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Insulin regulation of glucose metabolism in HT29 colonic adenocarcinoma cells: activation of glycolysis without augmentation of glucose transport

Christopher C. Franklin, Patricia C. Chin, John T. Turner and Hyun Dju Kim

Department of Pharmacology, School of Medicine, University of Missouri-Columbia, Columbia, MO (U.S.A.)

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The effects of insulin on glucose transport and metabolism were examined in cultured HT29 human colonic adenocarcinoma cells. The presence of glucose transporters was verified by D-glucose displaceable [^{3}H]cytochalasin B binding. The K_d and B_{max} values from cytochalasin B binding studies were 190 ± 30 nM and 8.4 ± 1.4 pmol/mg protein, respectively. Glucose transport determined with 3-*O*-methylglucose showed saturable kinetics with a K_m of 5.8 ± 0.4 mM and a V_{max} of 0.047 ± 0.003 μmol/mg protein per min at 25 °C. Moreover, in HT29 cells, two classes of insulin binding sites were detected in radioligand binding experiments. Although insulin failed to stimulate glucose transport, it was found to activate glycolysis in HT29 cells. Glucose consumption increased from 0.33 ± 0.03 μmol/mg protein per h to 0.49 ± 0.05 μmol/mg protein per h and lactate production was augmented from 0.67 ± 0.04 μmol/mg protein per h to 0.87 ± 0.06 μmol/mg protein per h in response to 10^{-7} to 10^{-5} M insulin. Insulin also enhanced mannose metabolism. Apart from these two hexoses, HT29 cells exhibited a surprisingly narrow substrate specificity. With the possible exception of glyceraldehyde, little lactate was produced from alternative substrates, including adenosine, inosine, ribose, deoxyribose, dihyroxyacetone, galactose and fructose either with or without insulin. Despite its limited utilization by the glycolytic pathway, adenosine was readily salvaged for de novo synthesis of adenine nucleotides. These findings suggest that insulin directly influences substrate utilization through the glycolytic pathway in HT29 cells without activating the glucose transport pathway.

Introduction

HT29 cells derived from a human colon adenocarcinoma were first established in culture by Fogh [1]. Since then, HT29 cells have been widely used as a cultured epithelial cell model in which to study a variety of plasma membrane receptors including α_2-adrenergic [2–3], vasoactive intestinal peptide [4–5] and insulin receptors [6]. HT29 cells share a common trait with other cancer cells in that glucose metabolism, culminating in lactic acid production, is impressively high [7–9]. A metabolic feature of HT29 cells which may distinguish them from other cancer cells is that the removal of glucose from the culture media does not result in the death of all the cells in the culture. A subpopulation of HT29 cells can be maintained in permanent culture without glucose

Abbreviations: VIP, vasoactive intestinal peptide; DMEM, Dulbecco's modified Eagle's medium; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; FBS, fetal bovine serum; IGF, insulin growth factor.

Correspondence: H.D. Kim, Department of Pharmacology, School of Medicine, University of Missouri-Columbia, Columbia, MO 65212, U.S.A.

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[8,10]. Unlike HT29 cells, which ordinarily exhibit undifferentiated morphology in the presence of glucose, the cells which survive in the absence of glucose display enterocytic differentiation after confluency. While the identity of energy source(s) adopted by glucose-deprived HT29 cells is presently unknown, a functioning gluconeogenic pathway has been demonstrated [11]. These findings led the authors to postulate that amino acids present in the culture media are the most likely candidates to serve as precursors for gluconeogenesis.

Although it is clear that HT29 cells have a complex metabolic machinery, relatively little is known about hormonal regulation of the metabolic carbon flow. Denis et al. [12] found that fructose 2,6-bisphosphate, which plays a pivotal role in the control of glycolysis and gluconeogenesis [13], is under the dual influence of VIP and α_2-adrenergic receptor activation through modulation of cAMP content in HT29 cells. As stated earlier, HT29 cells exhibit two classes of insulin-binding sites [6]. More recent work suggests that insulin acts as a growth factor for HT29 cells [14]. Thus, insulin receptors present on HT29 cells are apparently capable of mediating the biological effects of insulin. In this communication, we report the results of an investigation on the physiological role of insulin in HT29 cells. In particular, we examined glucose transport and metabolism. We found that, in accord with a general lack of insulin action on glucose transport across gastrointestinal epithelia [15], insulin fails to stimulate glucose transport in HT29 cells. However, insulin has been found to play a significant role in modulating metabolism by directly stimulating glycolysis in HT29 cells.

