BBA 29380

INDUCTION OF TYROSINE AMINOTRANSFERASE AND AMINO ACID TRANSPORT IN RAT HEPATOMA CELLS BY INSULIN AND THE INSULIN-LIKE GROWTH FACTOR, MULTIPLICATION-STIMULATING ACTIVITY

MEDIATION BY INSULIN AND MULTIPLICATION-STIMULATING ACTIVITY RECEPTORS

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(Received May 9th, 1980)

Summary

Insulin stimulates a 2-fold increase in the amount of tyrosine aminotransferase and a 5—10-fold increase in the rate of amino acid transport in dexamethasone-treated rat hepatoma cells. In order to determine whether these effects are mediated by insulin receptors or receptors for insulin-like growth factors, we have examined the binding of ^{125}I-labeled insulin and ^{125}I-labeled multiplication-stimulating activity, a prototype insulin-like growth factor, and compared the biological effects of these polypeptides. Insulin and multiplication-stimulating activity cause an identical increase in transaminase activity and transport velocity; half-maximal biological effects were observed at 35 ng/ml (5.5 nM) insulin and 140 ng/ml multiplication-stimulating activity. The hepatoma cells display typical insulin receptors of appropriate specificity; half-maximal displacement of tracer insulin binding occurred at 33 ng/ml unlabeled insulin, but only at 2500 ng/ml unlabeled multiplication-stimulating activity. Specific multiplication-stimulating activity receptors also were demonstrated with which insulin did not interact even at 10 μg/ml. Half-maximal displace-

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ment of tracer multiplication-stimulating activity occurred at 200 ng/ml unlabeled multiplication-stimulating activity. We conclude that insulin cannot act via the multiplication-stimulating activity receptor and presumably acts via typical insulin receptors. The effects of multiplication-stimulating activity on enzyme induction and amino acid transport are probably mediated primarily via the multiplication-stimulating activity receptor.

Introduction

Adrenal steroid hormones induce a 5—10-fold increase in the cellular concentration of tyrosine aminotransferase (EC 2.6.1.5) in HTC cells, an established line of rat hepatoma cells in tissue culture. This induction reflects a similar increase in the rate of synthesis of this enzyme [1]. The addition of insulin to HTC cells previously induced with dexamethasone causes a rapid, 2-fold further increase in the amount of this enzyme [2,3]. The induction by insulin occurs without a lag and is maximal after approximately 4 h incubation with this hormone. We have reported previously that the induction by insulin is the result of a selective, 2-fold decrease in the rate of transaminase degradation [4]. Glucocorticoids also cause a rapid and reversible 90% decrease in the initial rate of transport of the non-metabolized amino acid, α-aminoisobutyric acid. Addition of insulin to such dexamethasone-treated cells causes a 5—10-fold increase in the rate of α-aminoisobutyrate transport, which is maximal within 2 h incubation with insulin [5—7].

Because these effects were usually obtained using pharmacologic concentrations of insulin (more than 1 μg/ml), it was possible that they were mediated not by insulin receptors, but rather by receptors for insulin-like growth factors with which insulin can interact. The insulin-like growth factors (IGFs) are a family of polypeptides that include IGF I [8], IGF II [8], somatomedin A [9], and somatomedin C [10], isolated from human plasma, and multiplication stimulating activity (MSA) synthesized by a rat liver cell line in culture [11—13]. IGF I and IGF II have remarkable chemical similarity to insulin: over 40% of the amino acid residues in the regions corresponding to the A and B chains of insulin are identical in IGF I, IGF II and human insulin [14,15]. Not unexpectedly, insulin and the family of IGFs have a common spectrum of biological activities. In addition, although distinct cell surface receptors exist for insulin and the IGFs, IGFs cross-react weakly with all insulin receptors, and insulin cross-reacts to a major extent with some types of IGF receptors [11, 16—18].

In order to determine whether the insulin induction of tyrosine aminotransferase and the stimulation of amino acid transport are mediated by insulin receptors, we have compared the effects of insulin and the insulin-like growth factor, MSA, on the induction of transaminase activity and the stimulation of α-aminoisobutyrate transport. We have used ^{125}I-labeled insulin and MSA to determine if receptors for both polypeptides are present, to determine their specificities, and to attempt to resolve which receptors mediate the effects of these two polypeptides on α-aminoisobutyrate uptake and tyrosine aminotransferase induction.

