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Hormone Receptors and Receptor Diseases

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HORMONE RECEPTORS AND RECEPTOR DISEASES

Proceedings of the International Symposium on Hormone Receptors and Receptor Diseases, Kyoto, August 29 and 30, 1982

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PREFACE

Many important advances have been made in the past several years in the study of hormone receptors. Although the primary structure of hormone receptors has not been elucidated yet, recent studies with a variety of sophisticated techniques are unveiling the structure of hormone receptors and their mechanism of action. Much progress has also been made in the understanding of post-receptor events. In particular, the mechanism of hormone action on genes is one of the most rapidly growing areas because of the advances of molecular biology and gene engineering. Progress in the basic aspects of receptor research has contributed very much to the study of several disorders due to abnormalities of hormone action. New disease entities, such as insulin receptor defect, have been established. An old disease, Graves' disease, has been studied from the viewpoint of hormone receptors, and the concept of thyroid stimulation by TSH receptor antibodies as the cause of Graves' disease has been gradually accepted. A new disease group, called receptor diseases, the etiologies of which are explained by either congenital or acquired abnormalities of hormone receptors, has been advocated by some investigators. However, there are several diseases characterized by peripheral resistance to hormones of unknown etiology and either receptor or post-receptor defect is considered as the pathogenic factor in such diseases.

This volume contains the papers presented at the International Symposium on Hormone Receptors and Receptor Diseases. The aim of this symposium was to summarize recent advances in basic and clinical aspects of studies on membrane and intracellular receptors and post-receptor information transmission systems. Obviously, there are still many unsolved problems in this field but papers in this book will give a new insight and reinforced impetus to further studies on hormone receptors. It is hoped that the multidisciplinary nature of this book will be of help to investigators and clinicians with different scientific interests for understanding recent advances in this field.

On behalf of the organizing committee, I thank all those who contributed chapters to this book for their valuable cooperation and all those who participated in the symposium. Thanks are also due to President M. Yamamoto and other members of the Japan Medical Research Foundation for sponsoring this fruitful symposium.

Hiroo Imura, M.D.
Chairman, Organizing Committee

September 24, 1982

OPENING REMARKS

Ladies and gentlemen,

On behalf of the organizing committee for the International Symposium on 'Hormone Receptors and Receptor Diseases', I would like to extend our hearty welcome to all participants from abroad and from Japan to this symposium. This symposium is held under the auspices of the Japan Medical Research Foundation. The Japan Medical Research Foundation was founded to promote medical sciences, especially basic and clinical researches on the etiology, diagnosis and treatment of various intractable diseases. The Foundation holds 2 to 3 international symposia every year and, this year, this symposium on 'Hormone Receptors and Receptor Diseases' was chosen as one of the international symposia. The reason why this topic was chosen would be the remarkable progress in the study of hormone receptors and their disorders in recent years.

The endocrine system is composed of endocrine cells that produce and secrete hormones and their target organs. Endocrinology in the past was devoted mostly to the study of endocrine cells. That is, characterization of hormones, regulation of hormone secretion and their disorders. However, for the complete understanding of the endocrine system, the mechanism of hormone action and its abnormalities in target cells must be clarified also. In the past two decades, a great progress has been made in the study of hormone receptors, the initial step of hormone action. These studies have given an insight into the pathogenesis of certain diseases and contributed to the establishment of new disease entities which are caused by the abnormalities of hormone receptors. Moreover, a new light has been shed over old, well-known diseases such as Graves' disease, to elucidate their pathogenesis from a viewpoint as disorders of hormone receptors. This symposium is focused on recent advances in basic studies of hormone receptors and clinical aspects of receptor abnormalities. We are very pleased to have nine participants from abroad who have opened a new horizon in the study of hormone receptors by their pioneering works in this field. In addition, fifteen Japanese doctors are also going to participate in this symposium with their recent new studies. I expect, therefore, that this symposium will be the best symposium in this field that has ever been held in the world. I hope that all participants will keep strictly to the time allotted for their presentation, so as to leave enough time for free and hot discussions.

I would like to finish my opening remarks expressing my heartiest gratitude to the Japan Medical Research Foundation for the sponsorship and to all speakers and chairmen for participating in this symposium.

Thank you very much.

Hiroo Imura, M.D.
Chairman of the Organizing Committee
of the Japan Medical Research Foundation
Symposium on 'Hormone Receptors and
Receptor Diseases'

Professor of Medicine
Kyoto University Faculty of Medicine

CONTENTS

I. INSULIN RECEPTOR AND ITS DISORDERS

STRUCTURE, BIOSYNTHESIS AND PHOSPHORYLATION OF THE INSULIN RECEPTOR

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Insulin initiates its action at the cellular level by binding to its surface membrane receptor (Figure 1). This receptor serves two functions: First, to recognize, that is, bind, insulin with high affinity and specificity, and secondly to transmit a signal through the membrane to start the chain of events leading to insulin action. The final biological effect of insulin is a function of the number of insulin receptors, their affinity for hormone, and the efficiency with which they transmit the signal. In addition, at the intracellular level there are a number of processes required to complete this chain. Some of these are common steps in insulin action, such as generation of a second messenger (if one indeed exists), and some are along the branched pathways leading to specific biological responses.

Over the past 10 years, a large number of laboratories have studied the insulin receptor interaction and insulin action at the cellular level. These studies have revealed the importance of this series of reactions in many physiologic and pathophysiologic states. A variety of factors have been shown to regulate these processes, and it is now possible to classify their site(s) of action (1).

