Vol. 137, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1986 Pages 552-558

CHARACTERIZATION OF THE INSULIN AND INSULIN-LIKE GROWTH FACTOR RECEPTORS AND RESPONSITIVITY OF A FIBROBLAST/ADIPOCYTE CELL LINE BEFORE AND AFTER DIFFERENTIATION

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Received March 31, 1986

TA1 cells, like 3T3-L1 cells, undergo a differentiation process in vitro from a fibroblast to an adipocyte phenotype. The TA1 pre-adipocytes were found to have low numbers of insulin receptors but high numbers of receptors for insulin-like growth factors (IGF) I and II. Also, the pre-adipocytes were more responsive to IGF than insulin as measured by either stimulation of glucose or amino acid uptake. After differentiation, the adipocytes had higher numbers of insulin receptors and a better responsitivity to insulin than to IGF-I. These results indicate that insulin-like growth factors are the primary regulators of the pre-adipocytes whereas insulin regulates the adipocytes.

Cell lines which differentiate from a fibroblast phenotype (i.e. pre-adipocytes) to an adipocyte phenotype in culture have been extremely useful for studies of the hormonal regulation of the differentiation process as well as studies of the hormonal regulation of the differentiated adipocytes (1,2). One such cell line, called 3T3-L1, has been extensively studied as a model system for insulin action. After differentiation, there is an increase in insulin receptors and insulin responsitivity in these cells (3-5). These cells have therefore been extensively utilized to study the structure and synthesis of the insulin receptor (6,7). In addition, the differentiated adipocytes have been utilized to study both short and long-term biological effects of insulin (8-10). Finally, these cells have been utilized to study the mechanism of action of insulin (11-13).

In addition to its effects on the adipocytes, high concentrations of insulin have also been reported to stimulate the differentiation of the pre-adipocytes (14). Since insulin at high concentrations can interact with receptors for insulin-like growth factor-

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Abbreviations used: IGF, insulin-like growth factor; MSA, multiplication stimulating activity; and MAIB, methyl-aminoisobutyric acid.

0006-291X/86 $1.50

I (IGF-I) , it was not clear whether these effects of insulin were mediated through the insulin receptor or the IGF-I receptor (15-17). Although both human and rat fibroblasts have IGF-I receptors (18-20), one report suggested that the 3T3-L1 pre-adipocytes had only low levels of IGF-I receptor which increased after differentiation (21). In contrast, this report indicated that both pre-adipocytes and adipocytes had high levels of receptor for IGF-II (21), a receptor which is structurally quite dissimilar from the insulin receptor and which cannot bind insulin (15-17).

In the present studies we have therefore more fully examined the levels of insulin, IGF-I and IGF-II receptors in a cell line that differentiates from pre-adipocytes to adipocytes. In addition, we have tested the responsitivity of these cells to the three hormones. For these studies, we have utilized a newly described cell line, called TA1 cells, which differentiates in vitro from a fibroblast to an adipocyte phenotype (22). This differentiation process will spontaneously initiate when growth is arrested at high cell density; however, glucocorticoids and high concentrations of insulin will enhance the differentiation process (22). We have found in these cells a decrease in IGF receptor number and IGF responsitivity after differentiation whereas the number of insulin receptors and insulin responsitivity was found to increase.

MATERIALS AND METHODS

Cell Cultures - TA1 fibroblasts were plated in 24-well plates and fed every three days with Dulbecco-Vogt Modified Eagles' medium containing 10% fetal calf serum. Fibroblast cultures were utilized either at confluence (as pre-adipocytes) or allowed to become adipocytes. To obtain adipocytes, cells were allowed to differentiate for 12 to 14 days after reaching confluence. Alternatively, confluent cells were treated with 1 μM dexamethasone and 0.5 mM 1-methyl-3-isobutylxanthine for 3 days and normal medium for 3 days. Adipocytes derived from either treatment gave comparable results in binding and biological studies.

Binding studies - Insulin, IGF-I (a gift of Dr. James Merryweather, Chiron Corporation, Emeryville, CA) or rat IGF-II (single component MSA, MSA-III-1, a gift of Dr. P. Nissley, National Institutes of Health, Bethesda, MD) were labeled with Na ^{125}I (carrier-free, Amersham) by a chloramine T method to specific activities of 120, 100 and 100 μCi/μg, respectively (23). Cells were incubated with 20 to 80 pM labeled ligand for 16 hrs at 4°C in 0.5ml media (120 mM NaCl, 50 mM Hepes with 1% bovine serum albumin, pH 7.8) in the presence of the indicated concentrations of unlabeled ligand. Incubations were terminated by washing cells three times with 1 ml of ice cold Tris buffered saline, pH 7.6. Cells were then solubilized in sodium dodecyl sulfate, counted and the protein measured. Results are expressed as percent total counts bound per 2.5×10^5 cells. For pre-adipocytes and adipocytes, there was 55.8 and 60 μg protein per 10^5 cells, respectively and 1.4 and 2.6×10^5 cells per well, respectively.