Materials and Methods

Cell culture. HT29 human colonic adenocarcinoma cells were subcultured and grown as previously described [3]. Confluent cultures were grown in a humidified atmosphere of 5% CO_2/95% air in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum and 5% newborn bovine serum in 75 cm^2 tissue culture flasks. Cells were subcultured with 0.05% trypsin/0.01% EDTA. About $4.5 \cdot 10^5$ cells in 2.5 ml of growth medium were seeded in 35-mm dishes and grown to confluence with a replacement of growth medium at least every 48 h. Generally, cultures were used on days 6–9. For [^{3}H]cytochalasin B and ^{125}I-labeled IGF-I binding assays, 24-well multiwell dishes were used.

[^{3}H]Cytochalasin B binding assays. The growth medium was removed from confluent HT29 cells in 24-well multiwell dishes and the cells were washed twice with DMEM containing 20 mM Hepes (pH 7.4) (DMEM-Hepes). Cells were then incubated for 15 min at 25 °C in 0.25 ml of 10 mM phosphate buffer (pH 7.4), containing 5 mM KCl, 5 μM cytochalasin E, either 500 mM D-glucose or 500 mM L-glucose, about 30 nM [^{3}H]cytochalasin B and various concentrations of unlabeled cytochalasin B (none or 0.01–10 μM). At the end of the incubation, the binding medium was removed and the plates were quickly washed twice with ice-cold saline. Cells were extracted with 1 ml of 0.2 M NaOH. The lysate was transferred to a scintillation vial as was a subsequent 1 ml distilled water wash. The vial contents were neutralized with 0.2 ml of 1 M HCl, 18 ml of scintillation fluor (Budget-Solve, RPI Corporation, Mt. Prospect, IL) was added, and the radioactivity was quantitated by scintillation spectroscopy at an efficiency of 31%. Data were analyzed using the curve-fitting program cDATA (EMF Software, Knoxville, TN).

^{125}I-Insulin and ^{125}I-labeled IGF-I binding to intact attached HT29 cells. HT29 cells grown in 35-mm dishes or 24-well multiwell plates as described above were washed with 1 ml DMEM-Hepes followed by two 1 ml washes with buffer 1 (5 mM KCl, 136 mM NaCl, 10 mM sodium phosphate buffer, pH 7.4) containing 1% fetal bovine serum (buffer 2), the FBS being added to reduce nonspecific binding to the plasticware. 1 ml or 0.3 ml of buffer 2 containing approx. $2 \cdot 10^{-11}$ M ^{125}I-labeled insulin or ^{125}I-IGF-I, respectively, and various concentrations of unlabeled insulin (10^{-11} to 10^{-4} M) or IGF-I (10^{-10} to 10^{-7} M) was then added to each dish and the cells were incubated overnight (16–20 h) at 4 °C. The binding buffer was aspirated and the cells washed two or three times with 1 ml of ice-cold buffer 2. Cells were lysed with 1.0 ml of 0.2 M NaOH. The lysate and a subsequent 1 ml of distilled water

wash were transferred to 12 × 75 mm plastic test tubes. Bound radioactivity was determined by counting on a Tm Analytic 1191 Gamma Trac gamma counter with a counting efficiency of 77%. Specific binding was determined by subtracting nonspecific binding (the amount of binding in the presence of $1.5 \cdot 10^{-5}$ M unlabeled insulin or 10^{-7} M unlabeled IGF-I) from the total binding. Nonspecific binding was found to be about 5–20% of the total binding in both binding assays. All assays were performed in either duplicate or triplicate. ^{125}I-Insulin binding data were analyzed using cDATA with the assumption of similar affinities of ^{125}I-insulin and unlabeled insulin for the binding sites. The significance of the difference in fit of the data to one- and two-site models was determined by F test.

3-O-Methylglucose transport. Confluent HT29 cells in 35-mm dishes were washed and preincubated in 1 ml DMEM-Hepes for 90 min at 25 °C. Cells were then washed three times with 1 ml buffer 1 and pretreated as stated in the figure legends. A 1.0 ml solution of buffer 1 containing 1 μCi 3-*O*-methyl[^{3}H]glucose and 3.0 mM unlabeled 3-*O*-methylglucose was then added to each dish. The cells were incubated at 25 °C for the time periods indicated in the figure legends. 3-*O*-Methylglucose uptake was stopped by aspirating the medium and the cells were washed three times with ice-cold saline containing 2 mM $HgCl_2$ to inhibit the glucose transporter. The cells were harvested as described above. The mixture was then neutralized with 0.2 ml of 1.0 M HCl and 17 ml of Budget Solve scintillation fluor were added. Radioactivity was quantitated on a Beckman LS 2800 scintillation counter (efficiency was 31%). 3-*O*-Methylglucose uptake was calculated for each experiment by determining the specific activity of 3-*O*-methyl[^{3}H]glucose in the final incubation solution and reported as nmol/mg protein.

