Regular Review

Insulin receptors and the clinician

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Deficiency of insulin brings about rapid metabolic decompensation, but in several clinical states its action appears to be inadequate despite normal or raised serum concentrations of immunoreactive insulin. These insulin resistant states are characterised by raised circulating insulin concentrations in the face of normal or raised blood glucose concentrations. Table I lists some examples. Many of the metabolic abnor-

the physiology of insulin receptors.⁸ They observed that the affinity of insulin binding to leucocytes seemed similar to that shown by rat hepatocytes and adipocytes. It later became clear that monocytes accounted for most of the insulin binding capacity of mixed leucocyte preparations,^{9 10} though some insulin receptor activity was also present on lymphocytes, polymorphs, erythrocytes, and platelets. Table II

TABLE I—Insulin receptor binding data on human adipocytes and monocytes, and insulin action data on human adipocytes in insulin resistant states*

	Adipocyte insulin sensitivity	Adipocyte insulin binding	Monocyte insulin binding	References
Non-insulin-dependent diabetes	↓	N	↓	18,19,57
Insulin dependent diabetes (moderate or poor control)	↓	↓	↑ or N	15,58
Obesity	↓	↓	↓	59,60,61
Alcoholic cirrhosis	↓	↓	N	22,62,63
Chronic renal failure	N	N	↓	33,64
Acromegaly	—	—	N or ↓	65,66
Cushing's syndrome	—	—	N	67
Pregnancy (third trimester)	↓	↓	N	68,69
Extreme insulin resistance type A	—	—	↓ or N	49,70,71
Extreme insulin resistance type B	↓↓	↓↓	↓↓	71, R Taylor, unpublished data
Leprechaunism	—	—	↑	72

↓ ↑ N= Decreased, increased, normal.
*This is not an exhaustive list but includes most of the common states of insulin resistance and some of the more informative rare syndromes. Where discrepant data have been reported the majority view is stated. Insulin binding is quoted as maximum percentage specific binding compared with matched control data. Pitfalls in execution and interpretation of clinical studies of insulin binding have been reviewed.^{73 74}

malities might be expected to be reversed by the restoration of normal insulin action, and vast amounts of research have been done on insulin receptors in these states. This review will summarise the positive findings and attempt to dispel some of the associated myths.

Historical perspective

Specific tissue receptors for chemical messengers were first postulated by Langley, who suggested that drugs and hormones did not act directly on biological processes but that some receptive substance mediated their effects.^{1 2} Research on tissue receptors remained within the province of pharmacology for many years,^{3 4} but the advent of radiolabelled hormones of high specific activity opened up study.^{5 6} The basic work on the properties of insulin receptors used preparations of rat liver membrane and rat adipocytes.^{6 7} The discovery in 1972 by Gavin and coworkers of specific insulin binding to human leucocytes led to clinical investigation of

lists the cell types used for measuring insulin receptors. The discovery of specific insulin receptors on erythrocytes allowed their numbers to be measured without having to remove the large volumes of blood required for studies of monocytes.¹¹ Shortly after specific binding of insulin to blood cells was first described, it was suggested that such binding might reflect the insulin binding to metabolically important cells.¹² Data were presented which suggested a correlation between insulin binding to monocytes and adipocytes¹³ and it became widely believed that blood cell insulin binding mirrored the changes in target organ tissues.

Rise and fall of blood cell insulin binding

Clinical studies of insulin receptors have mostly examined abnormalities in their behaviour in insulin resistant conditions. For such studies to be clinically relevant they need to look at the main tissues responsive to insulin (liver, muscle, and fat). I have already referred to the assumption that

TABLE II—*Characteristics of cell types used for human insulin receptor studies*

	Adipocytes	Monocytes	Erythrocytes	Fibroblasts
Sampling	Surgical procedure; leaves scar	60-150 ml blood	15-25 ml blood	Skin biopsy
Cell isolation	Incubation with collagenase	Ficoll-Hypaque centrifugation	Ficoll-Hypaque centrifugation	Serial tissue culture
Cell suspension	Pure adipocytes. Wide range of cell sizes	10-30% monocytes, 70-90% lymphocytes. Neutrophils, platelets, red cells in small amounts	1% Granulocyte contamination	Pure
Particular practical problems	Expression of data to compensate for variability in cell size	Accurate and precise counting of percentage of monocytes	Mixture of red cells of differing ages	Studies on freshly isolated cells not possible
Suitability for measurement of insulin action	Excellent	None	None	Good
Relevance of cell to carbohydrate metabolism in vivo	Definitive but limited in terms of glucose disposal in vivo	None	None	Minimal but suitable for study of genetically determined factors
Non-specific binding as percentage of total binding at insulin tracer concentrations	0.8-4.0	5-30	10-20	At least 20

circulating blood cells faithfully reflect the insulin receptor state of target organ tissues. The study most widely quoted in support of this belief showed that insulin binding to both monocytes and adipocytes tended to be low in very obese patients. When data from obese and normal groups were pooled and analysed by linear regression a significant result ($r=0.54$) was obtained. Drawing linear regression lines between data points from two different groups of patients is of dubious validity, but the data were discussed with suitable caution in the original paper.¹³ Other workers, however, described the observed correlation between insulin binding to the two cell types as "excellent,"¹⁴ and this secondary opinion has been widely quoted.

