INSULIN ACTIVITY IN ISOLATED FAT CELLS WHICH ARE COMPLETELY PERMEABLE TO GLUCOSE

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Previous preliminary reports from this laboratory (MacLeod et al., 1960; Lynn et al., 1961; Lynn, 1962; Lynn, 1963) have suggested that the action of insulin on adipose tissue, under some conditions, is to increase glycolysis rather than to increase the rate of transport of glucose. The addition of insulin to whole tissue results in a decreased concentration of intracellular glucose (in spite of increased glucose uptake) and increased concentrations of glucose-6-phosphate and ATP (MacLeod et al., 1960; Lynn et al., 1961 and Lynn, 1962). When adipose tissue removed from fed rats was incubated with digitonin and hyaluronidase in the presence of large amounts of glucose, a condition in which glucose rapidly and completely equilibrates with tissue water, the addition of insulin caused an increased production of either lactate (anaerobically) or CO_2 and triglyceride (aerobically) from glucose, observable even after the first five minutes of incubation (Lynn et al., 1963). Furthermore, increased levels of hexokinase were observed in extracts of tissue previously incubated with insulin. Vester has also reported that incubation of the soluble fraction of rat liver homogenates with insulin greatly increases the glucokinase activity. However, liver homogenates from alloxan diabetic rats do not respond to insulin (Vester, 1964).

This report presents further data to show that insulin stimulates glycolysis under conditions where the possible effects of insulin on glucose transport have been virtually eliminated. This was done by using

isolated fat cells which are completely permeable to glucose. The cells were obtained by treatment of adipose tissue with collagenase (Rodbell, 1964).

METHODS

Adipose tissue was obtained from the epididymal fat pads of male Osborne-Mendel rats weighing from 160 to 200 grams each. Fat cell suspensions were prepared according to the method of Rodbell (1964) by treatment with collagenase (Worthington Biochemicals). The cell preparation was washed two times in 37°C buffer before aliquots were taken. These cells, like those prepared by Rodbell, were found to convert glucose to CO_2, triglyceride and lactate, and to respond to insulin, epinepherine and other hormones in a manner similar to that of whole tissue. Suspensions containing approximately 100 mg of cells in 0.5 ml of buffer were incubated in plastic tubes (obtained from Spinco, catalogue number - 303595) for 30 minutes in Krebs bicarbonate buffer (modified by replacing half of the NaCl concentration with KCl) with and without insulin (amorphous insulin, lot 192-235-B-188, Lilly Laboratories). The final concentration of insulin was 10 µg per 0.5 ml. After incubation the cells were immediately centrifuged for one to two minutes and the buffer removed from beneath the lighter cells by suction. The cells were not routinely washed after centrifugation since the permeability of these cells was so great. At this point cell wet weights were measured by weighing before and after drying to constant weight in a vacuum desiccator containing P_2O_5. In other duplicate experiments, 0.2 ml of chloroform-methanol 1:1 (v/v) was added immediately to the centrifuged cells (to prevent further oxidation of the intracellular glucose) and the tubes were shaken to insure rapid cell rupture. This homogenate was then partitioned between 1 ml of chloroform and 1 ml of water. Each layer was separated and analyzed.

Human serum albumin I^{131} (Abbott Laboratories, specific activity 0.5 millicuries per mg) and sorbitol-1-C^{14} (Volk Radio Chemicals, specific activity 4.55 millicuries per mg) were counted in a gas flow counter at infinite thinness. Inulin was measured colorimetrically by the method of Dische (1953). Glucose was determined enzymatically by the glucostat method (Worthington Biochemicals) and sodium and potassium were measured using a Zeiss Flame Photometer.

After collagenase treatment cell suspensions were checked microscopically and found to conform in appearance with those shown by Rodbell. To further insure that the isolated cells were intact and still contained nuclei, the deoxyribonucleic acid content of whole adipose tissue and isolated cells after collagenase treatment was measured (Webb and Levy, 1953) and found, on a wet weight basis, to be similar (47.4 - 71.3 ug DNA per 100 mg of whole tissue and 45- 50.2 ug per 100 mg of cells).

RESULTS AND DISCUSSION

Collagenase treated fat cell suspensions were found to equilibrate rapidly with all substances tested except serum albumin and sorbitol (Table 1.) Glucose space, under conditions of isotonicity, hypotonicity and hypertonicity was always the same as the total water space. Despite the fact that glucose concentration within the cell water was maximal, insulin still produced an increased rate of glucose utilization.

Since the action of insulin on isolated fat cells is apparently immediate (Fig. 1) it seems unlikely that insulin is exerting its effect by increasing the rate of synthesis of several glycolytic enzymes. Twenty-four hours are required to re-establish normal glucokinase activity after starvation, and 70 hours of starvation are required to decrease glucokinase activity by 90% (Salas et al., 1963).