Various incubation times (10 s to 5 min) were utilized for the saturation experiments depending on the concentration of unlabeled 3-*O*-methylglucose (0–20 mM) used. Values for K_m and V_{max} were determined as previously described [16].

Insulin effects on 3-*O*-methylglucose transport were studied as described above except that 1 μM insulin was present in the final incubation solution.

Adenosine transport. Adenosine transport experiments were carried out in a manner similar to that of the 3-*O*-methylglucose experiments except the incubation mixture contained 1 μCi [^{3}H]adenosine and 3.0 mM unlabeled adenosine. Adenosine uptake was terminated by aspirating the incubation solution and washing the cells three times with ice-cold saline containing 50 μM *S*-(*P*-nitrobenzyl)-6-thioinosine to inhibit the adenosine transporter. Specific experimental manipulations are described in the figure legends.

Insulin stimulated glycolysis in HT29 cells. HT29 cells were washed and preincubated in DMEM-Hepes for 90 min at 25 °C. Cells were then washed three times with buffer 1 and one ml of buffer 1 containing 3.0 mM glucose with or without insulin was then added to the dishes. The cells were then incubated at 37 °C for the indicated times (see figure legends). At the end of the incubation, cells were extracted with 0.3 ml of 2.5 M perchloric acid and the extract was neutralized to pH 6.9–7.3 with 6.5 M K_2CO_3. Lactate was determined on a perchloric acid extract by the procedure of Lundholm et al. [17]. Glucose was measured by the Sigma kit (No. 510-A). The same procedure was used to determine the effects of alternate substrates on HT29 glycolysis in the presence and absence of insulin. The concentration of these substrates was 3.0 mM unless otherwise stated.

Determination of purine compounds. Experimental conditions were similar to those described above for insulin stimulated glycolysis. Cells were washed and preincubated as described. 1 ml of buffer 1 containing either 3.0 mM adenosine or 3.0 mM glucose or with no additions was then added to the dishes. Cells were incubated at 37 °C for the indicated times (see figure legends). At the appropriate times, cells were extracted and neutralized as described above. The ATP content of the perchloric acid extract of the cells was then measured by HPLC as previously reported [18].

Materials. Dulbecco's modified Eagle's medium was purchased from K.C. Biologicals, Inc. (Lenexa, KS). Sera were obtained from Gibco (Grand Island, NY). 3-*O*-Methylglucose, *S*-(*P*-nitrophenyl)-6-thioinosine and ribose were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Adenosine was obtained from ICN Biochemicals (Cleveland, OH) and deoxyribose was purchased

from Calbiochem (San Diego, CA). The following compounds were purchased from Sigma Chemical Co. (St. Louis, MO): cytochalasin B, cytochalasin E, D-glucose, L-glucose, insulin (from porcine pancreas), mannose, galactose, fructose, glyceraldehyde and dihydroxyacetone. [^{3}H]Cytochalasin B (18.5 Ci/mmol) was obtained from New England Nuclear (Boston, MA), whereas [*iodotyrosyl*-^{125}I]insulin (89.9 Ci/mg), 3-*O*-methyl[^{3}H]glucose (50 Ci/mmol) and [^{3}H]adenosine (50 Ci/mmol) were purchased from ICN Radiochemicals (Irvine, CA). [3-*iodotyrosyl*-^{125}I]Insulin-labeled insulin-like growth factor-I ([Thr^{59}] (2000 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Recombinant insulin-like growth factor-I, [Thr^{59}] (purity > 99% as determined by HPLC) was obtained from AMGen Biologicals (Thousand Oaks, CA).

Results

Estimation of the number of glucose transporters in intact HT29 cells and the affinity of the transporters for cytochalasin B were determined by inhibition of [^{3}H]cytochalasin B binding by unlabeled cytochalasin B in the presence of cytochalasin E and either L-glucose or D-glucose. Results from a representative experiment are shown in Fig. 1. The difference between [^{3}H]cytochalasin B binding in the presence of D-glucose (closed circles) and that in the presence of L-glucose (open circles) was taken to represent binding by the glucose transporter. This difference at each concentration of unlabeled cytochalasin B is shown in the lower panel of Fig. 1 along with the theoretical curve obtained from computer-assisted curve fitting based on a one-site fit of the data. For the set of three experiments, the K_d and B_{max} values were 190 ± 30 nM and 8.4 ± 1.4 pmol/mg protein (mean ± S.E.), respectively.

To determine the kinetic properties of the glucose transport system, initial rates of sugar influx were measured by using various concentrations of the nonmetabolizable glucose analog, 3-*O*-methylglucose, ranging from 0.5 to 20 mM. A typical result is shown in Fig. 2. As expected, glucose uptake is a saturable process with a K_m value of 5.8 ± 0.4 mM and a V_{max} of 47 ± 3 nmol/mg protein per min at 25 °C ($n = 3$).