When the insulin receptors of circulating monocytes and adipocytes were examined critically in various normal and disease states no direct relation could be established.¹⁵⁻¹⁷ In severe obesity the numbers of insulin receptors were low in all tissues examined. Yet in other states such as non-insulin-dependent diabetes,¹⁸⁻¹⁹ insulin dependent diabetes,¹⁵⁻²⁰⁻²¹ cirrhosis,²² and thyrotoxicosis¹⁷⁻²³⁻²⁴ clear discrepancies were observed between monocyte and adipocyte insulin binding. Even comparisons between monocytes and erythrocytes have shown different characteristics of binding to the two cell types in a range of conditions.²⁵⁻²⁸ The lack of correlation between the insulin binding properties of blood cells and adipocytes does not merely reflect the differences in assay conditions: performing the adipocyte assay in the conditions necessary for the monocyte assay does not improve the correlation.¹⁵

Unfortunately, the assumed relevance of blood cell insulin receptors to carbohydrate metabolism has inhibited study of their physiological function on these cells. The teleological significance of insulin receptors on blood cells of any kind remains uncertain. Some isolated observations are of interest: insulin receptors on non-cytotoxic T lymphocytes are not expressed unless the cells are exposed to specific antigenic stimulation²⁹; insulin influences the fluidity of the cell membrane of erythrocytes³⁰; and further studies of the physiological relevance and mechanism of these findings will be of interest.

Receptor studies on insulin sensitive tissues

Many clinical studies have been carried out with adipocytes obtained by biopsy of adipose tissue under local anaesthesia.¹³⁻¹⁵⁻¹⁶⁻¹⁸⁻¹⁹⁻²²⁻²⁴ This procedure is well tolerated by patients, and the isolated adipocytes are easy to handle in the various assays of insulin binding and insulin action. Studies of adipocytes have the clear merit that the action of insulin

may be assessed at the same time as of the insulin receptors (table II). Nevertheless, of the two main extrahepatic insulin sensitive tissues—muscle and fat—the former is more important in the disposal of a glucose load.³¹

In interpreting the results of clinical studies of adipocyte behaviour we need to consider whether muscle cells and adipocytes are likely to exhibit parallel changes. No direct comparisons have been reported. These two cell types are exposed to similar hormonal and metabolic influences in the peripheral circulation. In obesity, insulin dependent diabetes, and hepatic cirrhosis the decreased numbers of insulin receptors in adipocytes as well as their insensitivity to insulin in vitro appear to parallel the observations in vivo on the sensitivity of muscle to insulin as assessed by the euglycaemic clamp technique.¹⁵⁻²²⁻³¹⁻³² We should not infer that adipocytes and myocytes always display similar changes in insulin sensitivity in all clinical states. Differential sensitivity of various metabolic pathways may be observed in the same cell type, suggesting that general comparisons should not be assumed in the insulin sensitivity of cells with disparate metabolic functions.¹⁵ For example, in patients with chronic renal failure the adipocyte insulin sensitivity is normal³³ but the insulin sensitivity of muscle is decreased.³⁴

Clinical studies of insulin receptors have tended to focus perforce on extrahepatic tissues. The lack of relation between the results of oral glucose tolerance tests and adipocyte insulin receptor studies has frequently been noted. This is likely to reflect the lack of concordance of insulin action in the liver and in peripheral tissues. Certainly the abnormality in glucose tolerance in hyperthyroidism is likely to result from hepatic abnormalities rather than from any changes in peripheral tissues.²³⁻²⁸⁻²⁹ Laboratory studies of human liver are ethically and practically difficult, but none the less one investigation has been reported of liver membrane insulin receptors from obese and normal patients.³⁵ The secrets of the hepatic insulin receptor—and any relevance to human disease—remain to be explored.

