Specificity of insulin or oxytocin stimulation of protein synthesis in adipose tissue

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Krahl, M. E. Specificity of insulin or oxytocin stimulation of protein synthesis in adipose tissue Am J Physiol 207(5): 1169-1172. 1964.—Insulin stimulates glucose uptake, fat synthesis, and incorporation of amino acids into protein of rat adipose tissue. Other agents having insulinnlike effects on glucose metabolism have now been tested for their ability to promote protein synthesis in this in vitro system. Rat epididymal fat pads were incubated in Krebs bicarbonate medium containing glucose or pyruvate, acetate-1-C14 as precursor for lipids, or (in separate experiments) histidine 2(ring)-C14 as precursor for protein. Glucose uptake was measured by the glucose oxidase method, and radioactivity of lipid and protein fractions was estimated. Synthetic oxytocin (Sandoz), 0.1-10 U/ml, stimulated glucose uptake, acetate incorporation into lipid, and histidine-C14 incorporation into protein when glucose was present; unlike insulin, oxytocin did not enhance protein synthesis when pyruvate replaced glucose in the medium. RNA (1 mg/ml), nicotinic acid (0.001 M), and protamine sulfate (1 mg/ml) each stimulated glucose uptake and acetate incorporation into lipid, but did not enhance histidine-C14 incorporation into protein. It is concluded that in adipose tissue insulin has a specific effect on protein synthesis which cannot be mimicked by other agents which stimulate glucose uptake or lipid synthesis.

Insulin enhances the incorporation of amino acids into the protein of isolated adipose tissue (3, 7, 10), and it also increases the glucose uptake (1, 9, 24). The question under test in the present experiments is whether an increase in glucose uptake by any means is a sufficient condition for stimulation of amino acid incorporation into protein. Other substances have been reported to have insulinnlike effects on glucose utilization of adipose tissue: oxytocin (18, 19, 22, 23), ribonucleic acid (RNA) (4), nicotinic acid (14), protamine (E. G. Ball and J. P. Flatt, personal communication); of these only oxytocin was found to mimic insulin in stimulating incorporation of amino acid into adipose tissue protein.

METHODS

Incubation medium. The basic medium was Krebs-Henselcit bicarbonate buffer (13) containing 0.2% gelatin; the gelatin was dissolved in 0.9% NaCl and the other components of the Krebs buffer were then added, followed by the agent to be tested. Oxytocin (2) and insulin (Lilly U-80) were each diluted with the gelatin solution and then added to the incubation mixture by Lang-Levy micropipettes to give the final concentrations shown.

Reagents. These were purchased from the following sources: RNA (needles, from yeast), Worthington Biochemical Corp.; nicotinic acid and protamine sulfate (N, 24%, sulfate, 17.5%), Nutritional Biochemicals; L-histidine-2(ring)-Cl4 (lot 21) and acetate-1-C14 sodium salt (lot 56), Nuclear-Chicago. The concentrations of nicotinic acid, protamine sulfate, RNA, and oxytocin were optimal for glucose utilization, as reported by previous investigators, or as established here.

Procedures. Male Holtzman rats were maintained on Purina laboratory chow and were used in the fasting or nonfasting states as indicated in the tables; stimulation of amino acid incorporation by insulin in presence of glucose is not significantly altered by fasting (12). The rats were killed by cervical section when they weighed 120-160 g. Four fat pads were used in each vessel, weighing 0.6-1.0 g together; of this, 10-20 mg were recovered as dry protein fraction at the end of the experiment. Twelve 20-ml beakers, each containing 5 ml medium (Table 2) or 2 ml medium (Tables 1, 3) were prepared. Epididymal fat pads from 24 rats were distributed so that each beaker contained the right fat pads from two rats and the left from two others. For amino acid incorporation studies, L-histidine-2(ring)-Cl4 was added by micropipette to all samples to give a final