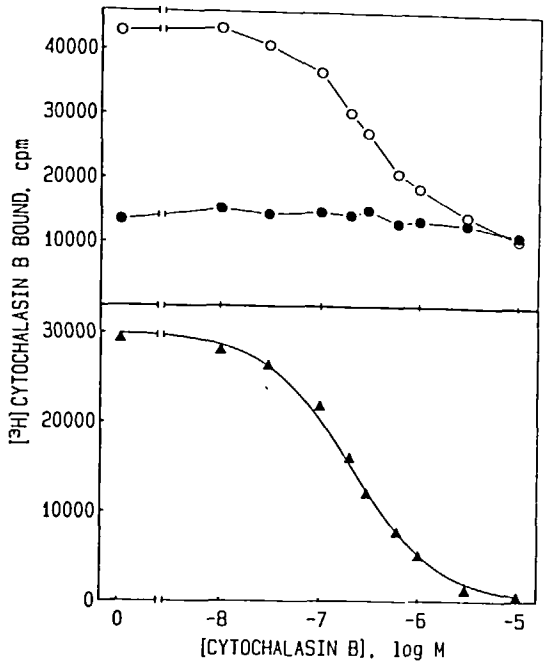

Fig. 1. Inhibition of [^{3}H]cytochalasin B binding by cytochalasin B. Cells in 24-well multiwell dishes were incubated for 15 min at 25 °C with approx. 30 nM [^{3}H]cytochalasin B and the indicated concentrations of unlabeled cytochalasin B in the presence of either 500 mM L-glucose (open circles) or 500 mM D-glucose (closed circles). The lower panel triangles represent the difference between radioligand binding in the presence of L-glucose and that in the presence of D-glucose. The lower panel curve is the computer-derived best fit of the data based on a single site model. The points shown are from a representative experiment performed in duplicate and repeated two times.

Results from experiments of insulin inhibition of ^{125}I-insulin binding in intact HT29 cells were analyzed by nonlinear regression of the untransformed data. A representative experiment is shown in Fig. 3. The fit of the data in each experiment to a two-site model was significantly better using an F test than to a one-site model ($P < 0.05$). A series of six experiments revealed a high-affinity ($K_d = 1.5 \pm 0.6$ nM, mean ± the S.E.), low-capacity ($B_{max} = 17 \pm 9$ fmol/mg protein) site and a low-affinity ($K_d = 57 \pm 25$ nM), high-capacity ($B_{max} = 180 \pm 70$ fmol/mg protein) site. In light of the presence of both glucose transporters and insulin receptors in HT29 cells, it was of interest to ascertain whether insulin exerts a stimulatory effect on glucose transport. The results shown in Fig. 4a reveal that insulin fails to augment glucose

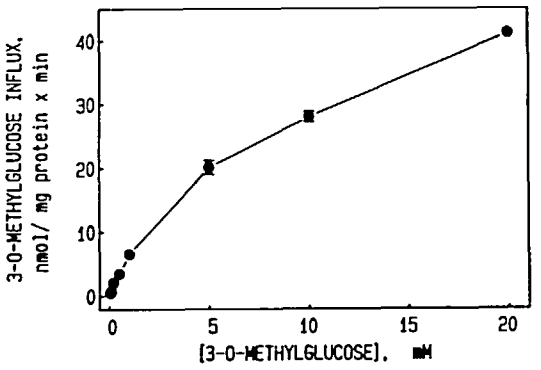

Fig. 2. 3-*O*-Methylglucose influx as a function of free 3-*O*-methylglucose concentration. HT29 cells were preincubated in 1 ml DMEM-Hepes for 90 min at 25 °C and then incubated at 25 °C in 1 ml of buffer 1 containing 1.0 μCi of 3-*O*-methyl[^{3}H]glucose and various concentrations of unlabeled 3-*O*-methylglucose. The initial influx rate was determined for each 3-*O*-methylglucose concentration as referenced in the Materials and Methods. Each point represents the mean ± S.E. of two or three experiments performed in duplicate.

transport in HT29 cells. Preincubation of the cells with insulin for up to 90 min before addition of 3-*O*-methylglucose was also ineffective in stimulating transport (Fig. 4b).