Cellular transmission of the insulin signal

In the 1970s almost all clinical papers on insulin binding opened with the comment that the first step in the action of peptide hormones consisted of interaction of the hormone with specific cell surface receptors. The underlying belief seemed to be that this first step was also the main regulatory step. Recognition of states of cellular insulin resistance characterised by normal insulin receptor numbers and affinity led to the concept of rate limiting steps in the intracellular pathways of insulin action.¹⁸ Conversely, in

hyperthyroidism the numbers of adipocyte insulin receptors are reduced while their sensitivity to stimulation of lipogenesis remains normal.²³

The linkage between the insulin receptor on the cell membrane and the regulatory enzymes concerned in the metabolic pathways has been the subject of recent intensive research. Intracellular second messengers for insulin have been postulated, including cyclic nucleotides,³⁶ free calcium,³⁷ and a peptide,³⁸ but no such simple mechanism seems likely to account for the known kinetics and stoichiometry of cellular insulin action. If only one molecule of a mediator was generated in the cytoplasm for each occupied insulin receptor then the concentration of the mediator in the cytoplasm would be less than 1 nmol/l; whereas in practice target enzymes (for example, cyclic adenosine monophosphate dependent protein kinase and pyruvate dehydrogenase phosphatase) are likely to be present at concentrations above 100 nmol/l.³⁹ Possibly an enzymatic cascade might provide the necessary amplification.

The insulin receptor consists of two α subunits sited on the outer surface of the cell membrane and two β subunits, each of which span the cell membrane.⁴⁰ Both subunits of the receptor are glycoproteins with complex side chains.⁴¹ The α subunits carry the binding sites for insulin, and the β subunits are probably concerned with the transmission of signals across the cell membrane. The interaction of insulin with the α subunit brings about phosphorylation of the β subunit.^{42 43}

This phosphorylation of the β subunit involves at least two separate processes. In crude or purified insulin receptor preparations insulin stimulates phosphorylation on tyrosine residues of the subunit,⁴⁴ indicating that tyrosine phosphorylation is an effect of a kinase activity intrinsic to the receptor itself. In intact cells, however, insulin stimulation causes phosphorylation of predominantly serine residues of the β subunit.⁴⁵ The serine kinase is likely to be a separate entity from the insulin receptor—but closely associated with it in the cell membrane and perhaps acting as a receptor regulator.⁴⁶

How kinase activity plays its part in transmitting the insulin signal is not definitely established, but several factors suggest that it may be the initial event in intracellular insulin action. Firstly, kinase activity is stimulated directly by binding of insulin to the receptor. Secondly, the kinetics of receptor phosphorylation/dephosphorylation are consonant with the time course of insulin action.^{47 48} Thirdly, tyrosine kinase activity is rare in cells, being restricted to those proteins that are important in the regulation of metabolism or cell growth. Finally, altered kinase activity has been shown in several insulin resistant states.⁴⁹⁻⁵¹

The precise way in which tyrosine kinase activation relays the insulin signal is also uncertain. The most obvious possibility is that the receptor kinase phosphorylates one or more proteins with kinase or phosphatase activity, thus initiating a cascade of phosphorylation/dephosphorylation reactions. The immediate substrate(s) for the receptor kinase is uncertain, but among several likely candidates a distinct

intracellular protein of molecular weight around 110 000 daltons has recently been described.^{52 53}

Possibly the transmission of the insulin signal may follow a branching pathway, allowing separate controlling influences to act on different limbs and hence to regulate separately the several different cellular actions of insulin. A single cascade of phosphorylation/dephosphorylation steps—or, indeed, a single mediator of insulin action—is probably insufficient to account for the variety of insulin effects. The current confusion of reports on insulin mediators with different biological and physical characteristics may be partly related to diversity in signal transmission. Finally, some actions of insulin may possibly not be mediated by the β subunit kinase. The effects of insulin on the cell membrane, such as the changes in sodium and potassium flux, might fall into this category, and glucose transport may be stimulated without receptor phosphorylation in certain limited conditions.⁵⁴ Insulin stimulation of glucose transport is not, however, mediated by the cell membrane alone but depends on translocation of glucose transporters from an intracellular pool.⁵⁵

The precise biochemical events surrounding insulin receptor function are slowly becoming clearer, and simultaneously the molecular details of the activation of the key insulin regulated enzymes are emerging (P Cohen, data presented to the Biochemical Society, 1985).⁵⁶ Links should soon be forged between these separate but converging areas of research.

Conclusions

The explosion of research into insulin receptors and their function continues unabated. The relevance of these studies to the pathophysiology of disease will need to be explored with caution in future. The old, outdated concept of blood cells as indicators of the insulin receptor state of "homogenised man" continues to be quoted as the justification for studies; established concepts die hard. The belief that the insulin receptor is the main regulatory step in cellular insulin action has now been replaced by an understanding of some of its functions in the context of a multistep intracellular pathway of hormone action. The early expectation that insulin receptor binding would prove to be the key to understanding common clinical states of insulin resistance has not been realised. The next few years are likely to provide exciting advances in the elucidation of insulin action within the cell, and it remains to be seen whether this will lead to more appropriate treatment for non-insulin-dependent diabetes, obesity, and other insulin resistant states.

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