Methods

Cells. Stock cultures of HTC rat hepatoma cells were maintained in either monolayer or spinner culture in antibiotic-free Eagle's Minimal Essential Medium supplemented with 5% calf serum and 5% fetal calf serum, and buffered with 50 mM Tricine and 0.5 g/l NaHCO$_3$ [19].

Peptides and reagents. Porcine zinc insulin, porcine proinsulin (lot 615-D63-267), desoctapeptide insulin, and crude bonito tuna fish insulin (lot 615-D63-300) were gifts from Dr. R.E. Chance, Eli Lilly Company. Dexamethasone was a gift from Dr. Walter Gall of Merck and Company. MSA was purified from medium conditioned by the rat liver cell line BRL 3A by Dowex chromatography and acid gel filtration on Sephadex G-75 [11,20]. Sephadex G-75 Peak II MSA was used in these studies. As described in detail [20], this preparation contains four protein bands, each of molecular weight 8700, and each possessing equivalent biological activity, receptor reactivity and immunologic reactivity. The heterogeneity in this preparation appears to result from species containing interruptions in the polypeptide chain in non-critical portions of the molecule. (Denaturation in the presence of thiol reagents causes a reduction in size of some of these closely related forms of MSA.)

Epidermal growth factor was purchased from Collaborative Research. Human growth hormone was a gift of the National Pituitary Agency. Trasylol and Tricine were obtained from Calbiochem. α-Amino[1-^{14}C]isobutyric acid (specific activity 52 mCi/mM) was purchased from New England Nuclear and [G-^{3}H]inulin (800 mCi/mM) was purchased from Amersham.

Insulin binding assay. Insulin was iodinated with Na^{125}I by a stoichiometric chloramine-T method to a specific radioactivity of approx. 170 Ci/g [18]. HTC cells in logarithmic phase of growth were collected by centrifugation, washed several times, and resuspended at a cell concentration of $2-5 \cdot 10^6$/ml in a total volume of 0.5 ml Hepes binding buffer, pH 8. Binding buffer consisted of 0.1 M Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 0.12 M NaCl, 1.2 mM MgSO$_4$, 2.5 mM KCl, 10 mg/ml bovine serum albumin and 10 mM glucose. ^{125}I-labeled insulin was used at approximately 100 pg/ml with or without unlabeled insulin as specified in the legends to the figures. The protease inhibitor Trasylol (800 kallikrein inhibitor units per ml) was added to the assay mix in order to prevent degradation of the ^{125}I tracer. At the end of the incubation 0.2 ml portions of the assay mix were layered over 0.2 ml cold (4°C) binding buffer and centrifuged for 1 min in a Beckman model 152 microfuge. Cell-associated radioactivity was determined in an autogamma spectrometer. Specific binding represents binding which can be prevented by large amounts of unlabeled insulin (10 μg/ml).

MSA binding assay. MSA was iodinated to a specific activity of 80—190 Ci/g, using the lactoperoxidase method [21]. The initial separation of iodinated peptides from free iodide was by Sephadex G-25 gel filtration in 1 M acetic acid containing 1 mg/ml bovine serum albumin. The tracer was purified from ^{125}I-labeled bovine serum albumin and aggregates before use by gel filtration on Sephadex G-75 in Krebs-Ringer-phosphate buffer, pH 7.4, containing 1 mg/ml bovine serum albumin; 0.5 ml was applied to a 50 × 1 cm column. The properties of these ^{125}I-labeled MSA preparations were similar to those previously

described for ^{125}I-labeled MSA prepared using chloramine-T and MSA preparations of comparable purity [18].

MSA binding was assayed in Hepes binding buffer, as described above for insulin. ^{125}I-labeled MSA (20 000—30 000 cpm) with or without competing unlabeled peptides was added to 0.5 ml of cell suspension (2.5—5 · 10^6 cells). Radioactivity was assayed as described for insulin binding, with an efficiency of 85%. Over the pH range of 7 to 8, binding was largely independent of pH.