Although insulin is without effect in stimulating glucose transport, it still controls energy metabolism of HT29 cells by directly stimulating glycolysis. Table I summarizes the effects of in-

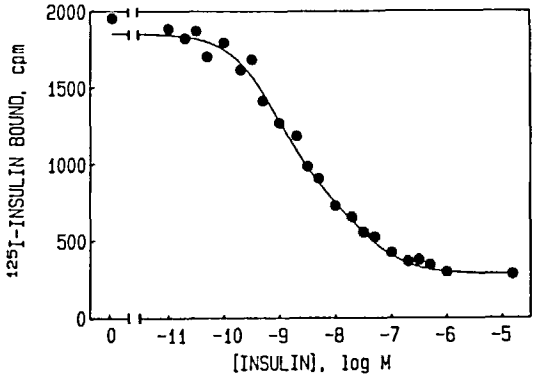

Fig. 3. Inhibition of ^{125}I-insulin binding by unlabeled insulin in intact HT29 cells. Confluent cultures in 35-mm dishes were incubated with approx. 20 pM ^{125}I-insulin and the indicated concentrations of unlabeled insulin for 16–20 h at 4 °C. The points shown are from a representative experiment performed in duplicate and repeated five times. The curve is the theoretical best fit of the data to a two-site model (see text).

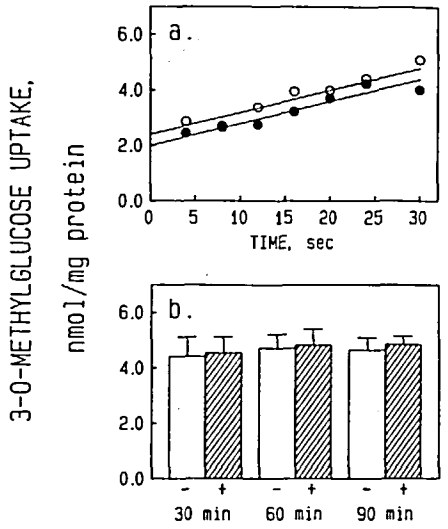

Fig. 4. (a) Effect of insulin on 3-*O*-methylglucose influx. HT29 cells were preincubated in 1 ml DMEM-Hepes for 90 min at 25 °C and then incubated at 25 °C for the times indicated in 1 ml of buffer 1 containing 1 μCi 3-*O*-methyl[^{3}H]glucose and 3 mM unlabeled 3-*O*-methylglucose in the presence (closed circles) or absence (open circles) of 10 μM insulin. The points shown are from a representative experiment performed in duplicate and repeated two times. (b) Effect of insulin preincubation on 3-*O*-methylglucose influx. Experimental conditions are similar to those in a except cells were preincubated in DMEM-Hepes for 90 min followed by a 30, 60 or 90 min preincubation in buffer 1 in the presence (hatched) or absence (open) of 1 μM insulin. 3-*O*-Methylglucose influx was then determined, as described above, for 30 s. Data are the mean ± S.E. of four experiments performed in triplicate.

TABLE I

EFFECTS OF INSULIN ON HT29 GLYCOLYSIS

Cells were incubated at 37 °C in buffer 1 for a period of 1 h. Cells were extracted with perchloric acid for the determination of glucose and lactic acid. When insulin was present, its concentration was 10^{-7} to 10^{-5} M. Values shown are the mean ± S.E. of nine experiments.

	Glucose consumption (μmol/mg protein per h)	Lactic acid production (μmol/mg protein per h)
Control	0.33 ± 0.03	0.67 ± 0.04
Insulin	0.49 ± 0.05 *	0.87 ± 0.06 *

* Values are statistically different from control ($P < 0.005$; two-tailed Student's *t*-test).

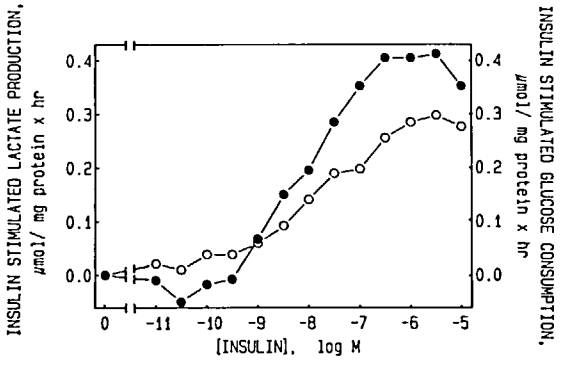

Fig. 5. Dose-dependent insulin stimulation of glycolysis in HT29 cells. Cells were preincubated in 1 ml DMEM-Hepes for 90 min at 25 °C and then incubated in 1.0 ml of buffer 1 containing 3.0 mM D-glucose and the indicated concentrations of insulin for 1 h at 37 °C. The points shown are from a representative experiment performed in triplicate and repeated two times. Glucose consumption, open circles; lactate production, closed circles.

sulin on glucose consumption and concomitant lactate production by HT29 cells. While glucose consumption was increased by approx. 0.16 µmol/mg protein per h, lactate production was stimulated by only 0.20 µmol/mg protein per h, implying that phosphorylated intermediates must be accumulating in response to insulin stimulation. Fig. 5 shows a dose-response curve of insulin-stimulated glycolytic activity. There is no detectable activation at 10^{-11} M insulin, while maximum activation takes place at insulin concentrations above 10^{-7} M with an EC_{50} value of approx. 8 nM. This dose response closely parallels that for insulin inhibition of ^{125}I-insulin binding.

