Insulin-like and insulin-inhibitory effects of monoclonal antibodies for different epitopes on the human insulin receptor

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Monoclonal antibodies previously shown to react with five distinct epitopes on the human insulin receptor were tested for their metabolic effects on isolated human adipocytes. Two antibodies which reacted with receptor α -subunit and completely inhibited ^{125}I -insulin binding mimicked the actions of insulin to stimulate lipogenesis from ^{14}C glucose and to inhibit catecholamine-induced lipolysis. On a molar basis, these antibodies were comparable in potency with insulin itself. Two other antibodies which decreased insulin binding only slightly or not at all also mimicked these metabolic effects of insulin. One of these antibodies reacted with receptor β -subunit. In contrast, a further antibody which reacted with α -subunit and inhibited insulin binding did not affect basal lipogenesis or catecholamine-induced lipolysis, but was able to antagonize the effects of insulin on these processes. The same antibody antagonized the insulin-like effect of another antibody with which it competed in binding to insulin receptor, but not the effect of an antibody which bound independently to the receptor. It is concluded that binding of ligand at or close to the insulin-binding site is neither necessary nor sufficient to trigger insulin-like metabolic effects, which may rather depend on some general property of antibodies, such as their ability to cross-link and aggregate receptor molecules.

INTRODUCTION

Many details of the molecular mechanism of insulin action remain obscure (Kahn, 1985; Czech, 1985). In particular, the nature of the signal generated by the insulin receptor, which mediates effects on a diverse array of metabolic processes, is unknown. Various candidates for the role of a diffusible second messenger have been considered (Kahn, 1985), although more recently attention has focused on the role of the intrinsic tyrosine-specific protein kinase activity of the receptor, which is activated by insulin binding (Gammeltoft & Van Obberghen, 1986).

Important insights into insulin action have come from study of the insulin-like effects of anti-receptor antibodies. Such antibodies occur spontaneously in human serum in the rare autoimmune syndrome of extreme insulin resistance (Flier *et al.*, 1976; Kahn *et al.*, 1981) or may be produced by immunization of rabbits with purified receptor (Jacobs *et al.*, 1978, 1980). These antisera are able to mimic many of the metabolic effects of insulin on cells *in vitro* (Jacobs *et al.*, 1978; Kahn *et al.*, 1981), an observation which was important in confirming that the information necessary to initiate insulin action resides wholly in the insulin receptor and does not involve the intracellular participation of insulin or peptides derived from it (Kahn, 1985). The metabolic potency of different antibodies did not parallel their ability to inhibit insulin binding, but antibody bivalency was necessary in order to elicit insulin-like metabolic effects (Kahn *et al.*, 1978; Heffetz & Zick, 1986). It was thus suggested that receptor aggregation may be an important step in the generation of an intracellular signal. It has been claimed that some antisera which

mimic metabolic actions of insulin do not stimulate the receptor kinase activity (Simpson & Hedo, 1984; Zick *et al.*, 1984). The significance of these experiments is unclear, given the relatively low titre and polyclonal nature of the sera involved, and the likely requirement for much higher fractional occupancy of receptors in order to affect significantly kinase activity compared with the metabolic activity of whole cells. Moreover, other antisera, and even some of the same antisera in other hands, do stimulate receptor kinase activity (Roth *et al.*, 1983a; Zick *et al.*, 1984; Heffetz & Zick, 1986).

Polyclonal antisera inevitably contain a heterogeneous mixture of antibodies reacting with multiple epitopes. It is therefore difficult with such reagents to relate effects in different systems, which may reflect distinct subpopulations of antibodies. It is not possible to associate particular effects with a given epitope, nor to compare the consequences of reaction at different epitopes. We have therefore prepared a bank of monoclonal antibodies with the aim of examining in more detail the nature and consequences of antibody–receptor interactions. These antibodies react with at least ten distinct epitopes on both α and β subunits of the receptor and have a variety of effects on insulin binding (Soos *et al.*, 1986a). We report here the metabolic effects of some of these antibodies in lipogenesis and lipolysis in human isolated adipocytes. A preliminary account of some of this work has been published (Soos *et al.*, 1986b).

