Insulin Stimulation of Glucose Entry in Chick Fibroblasts and Hela cells

by

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Abstract: 3 to 5-fold stimulation by insulin of the rate of D-glucose entry into chick fibroblasts in culture is observed in cells transferred for several hours to medium containing little (50 to 100 μg/ml) or no D-glucose. No stimulation is obtained in cells transferred for the same period to "high" glucose (1 mg/ml) medium. The latter finding probably accounts for the consistent failure of earlier studies to demonstrate stimulation. Pyruvate alone is as stimulatory as insulin and reverses the D-glucose "repression". Hela cells are as sensitive to insulin as chick cells.

Since the classic demonstration of insulin action in mammalian physiology has centered upon the stimulation of glucose entry and metabolism by tissues (1, 2, 3, 4, 5) the inability to duplicate that hormonal activity in cultivated cells (6, 7, 8, 9) has compromised their acceptance as models for the study of insulin's regulatory role.

We wish to report that both primary cultures of fibroblasts and Hela cells develop striking sensitivity to insulin within a few hours of their transfer to medium lacking a carbon and energy source. The stimulation observed is considerably higher than that achieved with tissue slices or cells freshly removed from animals (1, 2, 3, 4).

Materials and Methods

Cell cultures. Chick embryo monolayer cultures were prepared by trypsinization of whole 10- to 12-day embryos (10). Culture vessels were inoculated to obtain initial cell densities of 10^4 to 5 x 10^4 cells per cm^2. After 72-96 hr the monolayers were washed with Hanks' balanced salt solution (11) and incubated in Eagle's basal medium (12)

supplemented with 3% calf serum or unsupplemented as indicated for each experiment.

Hela cells. The Hela cells used were a mycoplasma-free strain obtained from Microbiological Associates Inc., Bethesda, Md., and were checked periodically for mycoplasma contamination by radioautography with thymidine-^{3}H (13). The cells were routinely grown as confluent monolayers in Eagle's Minimal Essential Medium (MEM) (14) supplemented with 10% calf serum.

Assay of glucose entry. The trichloracetic acid (TCA)-soluble D-glucose extractable from cells after a 5-minute exposure of the monolayers to labeled (^{3}H) glucose was obtained and measured as reported earlier (10).

Insulin. Several lots of bovine insulin were obtained from Sigma Chemical Co., Schwarz-Mann and Calbiochem. Porcine insulin was obtained from Schwarz-Mann. The insulin was dissolved in dilute acid at a concentration of 400 μg/ml and neutralized upon dilution before use. Sterility was obtained by filtration through Millipore filters.

Chemicals. ^{3}H-D-glucose (540 mc/m mole) and ^{3}H-D-galactose (2.04 C/m mole) were obtained from the New England Nuclear Corporation. Sodium pyruvate was obtained from Sigma Chemical and Schwarz-Mann.

Results and Discussion

D-Glucose "Uptake" by Starved Cells: Insulin Stimulation. If the rate of entry of D-glucose into cells starved of glucose is compared to that of cells provided 1 mg/ml of D-glucose, the starvard cells are much more active (10). Enhanced uptake is a function of the time of starvation with a 10 to 20-fold difference commonly observed with cells starved for 24 hours. The entry rate continues to rise through at least 48 hours of starvation. If bovine insulin was included in the

starvation medium at "0" time, a further 3 to 5-fold increase in entry rate was evident at 24 hours (Table 1). Indeed a 2-fold stimulation was

TABLE 1

Insulin Stimulation of D-Glucose "Uptake"

Preincubation Conditions (24 hrs)	p moles ^{3}H-D-glucose / mg protein / 5 min		
	Exp. #1	Exp. #2	Exp. #3
BME - D-Glucose	118	46	28
BME + D-Glucose (1 mg/ml)	6.3	3.4	1.7
BME - D-Glucose + INS 10^{-2} U/ml	375	110	109
BME + D-Glucose (1 mg/ml) + INS 10^{-2} U/ml	8.7	ND	1.4

Cells grown for 72-108 hr. in Eagle's basal medium (BME) supplemented with 3% calf serum were washed and provided with fresh BME with and without D-glucose (1 mg/ml) and insulin 10^{-2} unit per ml. After an additional 24 hr at 37°C, the cells were washed and incubated in 1.0 ml of BSS containing ^{3}H-D-glucose (4×10^{-6} M, 2.0 μC/ml) for 5 min. Acid-soluble counts were determined in a scintillation counter after suitable washing.

Each value presented is the average of three monolayer cultures. Average deviations from the mean were in no case greater than 15%.

INS = Insulin

ND = Not Done.

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detectable as early as 2 hours after the initiation of starvation (unpublished data). Little or no change was observed as a consequence of insulin treatment of cells provided with D-glucose (1 mg/ml) during the 24-hour incubation.

D-Glucose Concentration and Insulin Stimulation. Whereas high levels of D-glucose appeared to block any effect of insulin, lower levels of the hexose permitted the expansion produced in glucose-free medium (Table 2).

TABLE 2

Effect of Glucose Concentration

on Insulin Stimulation

Preincubation Conditions (24 hrs)	p moles ^{3}H-D-Glucose / mg protein / 5 min	
	Exp. #1	Exp. #2
D-Glucose 1 mg/ml	11.8	6.3
+ INS 1.0 U/ml	11.2	ND
+ INS 10^{-1} U/ml	8.0	7.7
+ INS 10^{-2} U/ml	7.0	ND
D-Glucose 500 μg/ml	9.3	8.2
+ INS 10^{-1} U/ml	18.8	31.0
D-Glucose 100 μg/ml	18.0	22.4
+ INS 10^{-1} U/ml	47.4	67.0
No Glucose	104	118
+ INS 10^{-1} U/ml	479	509

Cells were grown and prepared for 24 hr preincubation as described for Table 1. All additions were made at time "0". Values are average of three monolayers. Average deviations from the mean were in no case greater than 12%.