Tyrosine aminotransferase induction. HTC cells were harvested from spinner culture, washed, and resuspended in induction medium. Induction medium is identical to the growth medium except that it lacks serum and is supplemented with 0.1% bovine serum albumin and 50 mg/l neomycin. The cells were incubated with hormones as described in the legends to figures, and tyrosine aminotransferase assayed in cell lysates as described by Spencer and Gelehrter [22]. Specific enzyme activity is reported as milliuniys (mU) per mg protein; 1 mU catalyses the formation of 1 nmol of the product, *p*-hydroxyphenylpyruvate, per min at 37°C.

α-Aminoisobutyric acid transport. HTC cells were incubated in suspension culture in induction medium as described above. Measurement of transport activity was carried out as described previously by Heaton and Gelehrter [19]. Cells were incubated in the presence of α-amino[1-^{14}C]isobutyric acid (0.25 μCi/ml, final concentration of α-aminoisobutyrate 0.51 mM) for 5—10 min, an interval during which the rate of transport is linear. The cells were then rapidly separated from the medium by centrifugation at 8000 × g for 45 s in an Eppendorf microcentrifuge. [^{3}H]Inulin was added to each incubation in order to measure trapped extracellular water. After appropriate corrections were made for extracellular water, velocity of transport was expressed as nmol substrate taken up/min per mg protein.

Results

Induction of tyrosine aminotransferase and stimulation of amino acid transport by insulin and MSA. Fig. 1 compares the time course and magnitude of induction of tyrosine aminotransferase (A) and stimulation of α-aminoisobutyrate transport (B) by insulin and MSA in dexamethasone-treated HTC cells. The cells were incubated in serum-free medium for 18 h with 0.1 μM dexamethasone, after which either insulin (1 μg/ml) or MSA (2 μg/ml) was added to the cultures. Insulin and MSA both stimulate amino acid transport and transaminase activity to the same extent and with the same time course. Simultaneous addition of MSA and insulin in maximally effective concentrations (2 μg/ml) does not produce any further increase in either enzyme activity (not shown) or amino acid transport velocity (Fig. 1B).

Concentration-dependence of insulin and MSA effects on enzyme induction and amino acid transport. We have examined the concentration-dependence of insulin, MSA, and proinsulin, with respect to their effects on transaminase induction and α-aminoisobutyrate transport. Because the dose-response curves obtained for both effects were similar, we have pooled these data. This can be done because the differences between these two effects in the extent of response and in the time required to reach maximal response is eliminated by
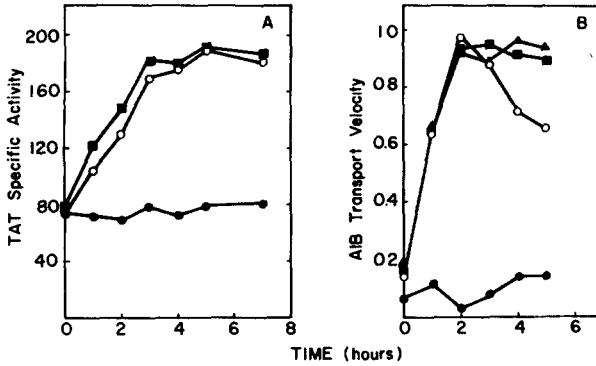
Fig. 1. Time course of induction of tyrosine aminotransferase (TAT) and α-aminoisobutyrate (AIB) transport by insulin and MSA. A. HTC cells were plated in monolayer culture in 35 mm tissue culture dishes at a density of 9 · 10⁵ cells per dish. Each monolayer was incubated in induction medium containing 0.1 μM dexamethasone for 18 h. At that time the medium was removed and fresh medium containing the appropriate additions was added. At the times indicated, the medium was removed from dishes, and the cells harvested by scraping with a rubber policeman. Samples were assayed for tyrosine aminotransferase activity. Tyrosine aminotransferase specific activity is reported as mU per mg protein. ●, 0.1 μM dexamethasone; ○, 0.1 μM dexamethasone + insulin (1 μg/ml); ■, 0.1 μM dexamethasone + MSA (2 μg/ml). B. HTC cells were incubated in suspension culture in induction medium containing 0.1 μM dexamethasone for 18 h. The cells were then divided into four portions, and additions made as stated below. At the times indicated samples were taken and α-aminoisobutyrate transport velocity (nmol α-aminoisobutyrate/minute per mg protein)determined. ●, 0.1 μM dexamethasone alone, ○, 0.1 μM dexamethasone + insulin (1 μg/ml); ■, 0.1 μM dexamethasone + MSA (1 μg/ml); ▲, 0.1 μM dexamethasone + insulin (1 μg/ml) + MSA (1 μg/ml).