EXPERIMENTAL

Materials

Human serum albumin (99% electrophoretically pure)

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was obtained from Hoechst (U.K.). Collagenase from *Clostridium histolyticum* (batch no. 504-22) was obtained from P-L Biochemicals. Crystalline pig insulin and [mono-¹²⁵I-Tyr^{A14}]insulin were supplied by Novo. D-[U-¹⁴C]Glucose (sp. radioactivity 270 mCi/mmol) was purchased from Amersham International.

Antibodies

The monoclonal antibodies used were obtained and characterized as described previously (Soos *et al.*, 1986a). Relevant properties of the antibodies determined previously, together with a summary of results of the present experiments, are given in Table 1. Cell lines were grown as ascites tumours in mice, and antibodies were partially purified from ascites fluids by precipitation with 40% -satd. (NH₄)₂SO₄ (Goding, 1983), before reconstitution to original ascites volume in phosphate-buffered saline. Total protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine γ -globulins as standard. The purity and concentration of antibodies was estimated by running samples on SDS/polyacrylamide gels under reducing conditions, staining with Coomassie Brilliant Blue and densitometric scanning with a Joyce-Loebl Chromoscan 3 instrument. Dilutions of a monoclonal IgG which had been highly purified by DEAE-cellulose chromatography were included on each gel as standards. The purity of the antibodies was generally 50–70%, and their concentrations in the stock solutions were 2–10 mg/ml.

Adipocyte isolation

Human adipocytes were prepared from subcutaneous adipose tissue (about 5 g, obtained with informed consent from the lower abdominal wall of patients aged 20–65 years undergoing gastroenterological surgery) by modification of the method of Pedersen *et al.* (1981). The subcutaneous tissue sample was conveyed to the laboratory in glucose/saline (5 mM-glucose/154 mM-NaCl). The tissue was finely chopped with scissors and then incubated with collagenase in a Hepes buffer, pH 7.4 [135 mM-NaCl, 4.8 mM-KCl, 0.2 mM-NaH₂PO₄, 1.0 mM-Na₂HPO₄, 1.7 mM-MgSO₄, 2.5 mM-CaCl₂, 10 mM-Hepes, containing collagenase (0.5 mg/ml), human serum albumin (25 mg/ml) and 5 mM-glucose] in a shaking water bath (120 rev./min) for 90 min at 37 °C.

The isolated adipocytes were then filtered through nylon mesh and washed extensively in Hepes buffer, pH 7.4, containing human serum albumin (50 mg/ml) but no glucose. The adipocyte suspensions were adjusted to a lipocrit of 5% (v/v) after spinning in a micro-haematocrit centrifuge to estimate packed cell volume. The coefficient of variation (C.V.) of the lipocrit determination was 2.5%.

Adipocyte insulin binding

Insulin binding to adipocytes was measured in duplicate after preincubation of 300 μ l portions of cell suspension with 20 μ l of antibody or buffer as indicated in each Figure legend. Mono[¹²⁵I]iodoinsulin (final concentration 16 pM) and 100 μ l of buffer or unlabelled insulin (final concentration 0.12–21 nM) were then added and incubation was carried out at 20 °C for 120 min or at 37 °C for 60 min in Hepes buffer, pH 7.4 [salts as above, but with human serum albumin (50 mg/ml) and 1 mM-glucose]. The binding reaction was stopped by adding 10 ml of chilled 154 mM-NaCl. Silicone oil (1 ml; density 0.97 g/ml) was then layered on the surface, the tubes were centrifuged at 1000 g for 3 min and the cell cake was harvested with a pipe cleaner (Gliemann & Sonne, 1978). Specific binding was calculated by subtracting the binding observed in the presence of 10 μ M-insulin from the total binding for each insulin concentration. The non-specific binding averaged $1.5 \pm 0.2\%$ of cell-bound insulin at a tracer concentration of 16 pM.

Stimulation of lipogenesis

Lipogenesis was determined by modification of the method of Pedersen *et al.* (1982). Triplicate 200 μ l batches of adipocytes in a glucose-free Hepes buffer, pH 7.4 (salts composition as above), containing human serum albumin (50 mg/ml) were preincubated at 37 °C for 45 min with or without different amounts of antibody or insulin. Then 0.4 μ Ci of D-[U-¹⁴C]glucose was added (final concn. 6 μ M) and the incubation was continued without shaking for 90 min. The reaction was stopped by adding 200 μ l of 1 M-H₂SO₄ and the tubes were left for 60 min before the total lipid fraction was separated by the procedure of Dole & Meinertz (1960). The amount of D-[U-¹⁴C]glucose incorporated into lipid for each tripli-

Table 1. Summary of properties of monoclonal antibodies

Data are taken either from Soos *et al.* (1986a)* or from the present paper†.