INS = Insulin
ND = Not Done

Minimal Effective Insulin Concentration. The lowest concentration of bovine insulin consistently stimulatory to glucose entry was 10^{-2} unit/ml (Table 3). A marginal effect was observed with 10^{-3} unit; intermediate concentrations were not tested. The effective insulin level did not vary whether low levels of D-glucose (50 μg/ml, unpublished data) or none at all was provided. The insulin stimulation observed with glucose was not apparent if D-galactose entry was monitored. Glucagon had

TABLE 3

Concentration of Insulin Required for Stimulation of Glucose

Entry: Lack of Stimulation of Galactose Uptake

Preincubation Conditions (24 hrs)	p moles ^{3}H - Hexose / mg protein / 5 min	
	^{3}H-Glucose	^{3}H-Galactose
No Glucose	28	13.8
+ INS 1.0 U/ml	142	20.5
+ INS 10^{-1} U/ml	94	17.0
+ INS 10^{-2} U/ml	74	15.2
+ INS 10^{-3} U/ml	39	10.2
+ glucagon 10 μg/ml	26	ND
+ glucagon 1.0 μg/ml	24	ND
+ glucagon 0.1 μg/ml	24	ND

Cells were grown and prepared for 24-hr preincubation as described for Table 1. All cells were starved for D-glucose.

Values average of three monolayers. Average deviations from the mean in no case greater than 10%.

INS = Insulin

ND = Not Done

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no effect on D-glucose uptake; nor did it interfere with the stimulation observed on starvation with or without insulin.

Insulin stimulation of D-glucose Uptake by Hela Cells. Hela cells subjected to D-glucose starvation exhibited essentially the same effects as chick fibroblasts (Table 4). In the absence of glucose the enhancement of uptake achieved levels about 20 times those characteristic of fed cells by 24 hours. 50 μg/ml of D-glucose permitted considerable expansion of transport during the same time period. D-Ribose (1 mg/ml) did not block the enhancement effected by starvation. Least expected

TABLE 4.

Stimulation of Glucose Entry into

Hela cells by Insulin

Preincubation (24 hrs) Conditions	p moles ^{3}H-D-glucose/mg protein/5 min
BME + D-glucose (1 mg/ml)	5.2
BME + D-glucose (50 μg/ml)	66
BME - D-glucose	118
BME + D-ribose (1 mg/ml)	101
BME - D-glucose + INS (10^{-1} U/ml)	275
BME - D-glucose + INS (10^{-2} U/ml)	221
BME + D-glucose (50 μg/ml) + INS 10^{-2} U/ml	106
BME + D-ribose (1 mg/ml) + INS 10^{-2} U/ml	209

Hela cells were trypsinized and plated to achieve a cell density of approximately 10^{4} to 10^{5} cells per cm^{2}. After 72-96 hrs of growth in MEM supplemented with 10% calf serum the cells were washed and incubated in BME with or without D-glucose for 24 hrs. Uptake by the preconditioned monolayers was assayed as described for Table 1.

All additions at time "0". Average deviations from the means in no case greater than 15%.

INS = Insulin

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was the further stimulation resulting from insulin in the starvation medium. Although the effect was less than 3-fold in several experiments, as little as 10^{-2} unit/ml proved active. Protein synthesis in this and other strains of Hela cells (15) is not stimulated by insulin which regularly stimulates protein synthesis in chick cells under the same conditions.

Pyruvate Stimulation. In the earlier report the effects of the inclusion of several sugars, both hexoses and pentoses, in the "star-

vation" medium were cited. Among those D-glucosamine and D-Ribose were included (10). When sodium pyruvate was substituted for D-glucose, the rate of glucose entry achieved was again 2 to 4-fold higher than cells simply starved of glucose (Table 5). Indeed the inclusion of sodium

TABLE 5

Pyruvate Stimulation of Glucose Entry

Preincubation (24 hrs) Conditions	p. mole ^{3}H-D-glucose / mg protein / 5 min Exp #1	Exp #2
BME + D-glucose 1 mg/ml	6.1	11.7
BME - D-glucose	129	70.4
BME + D-glucose 100 μg/ml	14	19.6
BME + Na pyruvate 1 mg/ml	283	133
BME + Na pyruvate (1 mg/ml) + D-glucose (1 mg/ml)	15.2	ND
BME + Na pyruvate (1 mg/ml) + D-glucose (100 μg/ml)	54.7	94
BME + D-Ribose (1 mg/ml)	108	91
BME + D-Ribose (1 mg/ml) + Na pyruvate (1 mg/ml)	242	ND

Conditions same as described for Table 1. All additions at time "0". Average deviations from the mean in no case greater than 8%.
ND = Not Done

pyruvate (1 mg/ml) in medium containing D-glucose at concentrations of either 1 mg/ml or 100 μg/ml alleviated partially the "repression" imposed by the glucose alone. The highest stimulation was observed with 100 μg/ml of D-glucose, at which concentration 1 mg of pyruvate produced a 4-fold increase in the rate of glucose uptake. The effects of insulin and pyruvate do not appear to be additive (unpublished data). Both actinomycin (0.5 μg/ml) and cycloheximide (5 μg/ml) prevent the insulin and the pyruvate stimulations (unpublished data). The implications for the mechanism of pyruvate interference with D-glucose "repression" are of considerable interest.

Whether the insulin stimulation of permeation capacity corresponds to a physiologic mechanism in situ remains to be examined. Having in hand the two assays for insulin activity in the same cells - stimulation of protein synthesis and of glucose uptake - we can now test the symmetry of these activities in chemically modified insulins.

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