insulin response at 1/500000 dilution. At this latter dilution 125I-insulin binding was inhibited by 30%. A 1/500000 dilution of antibody gave maximal stimulation of lipogenesis (85% that obtained with insulin) and >90% inhibition of 125I-insulin binding. These results suggest insulin and antibody 25-49 are exerting approximately equal effects on insulin receptors. Antibody 83-7 also stimulated lipogenesis but, unlike 25-49, had little effect on 125I-insulin binding. Half-maximal response for lipogenesis was obtained at 1/150000 dilution, a concentration at which 125I-insulin binding was inhibited by 15%. The highest concentration of antibody tested (1/500000 dilution) gave the same maximal lipogenic response as antibody 25-49 but only 30% inhibition of 125I-insulin binding. Antibody 47-9 was almost identical to 25-49 in its inhibitory effect on 125I-insulin binding (also showing half-maximal response at 1/250000 dilution and >90% inhibition at 1/50000 dilution) but it had a very different effect on lipogenesis. This antibody did not stimulate lipogenesis but antagonized the lipogenic effect of 10-10 M-insulin (a 10-fold higher concentration than that necessary for maximal response). A 1/500000 dilution of antibody inhibited the maximal insulin response by 50% while the highest concentration tested (1/500) gave 65% inhibition.

We have thus identified several monoclonal antibodies, recognizing distinct epitopes on the insulin receptor, which affect insulin binding and/or lipogenesis in human adipocytes. Antibodies which were presumed from inhibition of insulin binding to react close to the insulin-binding site either mimicked (25-49) or antagonized (47-9) metabolic effects of insulin. In addition, another antibody (83-7) mimicked the lipogenic effect of insulin by interaction at a distinct site which did not result in significant inhibition of insulin binding. Transduction of the signal mediating biological activity may thus occur with or without modulation of insulin binding and vice versa. These antibodies should be valuable reagents in correlating receptor function, particularly tyrosine kinase activity and autophosphorylation, with regulatory effects on intracellular metabolism.

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Molecular cloning and bacterial expression of a peptide with immunological similarity to the insulin receptor

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The insulin receptor is an integral plasma membrane glycoprotein, containing disulphide-linked subunits in an 
\( \alpha \beta \) structure. The \( \alpha \)-chain (M, 130000) contains the extracellular insulin-binding domain, and the \( \beta \)-chain (M, 90000) possesses an intracellular domain with tyrosine kinase activity, (Czech, 1985). The two chains are derived from a common precursor. We have attempted to use bacterial expression vectors to clone the gene for the insulin receptor. A major aim of this work was to obtain detailed information on the structure of the receptor and to relate this to functional domains defined by a panel of monoclonal antibodies (Soos et al., 1986).

A human liver cDNA library in the bacterial expression vector pEX-1 was prepared and cloned in Escherichia coli as described previously (Stanley & Luzio, 1984). The colony blot procedure (Stanley, 1983) was employed for immunological screening of expressed hybrid \( \beta \)-galactosidase protein, using a mixture of two rabbit antisera to purified human placental insulin receptor (Jacobs & Cuatrecasas, 1981; Fujita-Yamaguchi et al., 1983). Ten positive clones of 125I-insulin binding. These results (Soos et al., 1986). The reactivity of these clones was due to expression hybrid protein by testing the antisera on protein immunoblots (Burnette, 1981) of bacterial extracts fractionated by SDS/polyacrylamide-gel electrophoresis.

In order to discriminate false positives, the antibody-select procedure (Hall et al., 1984) was used to purify and determine the specificity of the antibodies reactive against each of the ten bacterially expressed hybrid proteins. The proteins were immobilized on nitrocellulose filters by protein blotting of polyacrylamide gels and used as substrates for affinity purification of the rabbit antisera. These purified antibodies were then tested for their reaction with protein immunoblots of other hybrid proteins and purified receptor. Only antibodies purified against hybrid proteins of two clones, designated pIR-3 and pIR-10, recognized insulin receptor. Both these antibodies reacted strongly with insulin receptor \( \alpha \)-chain on immunoblots, indicating the existence of an epitope common to the hybrid proteins and receptor. The same purified antibodies, unlike the unfragmented antisera, did not react with soluble, purified, radiiodinated receptor unless this was first denatured with SDS or urea, showing that the epitope was not accessible in native receptor. None of the antibodies purified on hybrid proteins of the other eight 'positive' clones, which represented five distinct epitopes, reacted with insulin receptor. It was concluded that in these cases the hybrid proteins were reacting with non-receptor antibodies present as contaminating activities in the antisera.