	Location of binding site (receptor subunit)*	Maximum inhibition of ¹²⁵ I-insulin binding (%)		Biological activity on adipocytes†	Concn. for half-maximal effect (nM)		
		IM-9 cells*	Adipocytes†		Inhibition of ¹²⁵ I-insulin binding†	Stimulation of lipogenesis†	Inhibition of lipolysis†
Insulin	α	100	100	—	0.3	0.05	0.02
47-9	α	92	98	Inhibits insulin	0.2	—	—
25-49	α	94	95	Insulin-like	0.1	0.05	0.01
83-14	α	80	90	Insulin-like	0.05	0.01	0.01
83-7	?‡	0§	10	Insulin-like	—	1.0	0.2
18-44	β	35	40	Insulin-like	0.2	0.3	0.01

‡ Antibody 83-7 did not react with isolated receptor subunits on immunoblots, so location of epitope could not be determined.

§ Antibody 83-7 increased ¹²⁵I-insulin binding to IM-9 cells by approx. 80%.

|| Preincubation of receptor with antibody 47-9 blocked subsequent binding to 25-49, but not to 83-14, 83-7 or 18-44 (Soos *et al.*, 1986a).

cate set of tubes was measured by scintillation counting and expressed as pmol incorporated/90 min. Results were further calculated as percentage of maximal insulin stimulation, 0% representing basal lipogenesis.

Inhibition of lipolysis

After preincubation of adipocytes with or without antibody or insulin in the Hepes buffer, pH 7.4 [containing human serum albumin (50 mg/ml) and 1 mM-glucose] at 37 °C for 45 min, caffeine (0.3 mM) and noradrenaline (1.7 μ M) were added. The reaction was stopped after 60 min by centrifuging the cells through silicone oil. Glycerol concentration was measured by an automated modification (COBAS-Bio; Roche Products, Welwyn Garden City, Herts., U.K.) of the assay of Wieland (1984), using glycerol-3-phosphate dehydrogenase, and the amount was expressed as pmol released/60 min. Results were further calculated as a percentage of maximally stimulated rates of lipolysis, 0% representing basal lipolysis.

RESULTS

Responsiveness of cells to insulin

The isolated adipocyte represents a convenient human cell system in which to assess *in vitro* the metabolic effects of antibodies. The cells are responsive to insulin in terms of stimulation of [14 C]glucose incorporation into lipid (lipogenesis) and inhibition of catecholamine-stimulated glycerol release (antilipolysis). In these experiments, the basal rate of lipogenesis was 32 ± 6 pmol of glucose incorporated/90 min per 15 μ l of packed cells (mean $\pm$ S.E.M., six experiments). This was stimulated approx. 2–3-fold by insulin, a half-maximal response being obtained with approx. 50 pM-insulin. The basal rate of lipolysis was 13 ± 3 pmol of glycerol released/h per 20 μ l of packed cells (mean $\pm$ S.E.M., five experiments). This was increased to 48 ± 8 pmol/h per 20 μ l of cells by noradrenaline plus caffeine, and preincubation of cells with insulin inhibited the effect of catecholamine by a maximum of 70%, with half-maximal effect of insulin at approx. 20 pM. Insulin-binding assays were carried out at both 20 °C and 37 °C, although at the latter temperature particularly there will certainly be significant internalization of insulin within the period of an experiment, and it is recognized that the results do not therefore represent surface binding alone. Nevertheless it was considered important to assess binding of insulin under conditions similar to those of metabolic studies (37 °C), as well as under conditions that will more nearly produce steady-state surface binding (20 °C). The maximum specific binding of 125 I-insulin (16 pM) was $2.9 \pm 0.1\%$ at 20 °C, and $1.0 \pm 0.1\%$ at 37 °C. Binding of 125 I-insulin at 20 °C was half-maximal in the presence of 300 pM unlabelled insulin.