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concentration of 0.01 μmoles (8 X 10^4 counts/min)/ml. For the lipid studies, acetate-1-C^14 instead of histidine-C^14 was added by micropipette to give a final concentration of 1.3 μmoles (2.7 X 10^4 counts/min)/ml. All samples were shaken in a Dubnoff incubator at 37 C for 2 or 3 hr as shown. Then the fat pads were lightly blotted on hard filter paper, weighed, and dropped into 25 ml ethanol-ether (3:1 v/v). The protein fraction was isolated as previously described (12). The lipids contained in the two initial hot ethanol-ether extractions of the protein isolation procedure were evaporated to dryness, after addition of 1 ml 7 N KOH to each sample. The saponified lipids were extracted by the isopropanol-heptane method (5); a 1-ml aliquot of the heptane layer was plated on a stainless steel planchette; the radioactivity of the deposited lipid was estimated on a Nuclear-Chicago D-47 thin-window gas counter having an efficiency of 35-40%. All counts for protein and lipids are corrected for self-absorption, but not for counter efficiency. About 1-2% of the radioactivity of the histidine in the medium was taken into the protein fraction, and about 20% (without insulin) to 60% (with insulin) of the acetate-1-C^14 was incorporated into lipid.

Glucose disappearing from the medium during incubation was estimated after dilution of 0.2 ml of the medium with 2 ml H_2O, and addition of 1 ml of Ba(OH)_2 and 1 ml ZnSO_4 precipitant (21); 0.5 ml of the filtrate subjected to analysis with the Glucostat glucose oxidase reagent (Worthington Biochemical Corp.).

RESULTS

Glucose uptake, lipid synthesis. In confirmation of previous reports, each of the substances tested was found to stimulate glucose uptake and lipid synthesis from acetate-1-C^14 to various degrees (Table 1). RNA and nicotinic acid appeared to enhance lipid synthesis with only a marginal increase in glucose uptake.

Incorporation of histidine-C^14 into protein. Oxytocin stimulated both glucose uptake and histidine-C^14 incorporation into protein. The effect of 1 μU/ml oxytocin was somewhat less than that of 50 μU/ml insulin (Table 2); in other experiments, results for 0.1 or 10 μU/ml oxytocin were the same as for 1 μU/ml. However, unlike insulin (12), oxytocin, even at the optimal concentration for lipid synthesis, could not increase histidine-C^14 incorporation when pyruvate was substituted for glucose in the incubation medium (Table 2).

None of the other substances tested could enhance histidine-C^14 incorporation into protein (Table 3) even though one of them, protamine, had an effect on glucose uptake comparable with that of oxytocin (Table 2).

DISCUSSION

Following the initial experiments of Levine and co-workers (15), a considerable body of evidence has now accumulated to indicate that insulin enhances the permeability of responsive cells to glucose (see 2, 8, 20). In adipose tissue, the entrance of glucose, rather than its phosphorylation, appears to be the limiting factor in

TABLE 1. Glucose uptake compared to incorporation of radioactivity of acetate-1-C^14 into lipid fraction of epididymal adipose tissue from normal fed rats; effect of insulin and other agents

<table>
<thead>
<tr>
<th>Addition to Medium, Conc. per ml</th>
<th>Glucose Uptake</th>
<th>Radioactivity of Acetate-1-C^14 Incorporated into Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/hr</td>
<td>Counts/min per mg lipid</td>
</tr>
<tr>
<td>None, control</td>
<td>0.34±0.03</td>
<td>165±27</td>
</tr>
<tr>
<td>Nicotinic acid, 1 μmole</td>
<td>0.45±0.04</td>
<td>352±25</td>
</tr>
<tr>
<td>Protamine SO_4, 1 mg</td>
<td>1.05±0.06</td>
<td>580±74</td>
</tr>
<tr>
<td>RNA, 1 mg</td>
<td>0.45±0.04</td>
<td>305±20</td>
</tr>
<tr>
<td>Oxytocin, 1 unit</td>
<td>0.89±0.07</td>
<td>336±32</td>
</tr>
<tr>
<td>Insulin, 0.01 unit</td>
<td>1.89±0.08</td>
<td>600±59</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 experiments. P = probability of differences occurring by chance according to Student’s t test. In each experiment 4 fat pads were incubated in 2 ml Krebs-bicarbonate medium containing 0.2% gelatin and 200 mg/100 ml glucose for 2 hr at 37 C; for isolation of products, see methods.