Recent reports indicate the presence of both insulin and IGF receptors in a variety of cells [29–31]. There appears to be a heterologous interaction of insulin and the insulin-like growth factors for these receptors as determined by both radioligand-binding studies and functional assays. To clarify further which receptor is responsible for the insulin-stimulated responses seen in HT29 cells, we performed competition radioligand-binding experiments using ^{125}I-IGF-I. HT29 cells were found to possess IGF-I-binding sites. Both IGF-I and insulin were found to competitively inhibit ^{125}I-IGF-I binding. IGF-I inhibited ^{125}I-IGF-I binding with an IC_{50} of approx. 6 nM, while insulin inhibited binding with an IC_{50} of about 1 µM as seen in Fig. 6. These values are in general agreement with previously reported values for several other cell types [29–31]. It should be noted that there is no inhibition of ^{125}I-IGF-I binding at insulin concentrations (0.1 µM) that maximally stimulate glycolysis (Figs. 5 and 6). This suggests that the IGF-I receptor probably is not involved in the insulin-induced glycolytic response.

We found that HT29 cells have a limited substrate specificity for glycolysis. Of the six carbon substrates studied, only glucose and mannose were metabolized by HT29 cells as shown in Fig. 7. Insulin stimulated mannose utilization to the same extent as it did glucose. Since glutamine is known to augment insulin-stimulated glucose metabolism in other cancer cells [19], we examined the possible role of glutamine in HT29 cells. We found that glutamine did not increase lactate production from glucose either with or without insulin (data not shown). Moreover, little or no lactate was produced from a number of alternate substrates which enter at various steps of the glycolytic pathway, including adenosine, inosine, ribose, deoxyribose, dihydroxyacetone and glyceraldehyde. Apart from glucose and mannose, glyceraldehyde was the only other carbohydrate substrate which resulted in lactate production. However, compared to glucose, glyceraldehyde was metabolized to a much lesser extent (Fig. 8). None of the substrates tested in Fig. 8 except glucose responded to insulin activation.

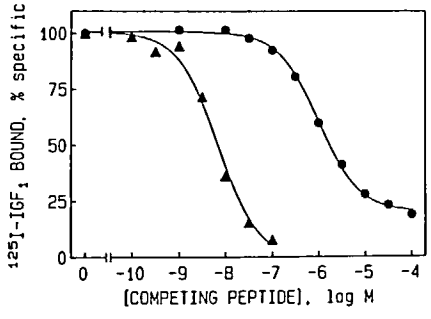

Fig. 6. Inhibition of ^{125}I-IGF-I binding by unlabeled IGF-I and insulin in intact HT29 cells. Confluent cultures in 24-well multiwell plates were incubated with approx. 20 pM ^{125}I-IGF-I and the indicated concentrations of unlabeled IGF-I (triangles) or insulin (circles) for 16–20 h at 4 °C. The points shown are from a representative experiment performed in triplicate and repeated two times. The curves are the theoretical best fit of the data to a one-site model.

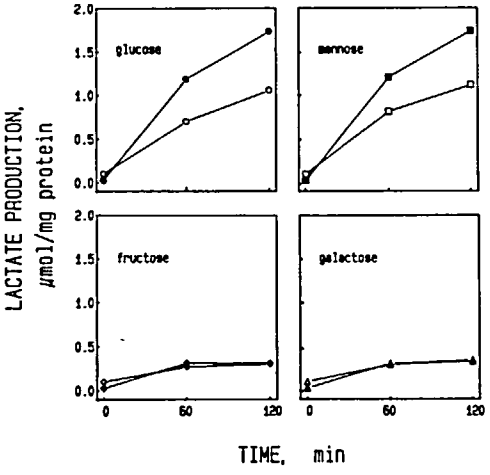

Fig. 7. Effects of insulin on hexose metabolism. Experimental conditions are the same as those described in Fig. 5. Cells were treated with (3.0 mM) D-glucose, galactose, fructose or mannose in the presence (closed symbols) or absence (open symbols) of 1 µM insulin.