Glucose and Amino Acid Uptake - Cells were incubated with hormone for 15 min or 6 hrs for glucose or amino acid uptake responses, respectively. Buffer was 140 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 1.47 mM KH_2PO_4, 1.47 mM KH_2PO_4, 8.06 mM $NaHPO_4$, 0.49 mM $MgCl_2$, pH 7.4 containing either 0.1% bovine serum albumin (for

glucose uptake) or 2% bovine serum albumin with 20 mM glucose (for amino acid uptake). The cells were then pulsed for 5 min or 10 min with either 0.1 mM 2-[1,2-^{3}H(N)]-deoxy-D-glucose (0.2 µCi/ml) (New England Nuclear) or 20 µM α-[methyl-^{3}H] aminoisobutyric acid (0.2 µCi/ml) (New England Nuclear), respectively. Cells were then rinsed three times, solubilized in sodium dodecyl sulfate and aliquots were counted and protein measured.

RESULTS

Binding studies. Both pre-adipocytes and adipocytes were found to specifically bind ^{125}I-IGF-I and ^{125}I-IGF-II (Fig. 1). However, the amount of IGF-I and II bound per cell in the pre-adipocytes was 3 and 6 times greater than that bound by the adipocytes (Fig. 1). The binding of ^{125}I-IGF-II was predominately via the type II IGF receptor since insulin, even at 10 µM, did not inhibit its binding (Fig. 1). In contrast, the binding of ^{125}I-IGF-I was primarily to the type I IGF receptor (16) since this binding was inhibited by insulin at concentrations 30 to 100 times greater than the concentrations of IGF-I required to inhibit binding (Fig. 1).

In contrast to the decrease in IGF binding observed with differentiation, insulin binding increased (Fig. 1). In adipocytes, 1.3% of the ^{125}I-insulin was bound specifically

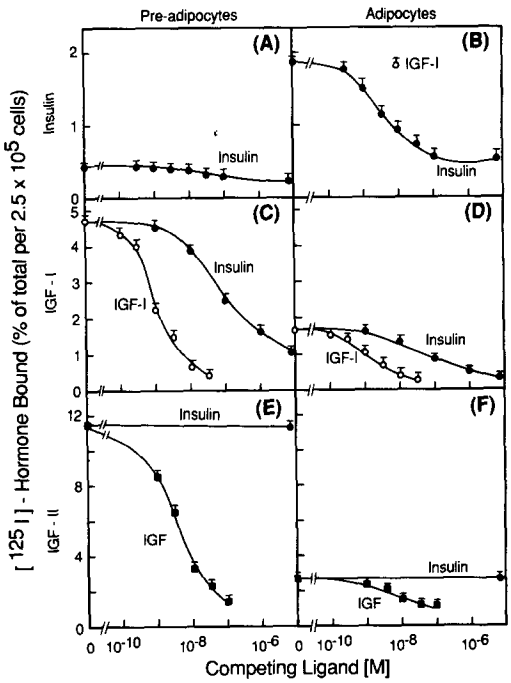

Figure 1 Receptors for insulin, IGF-I and IGF-II on TA1 cells. Either pre-adipocytes (left) or adipocytes (right) were incubated with labeled hormone in the presence of the indicated concentration of competing ligand and the amount of bound radioactivity determined. Each value is the mean ± SD of triplicate determinations.

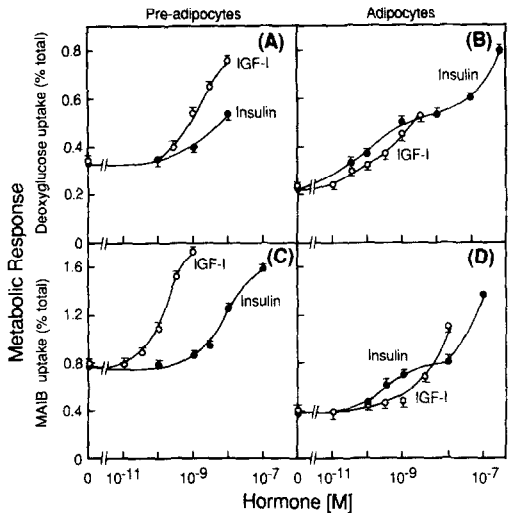

Figure 2 Stimulation of glucose and amino acid uptake in TA1 cells by insulin and IGF-I. Either pre-adipocytes (left) or adipocytes (right) were incubated with the indicated concentrations of hormone and then the cells were pulsed with either [^{3}H] deoxyglucose or [^{3}H] MAIB. Each value is the mean ± SD of triplicate determinations.

per 2.5×10^5 cells and 2 nM cold insulin half-maximally inhibited this binding. In contrast, in the pre-adipocytes, seven times less specific ^{125}I-insulin binding was observed (0.2% per 2.5×10^5 cells). In addition the concentration of insulin that half-maximally inhibited this binding was 40 nM (Fig. 1), suggesting that this low level of insulin binding to the pre-adipocytes might be to the IGF-I receptor.