TABLE 2. Glucose uptake compared to incorporation of radioactivity of histidine-2(ring)-C^14 into protein of epididymal adipose tissue from normal fasting rats; effects of oxytocin and insulin

<table>
<thead>
<tr>
<th>Addition to Medium, Conc. per ml</th>
<th>Glucose Medium</th>
<th>Pyruvate Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose uptake, mg/hr</td>
<td>P for glucose incorporation into protein, counts/min per mg protein</td>
</tr>
<tr>
<td>None, control</td>
<td>0.55±0.05</td>
<td>0.006</td>
</tr>
<tr>
<td>Oxytocin, 1 unit</td>
<td>0.77±0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.05 μU</td>
<td>1.69±0.05</td>
</tr>
<tr>
<td></td>
<td>10 μU</td>
<td>1.63±0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE for six experiments. P = probability of differences occurring by chance according to Student’s t test; NS, difference nonsignificant. In each experiment 4 fat pads were incubated in 5 ml Krebs-bicarbonate medium containing 0.2% gelatin, and 150 mg/100 ml glucose or 20 mm sodium pyruvate for 3 hr at 37 C; these conditions were chosen to duplicate those of previous paper (12).
INSULIN AND OXYTOCIN

TABLE 3. Glucose uptake compared to incorporation of radioactivity of histidine-\(\text{\textsuperscript{14}}\)C into protein of epididymal adipose tissue from normal fed rats; effects of insulin and other agents

<table>
<thead>
<tr>
<th>Addition to Medium, Conc. per ml</th>
<th>Glucose Uptake</th>
<th>Histidine-(\text{\textsuperscript{14}})C Radioactivity Incorporated Into Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg&amp;g/hr</td>
<td>Counts/min per mg protein</td>
</tr>
<tr>
<td>None, control</td>
<td>0.30±0.03</td>
<td>517±22</td>
</tr>
<tr>
<td>Nicotinic acid, 1 amole</td>
<td>0.42±0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Protamine SO(_4), 1 mg</td>
<td>0.67±0.09</td>
<td>0.009</td>
</tr>
<tr>
<td>RNA, 1 mg</td>
<td>0.34±0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, 0.01 unit</td>
<td>1.69±0.02</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means + s for 6 experiments. P = probability of difference from control occurring by chance according to Student’s t test; NS, nonsignificant. The conditions of incubation are the same as for Table 1. * Inhibition.

Specific glucose uptake (6). The principal question posed in the present investigation was: is a change in the adipose tissue which is sufficient to cause increased glucose uptake or increased lipid synthesis also sufficient to ensure a stimulation of amino acid incorporation into protein? The answer is unequivocally negative. Insulin elicits responses from the adipose cell which are not only quantitatively, but also qualitatively, different from those evoked by other agents which stimulate lipid synthesis.

Of the various metabolic activities examined (Table 4) incorporation of histidine-\(\text{\textsuperscript{14}}\)C into a protein fraction, and conversion of glucose to glycogen (which has been tested only with insulin and oxytocin) are the most specific in their response to insulin (Table 4). Oxytocin has a limited ability to stimulate histidine incorporation when glucose is present in the medium but, unlike insulin, is not active when pyruvate is used instead of glucose. RNA, nicotinic acid, and protamine did not display insulinlike activity toward histidine-\(\text{\textsuperscript{14}}\)C incorporation, even though all did enhance glucose uptake or lipid synthesis to varying degrees.

The conclusion is that, in adipose tissue as in muscle (11, 16, 17, 25), insulin has effects on peptide synthesis which are not brought about by increase in glucose utilization.

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REFERENCES

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