Clones pIR-3 and pIR-10 were identical by various criteria. The two hybrid proteins were of the same size (peptide M, approx. 20000) and each reacted with the corresponding affinity-purified antibodies. The cDNA inserts were also identical in size (approx. 550 base pairs) and restriction map. The sequence of a 430-base section of the cDNA insert from clone pIR-3 was obtained (Sanger et al., 1977). This showed a single long open-reading frame for which the amino acid sequence was deduced.

Abbreviations used: SDS, sodium dodecyl sulphate; kb, kilobases.
Further experiments were conducted to determine the presence in human cells of protein related to the hybrid protein expressed by clone pIR-3, and of RNA complementary to pIR-3 DNA. Human lymphoid cell lines known to be either positive (IM-9) or negative (MOLT-4, H9, CCRF-CEM) for surface insulin receptors were tested in the protein immunoblot assay using antibodies purified on hybrid protein of clone pIR-3. When SDS-polyacrylamide gels had been loaded to their highest capacity in order to favour detection of low-abundance proteins, only one species co-migrating with insulin receptor α-subunit in IM-9 cells, but absent in the other cell lines, was observed to react with antibody. Attempts to repeat this experiment with human liver and placenta did not show any unequivocal reaction of purified antibody, though a weak reaction with a component similar in mobility to α-subunit was sometimes observed. It was concluded that the insulin receptor was the only detectable human protein which shared an epitope with hybrid protein from clone pIR-3.

When pIR-3 DNA was used to probe Northern blots of RNA extracted from human cells, a 4 kb species was identified which was present in IM-9 but not CCRF-CEM cells, and which was accumulated in response to mitogens in human tonsil lymphocytes with similar kinetics to the induced insulin receptor. A 1 kb species also reacted in both lymphoid cell lines and activated lymphocytes. It was concluded that the 4 kb RNA was consistent in size and distribution with the properties expected of insulin receptor mRNA.

Proof of identity of cDNA clones depends ultimately on comparison with known peptide sequence. When the sequence of the insulin receptor became available (Ebina, J. I. Davies,* P. Lonnr0th and U. Smith

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The anti-insulin effect of the catecholamines in intact animals includes an inhibition of peripheral glucose utilization (Diebert & DeFronzo, 1980). This can be seen in isolated adipocytes where the catecholamines are strongly adenosine-dependent (Taylor & Halperin, 1979; Green, 1983; Kashiwaga et al., 1983). Little attention has so far been given to the time-course of the observed responses and as a result the dynamic state of the system has not been fully appreciated.

When rat adipocytes were added to a medium containing a combination of insulin (200 units/ml), isoproterenol (1 μm) and adenosine deaminase (1 μg/ml), rapid changes occurred in their 3-O-[2C]methylglucose transport activity. The initial response was dominated by insulin, though its stimulatory effect developed more slowly than in the presence of insulin alone. After 10-20 min, glucose transport activity entered an exponential decline. Three inhibitory effects on the response to insulin were distinguishable: one, (A), which diminished the initial rise in glucose transport, another, (B), which initiated the exponential decline, and a third, (C), which reinforced the decline phase. Inhibition (A) was distinguishable by the fact that it could not be prevented by high concentrations of insulin (10 units/ml), which reversed inhibitions (B) and (C). Discrimination between inhibitions (B) and (C) was also possible since only the former was reversible by the β-adrenergic antagonist propranolol (10 μm).

In a recent report, Smith et al. (1984) demonstrated that the effect of isoproterenol plus adenosine deaminase on insulin-stimulated glucose transport is partly due to a change in the activity of transporters located in the plasma membrane. Inhibition (A) appeared to have the kinetics implied by such a mechanism, since it was not prevented by high concentrations of insulin despite the efficacy of the latter in stimulating glucose-transporter recruitment. The inhibition developed rapidly and, though apparent in the presence of adenosine deaminase alone, was enhanced by the additional presence of isoproterenol.

Inhibitions (B) and (C) both appeared to be associated with changes at the level of the insulin receptor. Steinfelder & Joost (1982) showed that the lowering of receptor affinity due to a variety of substances correlated with their lipolytic activity (see also Steinfelder & Joost, 1983). This effect appears to have a different origin from the initial loss of insulin binding (insulin concentration, 20 units/ml), which was rapidly reversed by propranolol (1/2 approx. 6 min). It is not clear whether this was due to

The kinetics of the insulin, catecholamine and adenosine interaction in the regulation of adipocyte glucose transport

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