For convenience in comparing results between different experiments, the effects of antibodies were calculated as percentage of the maximum insulin effect. It was confirmed that monoclonal mouse immunoglobulins of irrelevant specificity, either anti-(rat liver membranes) (Siddle & Soos, 1981) or anti-thyrotropin (Soos *et al.*, 1984), were without effect on any of the parameters studied, within a concentration range equivalent to that used for anti-receptor antibodies (results not shown).

Effect of antibodies on insulin binding

Several antibodies were selected for study on the basis of previous work, which demonstrated reaction with distinct epitopes (Soos *et al.*, 1986a), and preliminary experiments showing particularly potent effects of certain antibodies to stimulate or inhibit adipocyte lipogenesis. Antibodies 47-9, 83-14 and 25-49, which bind to the α -subunit of the receptor, inhibited binding of 125 I-insulin to adipocytes at 20 °C by more than 90% at molar concentrations comparable with those of insulin (Fig. 1, Table 1), as previously observed in binding assays with IM-9 lymphocytes at 15 °C or placental membranes at 4 °C (Soos *et al.*, 1986a). Concentrations for half-maximum inhibition of 125 I-insulin binding to adipocytes were 200 pM (antibody 47-9), 50 pM (83-14) and 100 pM (24-49). Similar results were obtained by incubating adipocytes at 37 °C (results not shown). Antibody 18-44, which binds to the β -subunit of the receptor, maximally inhibited 125 I-insulin binding by 40%. This partial inhibition, which was reproducibly demonstrated in four separate experiments, is in agreement with the slight inhibitory effect on insulin binding to IM-9 cells observed previously (Soos *et al.*, 1986a). Antibody 83-7, which did not react with isolated receptor subunits on immunoblots, did not significantly interfere with 125 I-insulin binding, again in agreement with previous observations on other tissues.

Effects of antibodies on lipogenesis and lipolysis

Antibodies were tested initially for their effects on adipocytes in the absence of insulin. Antibodies 83-7, 83-14, 18-44 and 25-49 all stimulated lipogenesis and inhibited lipolysis to approximately the same extent as a maximally effective concentration of insulin (Figs. 2 and 3). When insulin was added together with a maximally effective concentration of antibody, no additional stimulation of lipogenesis was obtained (results not shown). In terms of their effect on lipogenesis (Fig. 2, Table 1), antibodies 83-14 and 25-49 were comparable in potency with insulin on a molar basis (half-maximal effects on lipogenesis at 10 pM and 50 pM respectively),

Fig. 1. Effect of antibodies on insulin binding to intact adipocytes

Binding experiments were carried out at 20 °C in the presence of increasing concentrations of insulin (●) or antibody 83-7 (■), 18-44 (○), 47-9 (□), 25-49 (△) or 83-14 (▲). Different antibodies were assessed in separate experiments. Data points are means of duplicate incubations.

Fig. 2. Insulin-like effects of antibodies on lipogenesis

Adipocytes were preincubated with insulin (●) or antibody 83-7 (■), 18-44 (○), 25-49 (△) or 83-14 (▲) for 45 min at 37 °C before addition of [¹⁴C]glucose. Within each experiment, stimulation of lipogenesis above basal was calculated as a percentage of that given by a maximally effective insulin concentration (0.1 μM). Data points are means ± S.E.M. for three separate experiments (insulin) or means of triplicate incubations within a single experiment (antibodies).

Fig. 3. Insulin-like effects of antibodies on lipolysis

Adipocytes were preincubated with insulin (●) or antibody 83-7 (■), 18-44 (○), 25-49 (△) or 83-14 (▲) for 45 min at 37 °C before addition of noradrenaline plus caffeine. Within each experiment, stimulation of lipolysis above basal was calculated as a percentage of that given by noradrenaline plus caffeine in the absence of insulin or antibody. Data points are means ± S.E.M. for three separate experiments (insulin) or means of duplicate incubations within a single experiment (antibodies).

but antibodies 83-7 and 18-44 were somewhat less potent (half-maximal effects on lipogenesis at 1 nM and 0.3 nM respectively). With respect to inhibition of lipolysis (Fig. 3, Table 1), antibodies 83-14 and 25-49 were at least as potent as insulin, 18-44 of comparable potency (half-maximal effect at approx. 10 pM) and 83-7 again somewhat less potent (half-maximal at approx. 200 pM). The antibodies reproducibly showed a paradoxical slight decrease in anti-lipolytic effect at supramaximal concentrations (Fig. 3), as is observed with insulin itself in human (Fig. 3) and rat (Siddle & Hales, 1980) adipocytes.