Glucose Uptake. IGF-I stimulated 2-deoxy-D-glucose uptake 2 fold in the pre-adipocytes with a half-maximal effect at 1 nM (Fig. 2). In contrast, insulin was about 30 times less potent (10 nM caused a 50% increase in uptake), suggesting that it might be acting via the IGF-I receptor (Fig. 2). In contrast, in the adipocytes, 1 nM insulin stimulated deoxyglucose uptake 2.5 fold (Fig. 2). Additional stimulation of glucose uptake was seen at higher insulin concentrations, suggesting that at these higher concentrations insulin was acting via the IGF-I receptor. In the adipocytes the response to IGF-I was also biphasic (Fig. 2), suggesting that IGF-I was also stimulating glucose uptake via both receptors.

Amino Acid Uptake. Insulin stimulates the uptake of the non-metabolizable amino acid analog α-methyl-aminoisobutyrate in 3T3-L1 adipocytes (10). A similar effect was seen with the TA1 adipocytes (Fig. 2). However, the dose response curve

was again biphasic, suggesting that insulin was acting through both receptors. Support for this hypothesis came from the finding that IGF-I could also stimulate amino acid uptake in these cells (Fig. 2).

In contrast, in the pre-adipocytes, IGF-I was approximately 30 times more potent than insulin at stimulating amino acid uptake (Fig. 2). In addition, rat IGF-II, was approximately as potent as insulin at stimulating MAIB uptake (data not shown).

DISCUSSION

Since the purification and characterization of the insulin-like growth factors I and II, it has become clear that these hormones can regulate many of the same metabolic responses of cells as insulin (17). However, these molecules have their own distinct receptors (15,16). Much work has therefore been devoted to attempting to delineate which responses are mediated through which receptors. At first, it was proposed that insulin regulated acute metabolic responses of cells whereas IGFs regulated more long-term effects of cells (24). However, data now indicate that in some systems insulin can stimulate growth of cells through its own receptor (25) and the IGFs can stimulate short term responses of cells through their own receptors (19,26,27).

The present study indicates that the state of differentiation may also affect the responses of cells to these hormones. Thus, the TA1 pre-adipocytes have high levels of receptors for both IGF-I and II and are more responsive to IGF-I than to insulin in both an acute response (stimulation of glucose uptake) and a more delayed response (stimulation of amino acid uptake). In contrast, after differentiation, the levels of IGF receptors were found to decrease and the insulin receptors increased. These results differ from those previously reported for 3T3-L1 cells (21) in which it was reported that IGF-I receptors increased after differentiation. These different results could be due to a difference in these two cell types. In any case, the findings of a differential regulation of these two structurally similar receptors suggests that the mechanism regulating their expression must be different. In contrast, prior studies of the levels of these two receptors in patients with defects in the expression of the insulin receptor suggested that similar mechanisms regulated their synthesis (28, 29).

In addition to the increase in insulin receptor number, the TA1 adipocytes had an increase in insulin responsitivity. The adipocytes were thus more sensitive to insulin

than IGF-I. However, the cells were still quite responsive to IGF-I and thus the effects of IGF-I, at least at low concentrations, are probably mediated through its own receptor. In addition, the responses to insulin appeared biphasic. The effects of insulin might therefore be mediated at low concentrations through the insulin receptor and at high concentrations through the IGF-I receptor. A similar biphasic response to insulin was seen in a human hepatoma cell line (26).

In summary, the present results suggest that for the undifferentiated TA1 preadipocyte, IGF may be the primary regulator of various classical "insulin dependent" metabolic responses. In contrast, after differentiation, the role of insulin in regulating these same responses becomes more prominant. Recently similar findings have been reported for muscle cells, where the IGFs were found to be the primary regulators of the metabolic responses of the undifferentiated myoblasts whereas insulin is more potent at regulating the differentiated myotubes (27, 30). These results support the hypothesis that the IGFs primarily regulate undifferentiated cells, whereas insulin primarily regulates differentiated cells.

ACKNOWLEDGEMENTS

The authors thank Drs. R. Humbel, P. Nissley and J. Merryweather for their gifts of IGF, and G. Ringold for the TA1 cells. This study was supported by National Institutes of Health Research Grant AM 34926 and RCDA AM 01393.

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