presenting the data as the percent of maximal response. The results of 5—10 such experiments are summarized in Fig. 2. Induction of tyrosine aminotransferase or of amino acid transport is obtained at concentrations of insulin as low as 1.0 ng/ml, and half-maximal induction is usually achieved at concentrations between 10 and 60 ng/ml (250—1500 μU/ml, 2—12 nM). Maximal induction is obtained at insulin concentrations of greater than 200 ng/ml.

Proinsulin is considerably less effective than insulin, although at high concentrations it induces tyrosine aminotransferase activity and stimulates amino acid transport to the same extent as insulin. Half-maximal induction is obtained at approximately 350 ng/ml and maximal induction at approximately 2—4 μg/ml. Similar results were obtained in the presence of the protease inhibitor, Trasylol, suggesting that biological activity is intrinsic to proinsulin and not dependent on activation by proteolytic conversion to insulin or intermediate forms. Desoctapeptide insulin (results not shown) is even less effective; at concentrations of 4 μg/ml it produces approximately 50% of the maximal induction obtained with insulin. Thus, the relative potency of these insulin analogs is quantitatively similar to their relative ability to stimulate glucose utilization in adipocytes and to bind to fat cells and liver membranes [23].

MSA was intermediate in potency between insulin and proinsulin in inducing tyrosine aminotransferase activity and amino acid transport. Half-maximal induction was obtained at approximately 150 ng/ml and maximal induction at concentrations greater than 1 μg/ml.

Based on ten experiments with insulin, in which transaminase induction was measured in seven, and amino acid transport in three, and five experiments
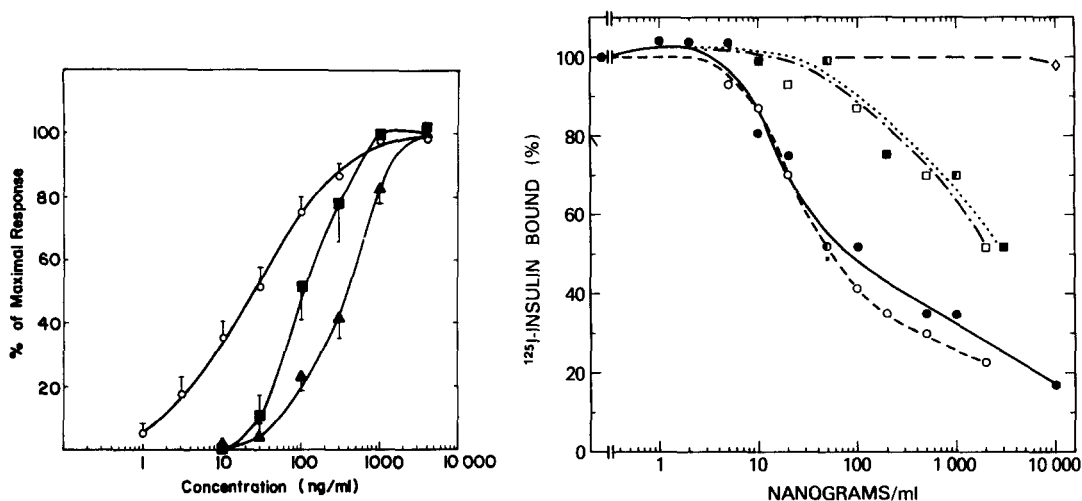
Fig. 2. Concentration-dependence for stimulation of tyrosine aminotransferase induction and α-amino-isobutyrate transport. HTC cells were incubated in suspension culture in induction medium containing 0.1 μM dexamethasone for 17—18 h. At that time, portions were transferred to flasks containing the appropriate amount of peptide to give final concentrations ranging from 1—4000 ng/ml. After 2- and 4-h incubation, samples were removed for assay of α-aminoisobutyrate transport and tyrosine aminotransferase specific activity. Maximal response and the percent of maximal response attained at each concentration was determined in each individual experiment. Each data point represents the mean ± S.E. of results of ten experiments for insulin and five experiments each for proinsulin and MSA. ○, insulin; ■, MSA; ▲, proinsulin.