In contrast with all the other antibodies tested, antibody 47-9 did not on its own affect basal lipogenesis or catecholamine-stimulated lipolysis. However, when

Fig. 4. Insulin-inhibitory effects of antibody 47-9

Adipocytes were preincubated with antibody 47-9 alone (□) or together with insulin at submaximally (●) or maximally (○) effective concentrations for 45 min at 37 °C before lipogenesis or lipolysis was assessed. For lipogenesis, insulin concentrations of 0.14 nM and 14 nM were used, but concentrations of 14 pM and 140 pM were used in lipolysis experiments to avoid the paradoxical decrease in insulin effect at higher concentrations. Lipogenesis (a) and lipolysis (b) were studied in separate experiments, data points being means of triplicate or duplicate incubations respectively.

cells were incubated with antibody 47-9 together with insulin, the action of insulin on lipogenesis and lipolysis was inhibited by antibody in a concentration-dependent manner (Fig. 4).

Effect of antibody combinations

The insulin-inhibitory antibody 47-9 was tested for its ability to block the effects of insulin-like antibodies. Previous studies had shown mutual competition of antibodies 47-9 and 25-49 in binding to solubilized receptor, although the epitopes were clearly distinct by other criteria. In contrast, antibodies 83-7 and 18-44 bound to solubilized receptor independently of 47-9 (Soos *et al.*, 1986a).

Adipocytes were preincubated with antibody 47-9 before addition of other antibodies at concentrations chosen potentially to give near-maximal and just maximal stimulation of lipogenesis. Antibody 47-9 substantially inhibited the stimulatory effect of 25-49, moderately inhibited the effect of 18-44, but had only a

Table 2. Effect of preincubation with antibody 47-9 on subsequent stimulation by other antibodies

Adipocytes were preincubated with or without antibody 47-9 (20 nM) before addition of insulin or antibody 83-7, 18-44 or 25-49 at the indicated concentration and continuation of the preincubation for 45 min. [14 C]Glucose was then added for determination of the rate of lipogenesis over the next 90 min. Data for a representative experiment are shown, derived from a single adipocyte preparation and expressed as percentage of maximal insulin stimulation above basal. Values are means $\pm$ S.E.M. of triplicate incubations.

Addition		Relative stimulation of lipogenesis (%)	
	Concn. (nM)	No 47-9	+ 47-9
Insulin	0.14	78 $\pm$ 4	4 $\pm$ 3
	140	100 $\pm$ 3	74 $\pm$ 2
25-49	0.5	99 $\pm$ 1	15 $\pm$ 3
	5	105 $\pm$ 2	41 $\pm$ 2
83-7	3	93 $\pm$ 4	72 $\pm$ 5
	30	89 $\pm$ 6	89 $\pm$ 2
18-44	2	50 $\pm$ 6	29 $\pm$ 3
	20	83 $\pm$ 4	59 $\pm$ 4

small effect on stimulation by 83-7 (Table 2). Precise comparison of the degrees of inhibition is complicated by the difficulty of ensuring concentrations of stimulatory antibodies which give equivalent submaximal effects in the absence of 47-9. When the order of antibody additions was reversed, 47-9 again inhibited stimulation of lipogenesis when added after antibody 25-49, the maximum inhibitory effect of 47-9 being smaller in the presence of 25-49 or insulin within the same experiment (Fig. 5b). In contrast, the inhibition by antibody 47-9 of lipogenesis stimulated by 83-7 was much less than its antagonism of the effect of insulin (Fig. 5a). It is likely that the binding of antibody mixtures approached equilibration during a 45 min preincubation, irrespective of the order of addition, since 47-9 could not otherwise antagonize 25-49.

