Biological potencies of different insulins in isolated adipocytes

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Knowledge of the molecular basis of insulin action remains incomplete (Czech, 1981), and changes in insulin sensitivity are believed to take place at the level of the target tissue (Olefsky, 1981). For adipose tissue, the functional heterogeneity of insulin receptors has been emphasized (Olefsky & Chang, 1979), but, on the basis of lipogenic and anti-lipolytic effects of chemically modified insulins, it has been proposed that different effects of the hormone in adipocytes are mediated through the same set of receptors (Thomas et al., 1979). To study this problem of adipose-tissue insulin sensitivity further, we determined the dose–response relationships of different types of insulins on CO₂ production and fatty acid synthesis from [U-14C]glycerol in basal conditions and triacylglycerol glycerol formation from [1-14C]glycerol in the presence of adrenaline by isolated adipocytes. This latter parameter was selected because we previously had found (Dominguez & Herrera, 1976) that adrenaline decreased [14C]triacylglycerol glycerol synthesis from [1-14C]glycerol by adipose tissue in vitro, an effect significantly compensated for by insulin when glucose was absent from the incubation media. Thus insulin effects on glucose utilization and on glycerol conversion into triacylglycerol glycerol in the presence of adrenaline may be considered independent parameters.

Adipocytes were isolated with collagenase in the presence of ovomucoid trypsin inhibitor by a modification (Bellido & Herrera, 1978) of the method of Rodbell (1964) from epididymal fat pads of fed male Wistar rats. Isolated adipocytes were placed in vials containing 1 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with purified bovine serum albumin (10 mg/ml) and either [U-14C]glucose (1 µCi/ml; 5 mCi/mmol) or [1-14C]glycerol (1 µCi/ml; sp. radioactivity 31 Ci/mol) plus adrenaline bitartrate (1.4 µM). Bovine, pig and rat insulin (from Novo Industri A/S, Bagsvaerd, Denmark) and bovine insulin (from Kodama Ltd., Tokyo, Japan) were radioimmunoassayed against rat insulin standards and added to the corresponding incubation vials at concentrations of 0, 0.001, 0.01, 0.1, 1, 10, 100, 200 or 1000 µunits/ml. Incubations were performed for 120 min under O₂/CO₂ (19:1), and samples were processed as previously described (Bellido & Herrera, 1978; Dominguez & Herrera, 1976).

Insulin potency corresponding to the concentration of the hormone required to produce a half-maximal effect was estimated from the curves of log dose against the percentage response. Curves were linearized by using log-logit transformation by plotting log insulin dose on the abscissa versus percentage of the hormone required to produce a half-maximal effect, this difference being statistically significant (Fisher, 1972). We express our gratitude to Amparo Aguilar for her excellent technical assistance, to Caroline S. Delgado for her editorial help, and to Novo Industri A/S for providing the insulin preparations and the radioimmunoassay kits for rat insulin.

The present results support the hypothesis that once insulin is recognized by (and presumably bound to) its appropriate receptor, it produces an unique metabolic response in the adipocyte.

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Table 1. Biological potencies of different insulins in isolated adipocytes

<table>
<thead>
<tr>
<th>Substrate</th>
<th>[U-14C]glucose</th>
<th>[1-14C]glycerol</th>
<th>4C-labelled fatty acids</th>
<th>4C-labelled triacylglycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adipocytes</td>
<td>[14C]glycerol</td>
<td>[14C]glycerol</td>
<td>Means ± S.E.M.</td>
<td></td>
</tr>
<tr>
<td>Bovine</td>
<td>8.02</td>
<td>6.36</td>
<td>3.42</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>Pig</td>
<td>4.10</td>
<td>6.54</td>
<td>3.40</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>Rat</td>
<td>2.47</td>
<td>3.96</td>
<td>4.31</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>Bonito</td>
<td>15.92</td>
<td>13.60</td>
<td>26.96</td>
<td>18.8 ± 4.1</td>
</tr>
</tbody>
</table>

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* 600th MEETING, OXFORD


**Table 1. Biological potencies of different insulins in isolated adipocytes**

Values are the concentrations of the hormone (µunits/ml) required to produce half-maximal effect. Correlation coefficients of the log–logit transformations against O were always statistically significant, but F = 8.77 (d.f. 3; 62/group). Analysis of the variance among the three parameters was not significant, but F = 8.77 (d.f. 3; 62, P < 0.001) among values for bovine, pig and rat insulins versus bonito insulin.

Substrate ... [U-14C]glucose | [1-14C]glycerol

- Insulin [14C]glycerol | [14C]glycerol
- Means ± S.E.M.
- 4C-labelled fatty acids
- 4C-labelled triacylglycerol
- Bovine 8.02 6.36 3.42 6.3 ± 1.0
- Pig 4.10 6.54 3.40 4.7 ± 1.0
- Rat 2.47 3.96 4.31 3.6 ± 0.6
- Bonito 15.92 13.60 26.96 18.8 ± 4.1

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