Fig. 3. Competition for binding of ^{125}I-labeled insulin (^{125}I-insulin) to HTC cells. ^{125}I-labeled insulin (173 Ci/g, 124 pg/ml), $2 \cdot 10^6$ HTC cells, Trasylol (400 kallikrein inhibitor units) and unlabeled polypeptides at the indicated concentrations were incubated for 3 h at 15°C in a final volume of 0.5 ml. Polypeptides were: porcine insulin (●———●), tuna fish insulin (○- - - - -○), porcine proinsulin (□· — · — ·□), MSA (■. . . . .■), and human growth hormone (◇———◇). Maximum binding of ^{125}I-labeled insulin determined in the absence of competing unlabeled hormone corresponded to 1.73% of input radioactivity. The percent of maximum ^{125}I-labeled insulin bound is plotted against the concentration of unlabeled polypeptide.

each with proinsulin and MSA, in which transaminase induction was assessed in four and transport in one, the following concentrations (mean ±S.E.) were defined for the half-maximal biological effect of each hormone: insulin 35 ± 7 ng/ml; MSA, 139 ± 49 ng/ml; and proinsulin, 370 ± 24 ng/ml.

Insulin binding to HTC cells. Insulin binding was carried out using suspensions of HTC cells incubated at 15°C in the presence of the protease inhibitor Trasylol, which effectively prevented degradation of the ^{125}I-labeled insulin tracer without interfering with binding. As shown in Fig. 3, HTC cells bind insulin in a concentration-dependent fashion; unlabeled insulin at 65 ng/ml inhibited ^{125}I-labeled insulin specific binding by 50%. In four such experiments half-maximal displacement of labeled insulin was observed at concentrations of 33 ± 12 ng/ml (mean ±S.E.); similar results have been reported for rat liver membranes [23]. In the experiment shown in Fig. 3, crude bonito tuna fish insulin was as potent as porcine insulin in competing for ^{125}I-labeled insulin binding; in three such experiments tuna fish insulin was 69 ± 25% as potent.

Proinsulin was only 3 ± 0.2% (*n* = 3) as potent as insulin in competing for binding, and human growth hormone did not compete at all. MSA was 1.3 ± 0.3% (*n* = 4) as effective as insulin in competing for iodinated insulin binding. The potency of these various polypeptides as competitors for ^{125}I-labeled insulin binding to the insulin receptor of HTC cells is consistent with their relative potencies with respect to the insulin receptors on cultured IM-9 human lymphoblastoid cells [23,24]. In parallel experiments, tuna fish insulin was 23% as effective, porcine proinsulin 3.3%, and MSA 0.7% as effective as porcine insulin in competing for ^{125}I-labeled insulin binding to IM-9 cells. Thus, HTC cells appear to possess typical insulin receptors.

MSA binding to HTC cells. MSA receptors were studied in HTC cells using ^{125}I-labeled MSA. As shown in Fig. 4, ^{125}I-labeled MSA binds rapidly and specifically to HTC cells at either 15 or 22°C. At 22°C 50% of the maximum specific binding is reached within 30 min, and a plateau of binding occurs between 2 and 6 h. At 15°C, binding is somewhat slower and steady-state is not obtained even after 6 h. At steady-state, approximately 60—80% of the total ^{125}I-labeled MSA binding can be abolished by incubation with 1 μg/ml of unlabeled MSA, and is referred to as specific binding.