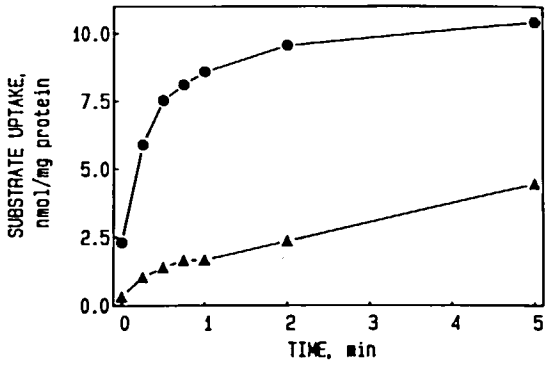

Fig. 9. A comparison of 3-*O*-methylglucose and adenosine uptake in HT29 cells. HT29 cells were preincubated in 1 ml DMEM-Hepes for 90 min at 25 °C and then incubated in 1.0 ml of buffer 1 containing either 1 µCi 3-*O*-methyl[^{3}H]glucose and 3.0 mM unlabeled 3-*O*-methylglucose (circles) or 1 µCi [^{3}H]adenosine and 3.0 mM unlabeled adenosine (triangles) at 25 °C for the time periods indicated. The reaction was terminated as described in Materials and Methods and the amount of cell-associated radioactivity was determined.

Of particular interest is the pathway by which HT29 cells utilize adenosine. Adenosine did not significantly participate in the glycolytic pathway to form lactate (Fig. 8). A limiting membrane permeability to adenosine could not account for this. Although adenosine uptake was slower than glucose uptake, HT29 cells were clearly permeable to adenosine (Fig. 9). Furthermore, there was no difficulty in converting adenosine to inosine by a reaction presumably catalyzed by adenosine deaminase (data not shown). Also shown in Fig. 8 is the lactic acid production resulting from inosine, the levels of which were comparable to those produced by substrate-free cells. Again, insulin was without effect in augmenting lactate production from either adenosine or inosine (Fig. 8). Thus, in terms of metabolic action of insulin, glucose and mannose were the only responsive

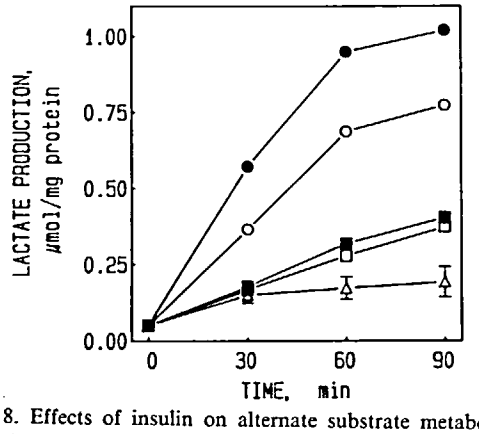

Fig. 8. Effects of insulin on alternate substrate metabolism. Experimental conditions are the same as those described in Fig. 5. Cells were incubated in 1.0 ml of buffer 1 containing (3.0 mM) D-glucose (circles), glyceraldehyde (squares), dihydoxyacetone, ribose, deoxyribose, adenosine or inosine (triangles) in the presence (closed symbols) or absence (open symbols) of 10 µM insulin (the ranges of insulin effects on dihydroxyacetone, ribose, deoxyribose, adenosine and inosine (triangles) -stimulated glycolysis are represented by the error bars).

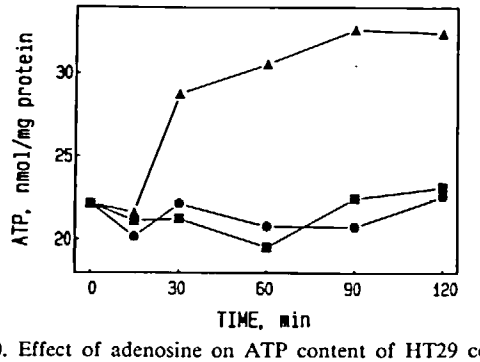

Fig. 10. Effect of adenosine on ATP content of HT29 cells. Cells were incubated at 37 °C in 1.0 ml of buffer 1 containing 3.0 mM adenosine (triangles) or 3.0 mM glucose (squares) or no addition (circles) for the times indicated. The reaction was terminated and ATP content was measured as described in Materials and Methods.

substrates. Even though little of the ribose moiety of adenosine was recovered as lactate, adenosine was readily salvaged by HT29 cells, as evidenced by a net synthesis of ATP content as shown in Fig. 10. These findings suggest that while the nucleoside phosphorylase activity is low, activities of adenosine kinase and adenylate kinase are high in HT29 cells.