DISCUSSION

We have demonstrated both insulin-like and insulin-inhibitory metabolic effects of anti-(insulin receptor) monoclonal antibodies on isolated human adipocytes. These results, together with other properties of the antibodies, are summarized in Table 1. Four antibodies, reacting with distinct epitopes, both stimulated lipogenesis (which under the conditions of these experiments probably reflected the rate of glucose transport) and inhibited catecholamine-stimulated lipolysis (Figs. 2 and 3). Two of these antibodies (83-14 and 25-49) inhibited insulin binding to adipocytes (Fig. 1) and to other human tissues, by binding to sites on the receptor α -subunit, although the respective epitopes were distinct in other respects (Soos *et al.*, 1986a). Of the other antibodies which showed insulin-like effects, 83-7 did not significantly interfere with the binding of insulin, or indeed of β -subunit-reactive antibodies, and 18-44 showed only a slight inhibition. These two antibodies therefore clearly

Fig. 5. Inhibitory effect of antibody 47-9 on lipogenesis

Adipocytes were preincubated with insulin (0.14 nM; ●) or with (a) antibody 83-7 (3 nM; ■) or (b) antibody 25-49 (0.5 nM; △) for 10 min before addition of the indicated concentrations of antibody 47-9. Preincubation was continued for 45 min before addition of [14 C]glucose. Data points are means $\pm$ S.E.M. for triplicate incubations. Basal lipogenesis was 15 pmol/15 μ l of cells per 90 min in both experiments, and maximal lipogenesis stimulated by 140 nM-insulin was 44 pmol/90 min per 15 μ l of cells in (a) and 49 pmol/90 min per 15 μ l of cells in (b).

bound to the receptor at some distance from the insulin-binding site, the epitopes being distinct and, at least in the case of 18-44, localized on the receptor β -subunit (Soos *et al.*, 1986a). The effect of 18-44 on insulin binding presumably reflected an influence on the affinity of the receptor for insulin binding, rather than a steric blockade of the site itself.

A fifth antibody (47-9) did not on its own affect lipogenesis or lipolysis, but did block both the binding and the metabolic effects of insulin, both in adipocytes (Fig. 4) and in a human hepatoma cell line (Soos & Siddle, 1985). In these respects the antibody was similar to that described by Roth *et al.* (1982). It appeared that antibody 47-9 acted primarily by blocking the binding of potentially stimulating ligands (insulin or other antibodies). Thus this antibody most strongly antagonized the stimulatory effect of the antibody with which it competed in binding to receptor (25-49), but had little effect on an antibody with which it did not compete for binding (83-7) (Table 2, Fig. 5). The partial inhibitory

effect of antibody 47-9 on lipogenesis stimulated by 83-7 and 18-44 was unexpected, given the lack of mutual interference between these antibodies in binding to solubilized placental receptor. The steric relationships in the respective epitopes may be different for adipocyte receptor *in situ*, or it may be that antibody 47-9 alters receptor conformation and thereby decreases its affinity for other antibodies without sterically blocking their binding. Alternatively, the possibility cannot be ruled out that 47-9 exerts a direct inhibitory effect on receptor function, and this aspect warrants further study. Interestingly, in other studies antibody 47-9 was not entirely passive in its binding to receptor, in that it markedly accelerated the dissociation of receptor-bound ¹²⁵I-insulin (M. Soos & K. Siddle, unpublished work) in a manner similar to unlabelled insulin (De Meyts *et al.*, 1976). A monoclonal antibody which induced down-regulation of receptors, although incapable of triggering insulin-like metabolic effects, was described by Roth *et al.* (1983b). It thus appears likely that the process of receptor activation by insulin involves separate events mediating metabolic action (activation of membrane transport and regulation of intracellular enzymes) and modulation of receptor function (negative co-operativity or down-regulation). Antibodies acting as partial agonists will be valuable reagents in attempting to define those activities of the receptor which are important in each of these pathways.

Polyclonal anti-receptor antibodies have been demonstrated to elicit a wide range of insulin-like effects on rat cells. This has been shown both with rabbit antibodies specifically raised against rat receptor (Jacobs *et al.*, 1978) and with human autoantibodies which cross-react with rat receptor (Kahn *et al.*, 1981). In the former case the antibodies did not inhibit insulin binding, but in the latter case they did inhibit, although because of their polyclonal nature it is not possible to ascertain whether the same sub-population of antibodies is responsible for binding inhibition and metabolic activation. By using monoclonal antibodies, the various effects arising from reaction at a defined epitope may be unambiguously determined. It should ultimately be possible to map the epitopes of these monoclonal antibodies in terms of the known primary sequence of the insulin receptor (Ullrich *et al.*, 1985; Ebina *et al.*, 1985) and thus to define the regions of receptor structure important in biological signal transduction.

