were, therefore, carried out for 2 h.

Addition of unlabelled hGH completed in a dose-related manner with ^{125}I-hGH binding. Specific binding per 10^6 fat cells reached saturation at hGH concentrations of 50 ng/ml (fig.1). Half-maximal binding was observed at 6–8 ng/ml of unlabelled hormone. Non-specific binding averaged $38 \pm 6\%$ (mean $\pm$ SE, $n = 11$).

A Scatchard plot of the data presented in fig.1 (inset) revealed a single class of binding sites with an apparent K_a of 2.1×10^9 M^{-1} and an estimated B_{max} of 7.300/cell.

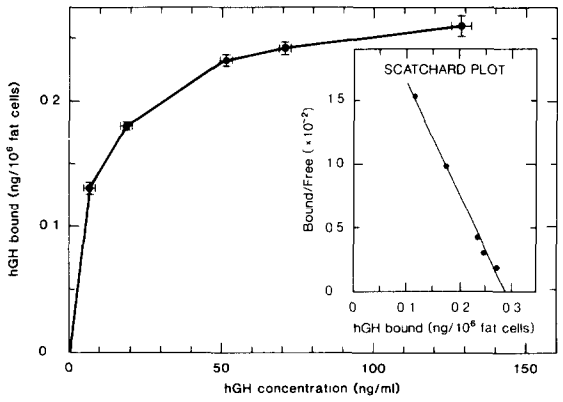

Fig.1. Specific binding of hGH to human fat cells after 2 h incubation at 24°C. The data represent the mean ± SE of triplicate samples from 11 subjects. The incubation flasks contained ^{125}I-hGH without or with increasing amounts of unlabelled hGH to achieve final hormone concentrations of 6–140 ng/ml. Inset shows the Scatchard plot of the same data.

Table 1 shows the ability of different peptide hormones to displace labelled hGH from adipocyte hGH receptors. It is clear from the lack of inhibition shown by rGH, bGH and oGH that the human adipocyte GH receptor failed to recognize GH from the other species tested. Furthermore, no significant inhibition of ^{125}I-hGH binding was found with hPRL or hPLH (table 1) or with other peptide hormones like hACTH, hTSH, or human IGFII (not shown).

3.2. *IGF binding*

No consistent specific binding of either human ^{125}I-IGFI or IGFII was found in fat cells from 9 adult individuals tested. Non-specific binding averaged 85 ± 15% of total binding. The lack of specific IGF binding sites in human fat cells was independent of temperature (24 or 37°C), incubation time (up to 3 h), addition of 1 mM KCN to prevent recycling of the IGFII receptors [9] or preincubation with a high insulin concentration (table 2).

In spite of the absence of specific binding sites, human IGFII stimulated both [U-^{14}C]glucose uptake (table 3) and 3-O-[^{14}C]methylglucose uptake (not shown) but exceedingly high concentrations were required (~100-fold higher than for insulin) suggesting that this effect may be mediated through the insulin receptors. This is supported by the finding that IGFII at these concentrations competed with the binding of ^{125}I-insulin to the insulin receptor (table 3).

Table 1

Inhibition of ^{125}I-hGH binding by GH from human or non-human species

Peptide hormones (3 μg/ml)	% inhibition of specific ^{125}I-hGH binding
hGH	100
rGH	1 ± 2
bGH	−6 ± 7
oGH	−1 ± 6
hPRL	−4
hPLH	−6

GH preparations from different species were incubated at 3000 ng/ml in the presence of 7.4 ng/ml ^{125}I-hGH for 2 h at 24°C. Each value represents the mean ± SE of triplicate determinations in 3 experiments. The results with human prolactin (hPRL) and placental lactogenic hormone (hPLH) are the means of one experiment performed in triplicates. Values shown indicate degree of inhibition of binding relative to that seen with unlabelled hGH (= 100%)

Table 2

Binding of human ^{125}I-IGFII to human fat cells

Additions to medium	Unlabelled IGFII, ng/ml				
	0	10	50	100	1000
None	100	100 ± 2	104 ± 4	98 ± 3	97 ± 2
Insulin (42 ng/ml)	102 ± 5	102 ± 5	105 ± 3	97 ± 4	93 ± 2