Discussion

It has long been known that a prominent mechanism by which insulin stimulates glycolysis is to augment the rate-limiting membrane permeability to glucose. Another metabolic action of insulin is to stimulate glycolysis directly, independent of its effect on glucose transport [20,21]. HT29 cells represent a good paradigm exhibiting the latter mechanism. The results described herein clearly demonstrate that HT29 cells are endowed with glucose transporters as evidenced by cytochalasin B binding. The affinity for cytochalasin B and the density of the transporters in HT29 cells are comparable to the values reported in a variety of other cell types [22].

Using the V_{max} of glucose transport depicted in Fig. 2 and glucose consumption under basal conditions reported in Table I, it is possible to estimate the efficiency with which the transport system delivers the substrate to the metabolic machinery. The glucose transport rate is at least 10-fold faster than the glycolytic rate in HT29 cells. Thus, it is not too surprising that glucose transporters in HT29 cells do not respond to insulin in a manner similar to that of the well-characterized system in erythrocytes which also lacks insulin activation. In view of the glycolytic activation seen in HT29 cells, the lack of insulin effect on glucose transport can not be attributed to defective or silent insulin receptors. Results of our binding studies with ^{125}I-insulin in intact HT29 cells are in general agreement with previous results obtained under different experimental conditions [6,14] and extend those results by demonstrating a statistically significant better fit of the untransformed inhibition binding data to a two-site model as compared with that of a one-site model. The binding of insulin to its receptor is thought to generate a transmembrane signaling by which glucose transporters are recruited to the plasma membrane surface from an internal storage site [23–24]. As in HT29 cells, insulin fails to stimulate carrier-mediated glucose transport in a variety of cells including erythrocytes, platelets and hepatocytes [32]. In light of the ubiquity of insulin receptors, these cells may simply lack recruitable carriers. The current study does not permit us to delineate the lesion responsible for the failure of insulin to enhance glucose transport.

In light of recent reports on the presence of both insulin and IGF receptors in a variety of cells [29–31], we investigated the possibility that insulin may exert its influence, in part, through the IGF-I receptor. As in other cells, IGF-I receptors were found to be present in HT29 cells. Both IGF-I and insulin competitively inhibit ^{125}I-IGF-I binding to these receptors. Whereas insulin inhibits IGF-I binding, this interaction apparently is not responsible for the increased glycolytic activity observed, since maximal stimulation of glycolysis occurs at insulin concentrations that do not inhibit ^{125}I-IGF-I binding (about 10^{-7} M). The dose response of insulin-stimulated glycolysis also correlates well with that of insulin-inhibitable ^{125}I-insulin binding, providing further evidence that one of the insulin-binding sites, not the IGF-I receptor, is responsible for the mediation of metabolic responses. Whether IGF-I can effect a similar glycolytic response by interaction with either the IGF-I or insulin receptor remains to be explored.

A wide range of glycolytic rates in HT29 cells have been reported in the literature [7–9]. Our rate of 0.3 μmol glucose consumed/mg protein per h is within the previously reported values of 0.3 to 0.61 μmol/mg protein per h. This is equivalent to approx. 35 μmol/ml cell water per h, which is two orders of magnitude higher than the glycolytic rates seen in erythrocytes [25]. In any case, compared to normal cells, HT29 cells have a remarkably high glycolytic capacity.

A surprising finding is the almost exclusive metabolic preference of HT29 cells for glucose and mannose, both of which are influenced by insulin. No other hexoses, pentoses, trioses or purine nucleosides examined provide a significant carbon flow through the glycolytic pathway. In addition, the unusual adenosine metabolism is noteworthy. Although little of the ribose moiety of

adenosine cycles through the pentose pathway, adenosine itself is readily salvaged to form adenine nucleotides (Fig. 10).

With respect to the mechanisms for activation of glycolysis, insulin has been found to stimulate a Na^+/H^+ exchanger, resulting in intracellular alkalinization [15,26]. Insulin is also known to activate Na^+/K^+-ATPase activity [15,27]. These findings suggest that augmented glycolysis may be a metabolic response to altered ion fluxes following insulin receptor activation. Although HT29 cells possess both a Na^+/H^+ antiport system and Na^+/K^+-ATPase activity, insulin does not stimulate either of the ion transport systems [28]. It is, therefore, likely that insulin directly stimulates one or more of the rate-limiting glycolytic enzymes. Using frog skeletal muscle, Özand and Narahara [20] found that insulin stimulated glycolysis by enhancing phosphofructokinase activity in muscle incubated without glucose. In addition, Beitner and Kalant [21] also showed an activation of phosphofructokinase activity in the rat hemidiaphragm under conditions in which glucose transport is unaffected by insulin. However, it should be pointed out that unlike HT29 cells, muscle tissues ordinarily possess glucose transporters which are stimulated by insulin. A detailed measurement of glycolytic intermediates is in progress to determine whether activation of phosphofructokinase or other enzymes explains insulin action in HT29 cells.

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