In conclusion, the inhibitory effect of TPA on histamine-stimulated aminopyrine accumulation is mediated by activation of protein kinase C and this enzyme may play a role in the regulation of acid secretion by the parietal cell.

Abbreviation used: SDS, sodium dodecyl sulphate.

Immunoaffinity purification of insulin receptor by use of monoclonal antibodies

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A considerable body of information is now available concerning gross structural features of the insulin receptor (Czech, 1985; Kahn, 1985), together with the complete amino acid sequence deduced from the clone receptor gene (Ebita et al., 1985; Ulrich et al., 1985). However, many important details of receptor structure remain to be elucidated including identification of sites of phosphorylation and glycosylation, and characterization of the insulin-binding site. These and other studies of receptor structure and function require the availability of purified, native receptor in some quantity. Published techniques of receptor purification, usually involving sequential affinity chromatography on lectin-agrose and insulin-agrose (Jacobs & Cuatrecasas, 1981; Fujita-Yamaguchi et al., 1983), take a considerable time and have not always yielded receptor preparations which are fully active with respect to insulin binding or endogenous protein kinase activity. With the availability of a panel of 28 monoclonal anti-receptor antibodies (Soos et al., 1985), and our previous experience in the application of anti-insulin receptor antibodies to affinity purification (Woodford et al., 1982), we sought to develop an immunoaffinity method for rapid purification of insulin receptor.

Monoclonal antibodies were partially purified from ascites fluids by precipitation with 40% (v/v) saturated ammonium sulphate. Immunoadsorbents were prepared by coupling of immunoglobulin fractions to amino-agarose (Habig & Woodford, 1982) and were used routinely in all further work because of its greater ease of production. Decreasing the pH to 4.5, or increasing salt concentration to 2.5 M, did not significantly improve the extent of elution by acetate/NaCl in a single incubation. Use of 1.5 M MgCl₂ in place of NaCl did give a greater elution of receptor but also produced a rapid loss of insulin-binding activity (50% within 30 min) unless the eluant was immediately diluted to prevent high protein and insulin concentrations. The pH of elution buffer was increased to pH 5.0/1 M NaCl/0.1% Triton was examined, this buffer having been used previously for elution of native receptor from insulin-agarose affinity adsorbent (Fujita-Yamaguchi et al., 1983). These conditions eluted 40–50% of receptor from just two antibodies (coded IR 18–28 and 18–42), and were ineffective (< 10% elution) with the remaining antibodies. Antibody IR 18–42 was used routinely in all further work because of its greater elution of receptor but also produced a rapid loss of insulin-binding activity (50% within 30 min) unless the eluant was immediately diluted to prevent high protein and insulin concentrations. No activity losses were apparent when using 50 mm-acetate pH 5.0/1 M NaCl, providing exposure of receptor to these conditions was kept to less than 15 min by neutralization of eluant immediately after removal of adsorbent. The initial eluant volume of 50 ml per placental preparation was reduced as necessary by ultrafiltration.

It was estimated from recoveries of protein and insulin-binding activity that a 40% yield of receptor, purified 2000-fold relative to placental membrane extract, was obtained within 4 h by this technique. Analysis of purified material by SDS/polyacrylamide-gel electrophoresis revealed two major protein bands of approx. 130 000 and 90 000 corresponding to the previously described α- and β-subunits of the receptor. The only other discrete bands visible, at much lower intensity, were of approx. 70 000, 50 000 and 25 000, the latter two presumably representing traces of finely divided immunoadsorbent not removed by centrifugation. Overall it appeared that the receptor was at least 50% pure. The binding of 125I-IGF-I and 125I-insulin by purified receptor was compared with that in crude placental extract. The relative titre was 2-fold in favour of insulin in the extract and this increased to 10-fold in purified material. This enrichment of specific insulin binding in immunoaffinity-purified material was in agreement with the known low cross-reaction of IR 18-42 antibody with IGF-I receptors. This
antibody was also known not to affect binding of insulin to its receptor, and the epitope was tentatively assigned to the extracellular domain of β-subunit.

