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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 CO_2 production and fatty acid synthesis from [U-^{14}C]glucose in basal conditions and triacylglycerol glycerol formation from [1-^{14}C]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 [^{14}C]triacylglycerol glycerol synthesis from [1-^{14}C]-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 1ml of Krebs–Ringer bicarbonate buffer, pH 7.4, supplemented with purified bovine serum albumin (10mg/ml) and either [U-^{14}C]glucose (1μCi/ml, 5mM) or [1-^{14}C]glycerol (1μCi/ml; sp. radioactivity 31Ci/mol) plus adrenaline bitartrate (1.4μM). Bovine, pig and rat insulin (from Novo Industri A/S, Bagsvaerd, Denmark) and bonito insulin (from Kodama Ltd., Tokyo, Japan) were radioimmunoassayed against rat insulin standards and added to the corresponding incubation vial at concentrations of 0, 0.001, 0.01, 0.1, 1, 10, 100, 200 or 1000μunits/ml. Incubations were performed for 120min under O_2/CO_2 (19:1), and samples were processed as previously described (Bellido & Herrera, 1978; Dominquez & 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 $\ln[(R_i-100)/(R_m-R_i)]$ on the ordinate scale, where R_i denotes response as a percentage of basal (100%, no insulin in the medium) and R_m denotes the maximal insulin response. Linear correlation coefficients against 0 were estimated by Student's t test, and comparisons of sensitivity values for the different insulins and parameters studied were performed by the analysis of variance for two factors and the Tukey t test (Sokal & Rohlf, 1969).

Insulin biological potencies found in the present study are summarized in Table 1. Compiled mean values of the effects of the four types of insulin used on formation of either CO_2 and fatty acid from [^{14}C]glucose or triacylglycerol glycerol from [^{14}C]glycerol in the presence of adrenaline were very similar and did not differ statistically, indicating that the sensitivity of all four insulins was the same for each of the three parameters studied. There were, however, differences among the insulins, the bonito type being the least active, because a greater concentration of it was required than of the other types to obtain a half-maximal effect, this difference being statistically significant (Table 1). There were, however, no differences in the observed biological potencies of rat, pig and bovine insulin.

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.

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 0 were always $P < 0.001$ (no. of pairs = 39–62/group). Analysis of the variance among the three parameters was not significant, but $F = 8.77$ (d.f. 3;6, $P < 0.05$) among values for bovine, pig, and rat insulins versus bonito insulin.

Substrate ...	[U-^{14}C]glucose		[1-^{14}C]glycerol	
Insulin	$^{14}CO_2$	^{14}C-labelled fatty acids	^{14}C-labelled triacylglycerol glycerol	Means ± S.E.M.
Bovine	8.02	6.36	4.52	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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