Under binding assay conditions, ^{125}I-labeled MSA does not appear to be degraded significantly by HTC cells; less than 7% of the total radioactivity in the medium had been degraded to low molecular weight material after 3 h
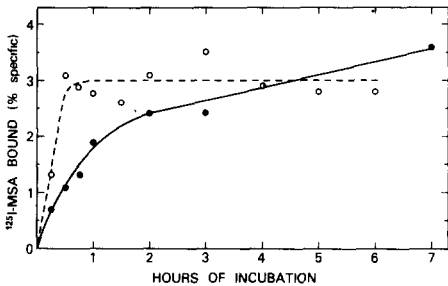

Fig. 4. Time course of ^{125}I-labeled MSA (^{125}I-MSA) binding to HTC cells. ^{125}I-labeled MSA was incubated with 2.2 · 10^6 HTC cells at 15°C (●———●) or with 3.0 · 10^6 HTC cells at 22°C (○-----○) for the indicated times. Nonspecific binding (determined in the presence of 1 μg/ml of unlabeled MSA) has been subtracted at each time. After 6 h incubation, the nonspecific binding was 1.9 and 1.2% of input radioactivity at 22 and 15°C, respectively. The percent of ^{125}I-labeled MSA bound specifically is plotted against the time of incubation.
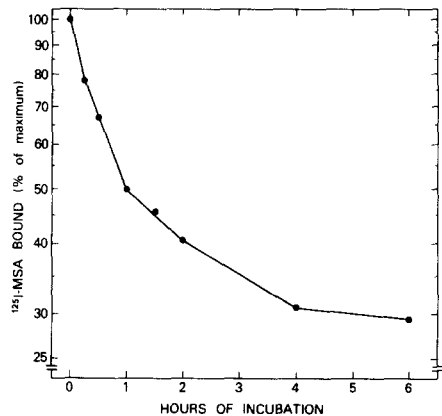
Fig. 5. Dissociation of ^{125}I-labeled MSA (^{125}I-MSA) from HTC cells. ^{125}I-labeled MSA was incubated with 6 · 10^6 HTC cells/ml for 3 h at 22°C. The cells were collected by centrifugation, resuspended in the same volume of Hepes binding buffer, pH 8.0, and the incubation continued at 37°C. At the indicated times, duplicate tubes were harvested and the cell-associated radioactivity determined. The percent of initial ^{125}I-labeled MSA bound is shown for different durations of incubation at 37°C. In parallel incubations of HTC cells for 3 h at 22°C, 5.2% of the input radioactivity was bound in the absence of unlabeled MSA, and 1.7% was bound in the presence of 1 μg/ml of unlabeled MSA.

incubation at 22°C (87% coincident with MSA, 6% larger forms (data not shown)). Furthermore, preincubation of HTC cells in binding buffer for 2 h at 22°C does not decrease their ability to bind ^{125}I-labeled MSA, suggesting that there is no apparent degradation of MSA receptors under these conditions (data not shown).

[125]I-labeled MSA bound to HTC cells can be dissociated in a time-dependent fashion, as shown in Fig. 5. In this experiment, ^{125}I-labeled MSA was incubated with cells for 3 h at 22°C. The cells were then collected by centrifugation and resuspended in the same volume of buffer without labeled hormone, and the incubation continued at 37°C. Approximately 50% of the initially bound radioactivity was released within 1 h, and 70% dissociated within 6 h. Approximately 60% of the radioactivity dissociated after 6 h eluted in the position of ^{125}I-labeled MSA after filtration on Sephadex G-75 in 1 M acetic acid; 15% appeared in larger aggregates and 25% as iodide or iodo-peptides (data not shown).