The mechanism whereby monoclonal antibodies activate the insulin receptor to mimic metabolic effects of insulin is not at present certain. Antibodies and insulin gave the same maximal effects, which were not additive, confirming that they acted ultimately through a common pathway. Several antibodies showed a potency on a molar basis comparable with or greater than that of insulin itself, suggesting that they, like insulin (Gammeltoft, 1984), elicited maximal metabolic effects at relatively low fractional occupancy of receptors, assuming comparable binding affinities. The titres of antibodies for inhibition of lipolysis and stimulation of lipogenesis at 37 °C were indeed generally higher than those for inhibition of insulin binding at 20 °C (Figs. 1 and 2; Table 1). Both insulin and insulin-like antibodies showed a somewhat higher potency on a molar basis for inhibition of lipolysis than for stimulation of lipogenesis. The different potencies of different antibodies presumably reflected their relative binding affinities. It is clear

that binding at or close to the insulin-binding site is neither necessary nor sufficient for antibodies to elicit insulin-like effects. It is unlikely that so many different antibodies, interacting with either the α - or β -subunit, could all act by directly triggering the same conformational change as is induced by binding of insulin at a specific bioactive site. Rather it appears possible that activation of receptor by antibodies may arise from some more general, epitope-independent, consequence of antibody binding, such as cross-linking and aggregation of dimeric $\alpha_2\beta_2$ receptor structures by bivalent antibody, given appropriate disposition of epitopes. In this context it will be interesting to investigate the biological effects of monovalent (Fab) and bivalent [F(ab)₂] antibody fragments.

Experiments with monovalent Fab fragments of polyclonal antibodies have previously suggested that antibody bivalency is necessary for biological activity (Kahn *et al.*, 1978; Heffetz & Zick, 1986). However, it could not be deduced whether all antibodies which cross-linked receptors were biologically active, regardless of individual epitopes. The availability of monoclonal antibodies for multiple epitopes will help to clarify this issue. It is apparent that monomeric insulin cannot itself cross-link receptors by bivalent binding, although it might spontaneously induce micro-aggregation secondary to a conformational change in the receptor, as has been observed for epidermal-growth-factor receptors (Zidovetzki *et al.*, 1986). Alternatively, insulin binding and receptor aggregation may be different ways of triggering a common receptor-activation event, be that activation of intrinsic protein kinase activity or generation of a soluble second messenger (Czech, 1985; Kahn, 1985). It is noteworthy that monoclonal antibodies for other polypeptide-hormone receptors have also been demonstrated to have biological activity, even though the likely mechanisms of action of different receptors vary considerably. Among antibodies which inhibit hormone binding, both agonist and antagonist antibodies have been identified for thyrotropin receptors (Valente *et al.*, 1982a,b), lutropin receptors (Podesta *et al.*, 1983), prolactin receptors (Djiane *et al.*, 1985) and epidermal-growth-factor receptors (Schreiber *et al.*, 1981; Gill *et al.*, 1984; Chandler *et al.*, 1985). In several cases bivalency of antibodies was necessary for biological activity (Schreiber *et al.*, 1983; Podesta *et al.*, 1983; Djiane *et al.*, 1985). However, for epidermal-growth-factor receptors, not all antibodies possess biological activity (Schreiber *et al.*, 1983; Richert *et al.*, 1983; Gregoriou & Rees, 1984; Gill *et al.*, 1984), and it may be that receptor cross-linking is not a sufficient trigger for activation in all cases.

The monoclonal antibodies for the insulin receptor that have been characterized in the present study promise to be valuable reagents in identifying aspects of receptor structure that are important for function, and in probing the mechanisms whereby ligand binding triggers cell activation and receptor regulation. In particular it will be of interest, using antibodies as partial agonists, to correlate effects on intracellular metabolic activity with rapid changes in the receptor itself, including micro-aggregation and autophosphorylation, in order to determine the significance of these events in intracellular signalling.

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