Isolated fat cells from 5 adult individuals were preincubated for 60 min at 37°C with 42 ng/ml insulin. After 55 min, 1 mM KCN was added to prevent recycling and 5 min later 0.3 ng/ml ^{125}I-IGFII was added and the vials were incubated at 24°C for an additional 60 min. All results (mean ± SE) are expressed relative to total binding obtained in the absence of unlabelled IGFII (= 100%)

Table 3

The effect of insulin and human IGFII on ^{125}I-insulin binding and glucose transport in human fat cells

Additions to medium ng/ml (M)			Glucose clearance (fl/cell per s)	^{125}I-insulin binding (% of control)
None			1.5	100
IGFII	0.8	(10^{-10})	1.4	98
	8	(10^{-9})	1.4	97
	80	(10^{-8})	2.3	87
Insulin	0.6	(10^{-10})	2.3	85
	6	(10^{-9})	2.8	39
	60	(10^{-8})	2.5	8

Isolated human fat cells were incubated with [U-^{14}C]glucose or ^{125}I-insulin for 60 min and with unlabelled hormone as indicated. The results are the means of duplicate determinations of a representative experiment

4. DISCUSSION

The present study demonstrates that human

adipocytes contain specific hGH receptors but, surprisingly, no receptors for the insulin-like growth factors I and II could be demonstrated. The half-maximal specific binding of hGH was reached at physiological hormone concentrations, indicating that these specific binding sites may indeed be functional receptors for the hormone. Human adipocytes, like rat adipocytes, are target cells for GH [2–7]. However, only the insulin-antagonistic effect of hGH has been demonstrated in human fat cells [5] while rat fat cells respond with both an initial insulin-like and a subsequent insulin-antagonistic effect [6,7].

It is not surprising that a hormone which produces biological effects is also specifically recognized and bound by the target cell. The binding kinetics for hGh to human fat cells are very similar to recent findings with human blood cells [17] but are both quantitatively and qualitatively different from those of rat adipocytes. The total number of binding sites appears to be lower in human than in rat fat cells [6]. The most significant difference, however, is the inability of growth hormone from non-primate species to interact with the hGH receptor sites on human fat cells. This is in clear contrast to the rat adipocytes which have been shown to recognize growth hormone from several species [6]. The lack of recognition by the human cells of other species of growth hormone is consonant with the known species differences in the biological action of growth hormone and may indeed be responsible for them.

The lack of specific binding sites for both IGFI and II in human fat cells is also in marked contrast to the findings with rat adipocytes where binding of both IGFI and II to the IGFII receptors has repeatedly been demonstrated [9]. Furthermore, in rat fat cells insulin markedly increases IGFII binding through the translocation of receptors from a large intracellular pool to the plasma membrane, similar to the effect of insulin on the glucose transporters [9]. However, in the human adipocytes no such effect of insulin could be demonstrated. The complete absence of both IGFI and II receptors excludes an important role of these growth factors in the metabolic effect of insulin in the mature human fat cell. It is interesting to note that human fibroblasts differ in this respect as specific binding of IGFII has clearly been demonstrated in these cells [18]. It is not clear whether this difference mirrors the different cells or the degree of maturation of the cells.

Table 4

Comparison of rat and human adipocyte membrane binding of growth factors

	Rat cells	Human
Specific binding of hGH	+	+
Recognition of non-primate GH by hGH receptor	+	–
Specific binding of insulin	+	+
Specific binding of IGFII	+	–
Effect of insulin in enhancing IGFII binding	+	–

The insulin-like effect of IGFII was demonstrated in human fat cells but the potency was around two orders of magnitude less than for insulin. Since IGFII has a low affinity for the insulin receptor in human fat cells (table 3) it is likely that this stimulatory effect is also mediated through the insulin receptors, analogous to findings with rat fat cells [8,18].

In conclusion, the demonstration of specific hGH binding sites in isolated human adipocytes may be useful for the exploration of the relationship between cellular binding, plasma levels and biological effects of this hormone during growth and development and in abnormal states such as short stature and acromegaly. Additionally, this may prove to be a useful system to evaluate structure, activity and binding characteristics of different GH preparations and fragments.

The salient differences between binding and action of GH and IGF in rat and human fat are summarized in table 4.

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