Because sodium equilibrates with all the tissue water of isolated

cells, it appears that these cells have lost their ability to maintain differential permeability to cations. These cells have also lost their semi-permeable properties, for rapid alteration in ionic strength by dilution with water or by addition of hypertonic salt concentrations

Table 1.

Glucose Utilization and Water Space of Fat Cells Incubated in Isotonic, Hypotonic, and Hypertonic Buffer With and Without Insulin

	% Glucose Utilized	Spaces Expressed as % of Water Space					
		Sorbitol 1-C^{14}	Inulin	Na	K	Glucose	Albumin I^{131}
0 Time		75.8 ±10.6	107.2 ±11.0	100.0	96.4 ±10.6	105.8 ±10.5	40.8 ± 9.2
Isotonic	12.6 ±4.9	81.2 ±12.0	103.4 ±5.6	100.0	102.4 ±10.0	101.4 ±4.0	40.6 ±4.6
Isotonic + Insulin	43.6 ±6.0	91.8 ±8.2	100.8 ±13.6	100.0	105.6 ±6.2	96.6 ±16.4	40.2 ±7.4
Hypotonic	13.3 ±5.3	83.0 ±7.0	106.4 ±2.4	100.0	112.6 ±15.7	95.8 ±15.0	38.0 ±10.0
Hypotonic + Insulin	43.4 ±7.6	97.4 ±5.4	100.8 ±9.2	100.0	96.8 ±12.0	105.2 ±9.2	37.8 ±10.6
Hypertonic	20.2 ±12.2	102.3 ±2.3	104.2 ±22.4	100.0	104.1 ±22.5	98.4 ±13.8	37.8 ±6.6
Hypertonic + Insulin	44.4 ±12.8	99.4 ±15.4	100.2 ±16.2	100.0	106.8 ±16.6	106.2 ±11.6	47.6 ±8.4

Values represent the mean of at least five experiments. Fat cells, weighing 100 mg were incubated for 30 minutes in 0.5 ml of Krebs bicarbonate buffer containing 4% bovine serum albumin (Armour), 4 μ moles of glucose, 250 μg inulin (raffinose space was found to equal inulin space), and 50,000 cpm of Sorbitol or I^{131} serum albumin per ml. In order to have measureable amounts of intracellular potassium, half the NaCl concentration was replaced by KCl. The hypotonic buffer contained one half the amount of NaCl + KCl and the hypertonic buffer one and a half times as much NaCl + KCl. Glycolysis and insulin response were not significantly affected by these concentrations of potassium. Buffer concentrations and cell content were analyzed (see Methods) after centrifugation of the cells and removal of as much buffer as possible by suction.

(KCl or NaCl) failed to produce any measurable shrinking or swelling, as observed microscopically. But, despite the fact that these cells are completely permeable to monovalent cations and glucose, they have not lost their ability to glycolyze or synthesize fat and are still responsive to hormones (insulin and epinephrine).

The action of insulin on this tissue under these conditions, therefore, cannot be mediated by an increased rate of glucose transport into cell water, since glucose is equilibrated with all cell water throughout the incubation. Morgan, Regan and Park (1964) have shown that insulin increases sugar transport in cardiac muscle and other tissues and the

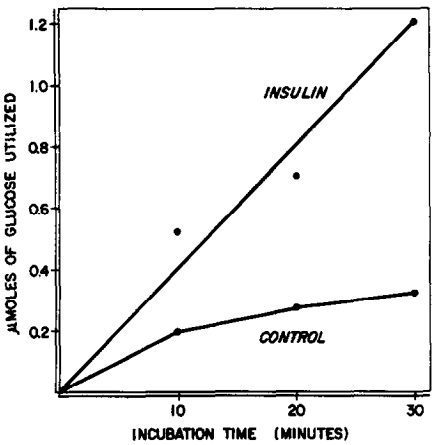

FIGURE 1.

Fat cells (100 mg) were incubated in 0.5 ml of Krebs bicarbonate buffer containing 4% albumin and 2.1 μ moles of glucose. Each point represents the average of 4 or more separate cell preparations.

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recent work of Salas, Viñuela and Sols (1963) and Viñuela et al. (1963) has shown that insulin is also necessary for the synthesis of glucokinase in liver and adipose tissue. Thus, it appears that insulin probably possesses

at least three apparently unrelated actions, i.e., stimulation of glycolysis, promotion of sugar transport (Morgan et al. 1964) and activation of protein synthesis (Salas et al. 1963; and Viñuela et al. 1963).

SUMMARY

Cell suspensions of collagenase treated adipose tissue, which are completely permeable to sodium, potassium, glucose, inulin, and other substances, respond to insulin in the same manner as intact cells. Thus the action of insulin under these conditions cannot be mediated via glucose transport, but must in some way exert its effect by increasing the rate of glycolysis.

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