The specificity of the receptor to which ^{125}I-labeled MSA binds is shown in Fig. 6. ^{125}I-labeled MSA binding to HTC cells was inhibited in a concentration-dependent fashion by unlabeled MSA. Inhibition of specific binding was observed at 50 ng/ml, and was half-maximal at approximately 200 ng/ml. Porcine insulin did not significantly inhibit ^{125}I-labeled MSA binding, even at concentrations as high as 10 μg/ml. Proinsulin at 1 and 10 μg/ml, epidermal growth factor at 5 and 10 μg/ml and human growth hormone at 10 and 20 μg/ml also did not inhibit ^{125}I-labeled MSA binding (data not shown). Thus, ^{125}I-labeled MSA appears to bind to an insulin-insensitive MSA receptor, similar to that present in rat liver plasma membranes and in the BRL 3A2 rat liver cell line [24, 25]. This MSA receptor is therefore clearly differentiable from the insulin-sensitive MSA receptor described in chick embryo fibroblasts and human skin fibroblasts [17,18,26].
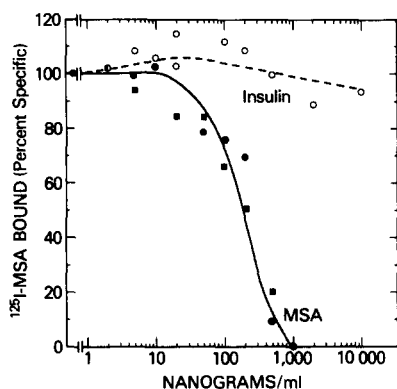

Fig. 6. Competition for binding of ^{125}I-labeled MSA (^{125}I-MSA) to HTC cells by unlabeled insulin and MSA. ^{125}I-labeled MSA, 5·10^6 HTC cells, and the indicated concentrations of unlabeled MSA (●————●) or porcine insulin (○-----○) were incubated for 6 h at 15°C. Percent of maximum specific binding is plotted against the concentration of unlabeled hormone (ng/ml). Total binding of ^{125}I-labeled MSA was 4.55% of input radioactivity; nonspecific binding of 1.1% has been subtracted. Results from a second competitive binding experiment with unlabeled MSA are also plotted (■). In this experiment, ^{125}I-labeled MSA was incubated with 4·10^6 HTC cells for 2 h at 22°C. Total binding was 5.43%, and nonspecific binding was 2.43%.

These results indicate that MSA can bind to the insulin receptor and compete weakly with insulin for binding; in contrast, insulin does not appear to bind to the MSA receptor at all.

Discussion

In this report, we have compared the effects of MSA and insulin on two biological activities in HTC cells: α-aminoisobutyrate transport and tyrosine aminotransferase. Both properties are stimulated by MSA and insulin to the same extent and with an identical time course. At maximally effective concentrations, the effects of MSA and insulin were not additive. For both activities, the order of potency was insulin > MSA > proinsulin. This contrasts with the order of insulin-like potencies of these polypeptides as determined in adipose tissue: insulin > proinsulin > MSA [23,24,27]. The different biological potencies raised the following mechanistic alternatives: (1) the effects in HTC cells might be mediated by insulin receptors exclusively, but these receptors would have an atypical specificity; (2) the effects might be mediated by both insulin receptors and MSA receptors, with the MSA receptors possibly capable themselves of interacting with insulin.

Using ^{125}I-labeled insulin, we have demonstrated that HTC cells possess insulin receptors of appropriate specificity. The concentration of unlabeled insulin that produced half-maximal displacement of tracer insulin was approximately 35 ng/ml (5.5 nM). Proinsulin was 3% as potent as porcine insulin in competing for binding, and MSA was 1.3% as potent, consistent with the relative potency of these polypeptides for binding observed in other experimental systems [23, 24]. In addition, using ^{125}I-labeled MSA, we have demonstrated that HTC cells also display receptors to which MSA binds specifically and reversibly. Unlabeled MSA at a concentration of 200 ng/ml displaced 50% of labeled MSA binding. Insulin and proinsulin, even at concentrations of 10 μg/ml, did not compete with labeled MSA for binding. Therefore, the MSA receptors defined on HTC cells are comparable to the insulin-insensitive receptors described on rat liver membrane preparations and on BRL 3A2 cultured rat liver cells, and unlike the insulin-sensitive MSA receptors described on chick embryo fibroblasts and human skin fibroblasts in culture [11].