The protein kinase activity of the purified receptor was examined under conditions described previously (Petruzelli et al., 1984). It was necessary to add 0.5–1 mm-dithiothreitol to elution buffer in order to retain maximal kinase activity, although this addition was without effect on the yield of insulin-binding activity. The purified receptor was active in both autophosphorylation and histone phosphorylation assays. Insulin stimulated 2–6-fold the autophosphorylation of receptor β-subunit measured over 1–5 min at 4°C.

It is concluded that immunoaffinity purification is a rapid, high-yield method for the provision of active insulin receptor for use in studies of receptor structure and function.

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

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The means by which insulin binding to its receptor initiates varied metabolic responses in target cells remains unclear. Rapid effects of insulin, which have been postulated as possible mediators of its biological activity, include aggregation of receptors, production of peptide mediators and stimulation of tyrosine and serine kinase activities, leading to autophosphorylation and phosphorylated intracellular components (Kahn, 1985). Although autoantibodies, which mimic most of insulin's biological actions, have proved useful probes in studying the functional domains of the receptor, their polyclonal nature restricts their use in associating discrete epitopes on the receptor with its various functions. We have produced a series of 28 monoclonal antibodies (Soos et al., 1985) recognizing different epitopes on the human insulin receptor as probes for investigating the relationship between insulin binding and subsequent events leading to a biological response. In this paper we describe preliminary studies on the effects of selected antibodies on lipogenesis in isolated human adipocytes.

Isolation of adipocytes from human subcutaneous fat and insulin-binding studies were performed as described by Pedersen et al. (1981). Incorporation of tritiated glucose into total lipids was assayed as described by Pedersen et al. (1982), except that carrier glucose was omitted, giving a final glucose concentration of 5.8 μM. Anti-receptor monoclonal antibodies were partially purified from ascites fluid by ammonium sulphate precipitation (40% v/v), followed by reconstitution in phosphate-buffered saline to the original ascites volume (approx. 2–5 mg of antibody/ml). The effect of antibodies on insulin binding and lipogenesis was determined after preincubation of adipocytes with antibody for 30 min at 37°C.

Under these experimental conditions insulin (10−8 M) gave a 2–3-fold stimulation of glucose incorporation into lipid. Preliminary experiments identified five antibodies which had a marked effect on lipogenesis at a 1/500 dilution, a concentration at which non-immune mouse antibody had no effect. Some antibodies (83-7, 83-14 and 25-49) were insulin-like, stimulating lipogenesis to approximately the same maximum as insulin. The effects of antibody and insulin were not additive. In contrast, antibodies 47-9 and 47-46 had no effect on basal lipogenesis but inhibited insulin-stimulated lipogenesis by 100% and 40% respectively.

The effects of antibodies on lipogenesis were then studied in parallel with their effects on binding of A14 monoiodinated insulin (16 PM). In the insulin-binding experiments cell-bound radioactivity (in the absence of antibody or unlabelled insulin) was 1.31% of the total. Non-specific binding, determined in the presence of 10−8 M-insulin, was only 3% of the total cell-bound radioactivity. Unlabelled insulin blocked 125I-insulin binding with a half-maximal response at 3 × 10−10 M and >90% inhibition at 10−8 M. In the lipogenesis experiments insulin (10−8 M) gave a 2.7-fold increase in the incorporation of D-[U-14C]glucose (0.4 μCi, 5.8 μM) into lipid. The basal and maximal rates of lipogenesis were 2.05 ± 0.3 and 5.5 ± 0.3 pmol/μl packed volume cells per 90 min respectively. Half-maximal stimulation was achieved by 5 × 10−11 M-insulin.

Three antibodies were studied in detail, which recognized different epitopes on the receptor as judged by species specificity and competitive binding assays. Antibody 83-7 did not compete with antibodies 47-9 and 25-49 for binding of 125I-receptor, indicating that its binding site on the receptor was distant from those for the other antibodies (K. Siddle & M. Soos, unpublished work). Antibody 25-49 behaved similarly to insulin and was very effective in both inhibiting 125I-insulin binding, with half-maximal response at 1/250,000 dilution, and in stimulating lipogenesis, giving 60% of the maximum