Human insulin receptor contains covalently bound palmitic acid

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The widespread occurrence of the covalent modification of proteins with long-chain fatty acids has become clear in recent years (Magee & Schlesinger, 1982; Magee & Courtneidge, 1985). Examples are a number of proteins believed to be involved in signal transduction such as the oncogene products pp60<sup>v-fgr</sup> and p21<sup>ras</sup> (Sefton et al., 1982) and several cell-surface receptors for extracellular ligands such as transferrin receptor (Omary & Trowbridge, 1981). During the course of a survey of receptors for growth factors we have observed the incorporation of palmitic acid into the β-subunit of the human insulin receptor.

The human hepatoma cell line HepG2 was chosen for these studies since it expresses a large number of insulin receptors of both high and low affinity (Soos & Siddle, 1985). Cells were labelled with <sup>[35S] </sup>methionine, <sup>[3H]</sup>myristic acid or <sup>[3H]</sup>palmitic acid and detergent lysates were immunoprecipitated with a rabbit polyclonal antiserum to the P-subunit of the human insulin receptor (Fujita-Yamaguchi et al., 1983) from human placenta. Some cross-reactivity of this antiserum with IGF-I receptor cannot be excluded. Fig. 1(b) shows that the mature insulin receptor subunits of 130 kDa and 90 kDa respectively were recognized by this serum, as well as a protein of 190 kDa which probably corresponds to the precursor as shown by pulse-chase labelling (not shown). <sup>[3H]</sup>Myristic acid, which is believed to be specific to proteins acylated at the amino terminus in amide linkage, did not label the insulin receptor subunits. However, <sup>[3H]</sup>palmitic acid specifically labelled the transmembrane β-subunit as well as the αβ-precursor (Fig. 1b), but not the extramembranous α-subunit. Labelling of the precursor suggested that acylation was an early event. This was confirmed since pretreatment of cells with the protein synthesis inhibitor emetine abolished labelling with fatty acid. Aciylation was also abolished by the drug cerulenin, which has been shown to inhibit the acyltransferase involved in addition of palmitate to viral membrane glycoproteins (Schlesinger & Malfer, 1982).

The linkage of the acyl group to the receptor was characterized as a thioester by its liability to mild hydroxylamine treatment at neutral pH (Magee et al., 1984). Examination of the published sequence of the human insulin receptor (Ebina et al., 1985) suggests that Cys-981 in the β-subunit located in a region near the transmembrane domain but cytoplasmic to it is a likely candidate for the acylation site by analogy with other acylated transmembrane proteins. This is consistent with the observation that the aberrant β'-subunit, which has probably lost the bulk of its cytoplasmic kinase domain but retains Cys-981, is also acylated (not shown).

Since the acyl moiety of the transferrin receptor has been shown to turn over faster than the polypeptide (Omary & Trowbridge, 1981), a phenomenon which may be related to receptor function and recycling, we performed pulse–chase studies on <sup>[35S] </sup>methionine- and <sup>[3H]</sup>palmitate-labelled receptor. The results indicate that in this case the fatty acid turns over with similar kinetics to the polypeptide backbone. Thus far we have been unable to demonstrate any differential effects on fatty acid turnover using agents which perturbed the endocytic cycle of the insulin receptor. The function of the acyl group thus remains enigmatic, although this system provides the potential to test for effects of acyl group removal or modification on receptor function.

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Fig. 1. Labelling of insulin receptor precursor and β-subunit with [3H]palmitic acid

Subconfluent cultures of HepG2 cells were labelled with 80 μCi of <sup>[35S] </sup>methionine/ml in medium containing 10% of the normal methionine concentration for 3 days (a, b) or with 150 μCi of <sup>[3H]</sup>palmitic acid/ml in medium supplemented with 5 mM-pyruvate for 4 h (c). Cells were washed, lysed in 20 mM-Tris/HC1/150 mM-NaCl/0.5% Nonident NP40/0.5% sodium deoxycholate/0.1% SDS/0.2 mM-phenylmethanesulphonyl fluoride 1% Trisylol, pH 7.5, and immunoprecipitated with non-immune rabbit serum (a) or rabbit-anti-human insulin receptor antiserum (b, c) and protein A-Sepharose beads. Immunoprecipitates were analysed by SDS/polyacrylamide-gel electrophoresis on a 5–15% gradient gel followed by fluorography.


The mechanism of synergistic actions of adrenaline and thrombin on human platelets

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The synergism of various agonists in their effects on the aggregation of blood platelets in platelet-rich plasma has been described many times, but there has been no satisfactory explanation. However, combined stimulation of the platelet with adrenaline and thrombin did not result in the appearance of increased metabolites of arachidonic acid, including thromboxane B2 and 12 hydroxyeyecosatetraenoic acid in ether extracts of platelet lipids which had been separated on Kieselgel 60 TLC plates in a solvent system of chloroform/methanol/acetic acid/H2O (90:8:1:0.8, by vol.). Similarly, no increases were observed when combined doses of these agonists, which together aggregated platelets, were used to determine the importance of arachidonic acid metabolism. The same lipid extracts were run on a separate TLC plate in petroleum ether/ether/acetic acid (70:20:4, by vol.) to isolate diacylglycerols. Unlike large doses of thrombin (Rittenhouse-Simmons, 1981) no increases in diacylglycerol synthesis were observed for the combined agonists 10 or 120s after addition of the second agonist, thrombin.

In separate experiments isolated platelets were prelabelled for 90 min with Na[32P] to label ATP and stimulated with adrenaline, thrombin or combined agonists. The platelet proteins (60μg) were separated on 13% polyacrylamide/sodium dodecyl sulphate gels and run at 45 mA in 25 mm-Tris/150 mm-glycine/1% SDS buffer, pH 7.4. The phosphorylation of protein bands was assessed by autoradiography and densitometry. Even at high doses, adrenaline had only small effects on 20kDa and 47kDa protein phosphorylation compared with thrombin. Sub-threshold doses of these agonists had little effect compared with the basal phosphorylation, but there was a modest increase 30s after stimulation by the combined agonists.

Finally, sub-threshold concentrations of adrenaline lowered cyclic AMP concentrations in forskolin-treated platelets to basal levels (measured by radioimmunoassay) whereas the effects of low doses of thrombin were slight. The combined effect of both agonists on cyclic AMP was the same as that for adrenaline alone.

The role of intracellular Ca2+ in the potentiation of the effects of low doses of thrombin by adrenaline remain unclear. There appears to be a sufficient increase in phosphorylation of the 20kDa protein after 30s to suggest an increase in [Ca2+] great enough to encourage aggregation and secretion.

The metabolism of arachidonic acid plays a negligible role in this synergism, which is not surprising since the effects of adrenaline on these pathways, even at higher concentrations, are minimal. The fact that optimal results are obtained when agonists are added in sequence suggest that these are post-receptor events and may involve a reduction in the activity of adenylate cyclase by adrenaline.

Abbreviation used: [Ca2+], intracellular Ca2+ concentration.

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