Since insulin does not appear to interact with the MSA receptor, it is clear that the biological effects of insulin in HTC cells cannot be mediated via the receptor for the insulin-like growth factor, MSA *. It appears most likely that insulin mediates its stimulation of tyrosine aminotransferase activity and α-aminoisobutyrate transport via typical insulin receptors. Consistent with this interpretation is the observation that half-maximal induction of transaminase and stimulation of α-aminoisobutyrate transport occur at the same concentration that gives half-maximal displacement of ^{125}I-labeled insulin binding. This

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* In extensive comparative studies, MSA and the purified human insulin-like growth factors (IGF-I, IGF-II), somatomedin A and somatomedin C cross-react with all known receptors for the other peptides [11,12,25,28,29]. We cannot exclude the possibility that insulin acts via a different IGF receptor in HTC cells, not identified by ^{125}I-labeled MSA binding, but consider this most unlikely.

latter finding is similar to the insulin stimulation of α-aminoisobutyrate transport in thymocytes [30] and human fibroblasts [31].

Proinsulin appears to produce its biological effects in HTC cells via the insulin receptor, as does insulin. Proinsulin fails to interact with the MSA receptor, and its potency relative to insulin is the same with respect both to binding to the insulin receptor and to stimulation of amino acid transport and enzyme induction.

It is unlikely, however, that MSA produces these biological effects via the insulin receptor. MSA is only 1% as potent as insulin in displacing ^{125}I-labeled insulin binding to the insulin receptor, but it is 25% as potent as insulin in inducing transaminase and stimulating α-aminoisobutyrate transport. In addition, MSA is somewhat less potent than proinsulin with respect to binding to the insulin receptor, but it is approximately 2.6 times more potent than proinsulin with respect to its biological actions. Thus, MSA does not appear to exert its metabolic effects via the insulin receptor in HTC cells.

Rather, it is more likely that MSA affects both amino acid transport and enzyme induction via an MSA receptor, although we cannot exclude the possibility that MSA is acting via both the MSA and insulin receptors. The same concentrations of MSA, 100—200 ng/ml, give half-maximal biological effect and half-maximal inhibition of labeled MSA binding. The shape of the biological dose-response curve (Fig. 2) for MSA appears to be different from that of insulin or proinsulin and is at least consistent with MSA interacting with a separate receptor. MSA receptor-mediated [32—34] as well as insulin receptor-mediated [30,31] stimulation of α-aminoisobutyrate transport have been described in other experimental systems.

In various other experimental systems, insulin and insulin-like growth factors such as MSA may produce their common biological effects via the insulin receptor alone, the IGF receptor alone, or via both receptors. In adipocytes, it has been demonstrated that both MSA and insulin stimulate glucose oxidation via the insulin receptor [35]. In fibroblasts the stimulation of thymidine incorporation into DNA by MSA and insulin does not involve the insulin receptor [35] and probably involves MSA receptors [8,17,18,26]. In contrast, both insulin and MSA receptors are involved in the stimulation of α-aminoisobutyrate transport and transaminase induction in HTC cells, and in the stimulation of hexose uptake in perfused rat heart muscle described by Meuli and Froesch [36,37].

The lack of additivity of the effects of insulin and MSA in HTC cells suggests a common step in their mechanism of action; and the observations discussed above would suggest that this common step occurs at a post-receptor level. The observations of Meuli and Froesch [37] also support such an interpretation. It is noteworthy that when HTC cells are incubated with high concentrations of insulin there is a time-dependent, complete loss of insulin responsiveness as assessed by the stimulation of amino acid transport and induction of transaminase [7]. Under these conditions, HTC cells are unresponsive to MSA as well as to insulin (unpublished results). These observations are consistent with desensitization occurring at a post-receptor step shared by MSA and insulin, in contrast to the 'down regulation' at the receptor level described in several other experimental systems [38].

Our observations indicate that the biological effects of insulin on HTC cells are mediated via typical insulin receptors. They suggest that MSA effects are mediated by an MSA receptor in this cell line. HTC cells provide a favorable experimental system for studying the MSA regulation of enzyme induction and amino acid transport since the regulation of these parameters by glucocorticoids and insulin has been extensively defined [2—7,39]. The HTC cell model system also allows one to study the regulation of plasma membrane receptors for these two polypeptides as well as the regulation of cellular responsiveness, using two independent responses. Correlation — or dissociation — of regulation of receptor concentration and regulation of hormone responsiveness may be important for an understanding of the cellular mechanisms of hormone action, and the interplay of insulin and insulin-like growth factors in the regulation of sensitivity to peptide hormones.

Acknowledgments

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