In the present paper, we would like to consider three recent aspects of our work which we hope will add in our understanding of the role of insulin receptors in health and disease. These are the structure, biosynthesis, and phosphorylation of the insulin receptor.

THE STRUCTURE OF THE INSULIN RECEPTOR

The structure of the insulin receptor has been approached using a variety of techniques. We have relied most extensively on a method involving biosynthetic or surface labeling followed by immunoprecipitation as shown in Figure 2. Using this technique, two major subunits of the insulin receptor can be identified (Figure 3, left) (2-4). These have molecular weights of 135,000 and 95,000 and have been referred to as the α and β subunits, respectively (5). These subunits label biosynthetically with either (^{3}H)-sugars or (^{35}S)-methionine (2,3), and

POSSIBLE MODEL FOR INSULIN ACTION

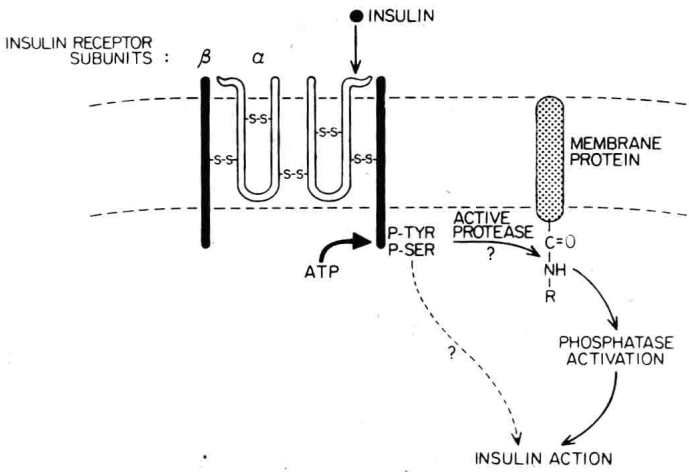

Figure 1. A possible model for the structure of the insulin receptor and the mechanism of insulin action.

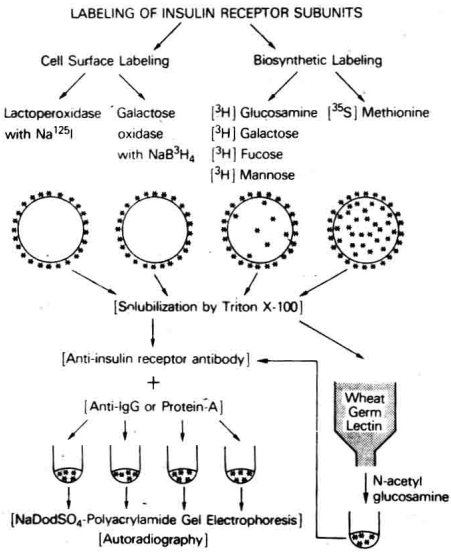

Figure 2. Method for identification of the insulin receptor subunits by cell surface or biosynthetic labeling.

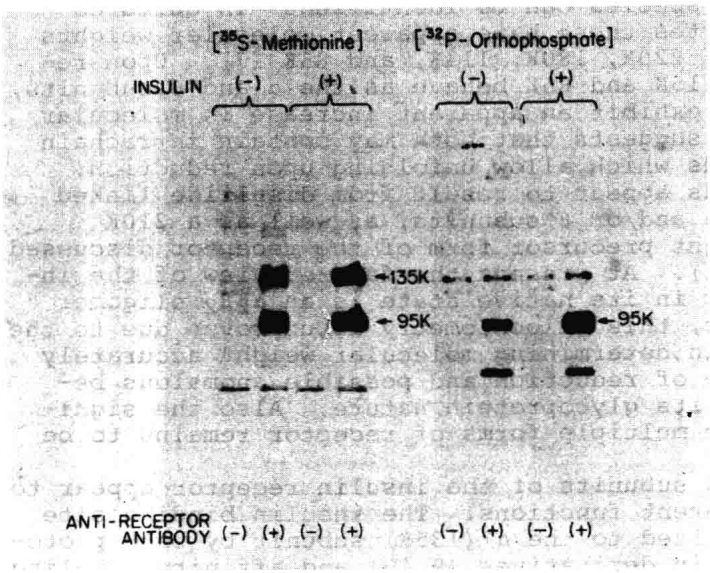

Figure 3. Autoradiogram of SDS gel of biosynthetically labeled insulin receptor in cultured hepatoma cells. (Left). The cells were labeled for 16 hr with ^{35}S-methionine and treated with 10^{-7} M insulin or buffer for 15 min prior to solubilization and isolation of receptors by sequential lectin chromatography and immunoprecipitation with control serum or serum containing anti-insulin receptor antibodies. (Right). The cells were labeled for 2 hr with ^{22}P-orthophosphate and then treated as described above. (Adapted from reference 20.)

both can be surface labeled by lactoperioxidase catalyzed iodination (6) or reduction with tritiated sodium borohydride (3), indicating that both are glycoprotein and both are expressed on the external surface of the cell.

Using modifications of these techniques more detailed structure can also be determined. For example, NaB^3H_4 will label rather specifically sialic acid residues, if glycoproteins are first oxidized with periodate. Using this approach, we find that only the β subunit of the receptor is labeled suggesting it contains more sialic acid (3). This is also consistent with its more acidic isoelectric point (7). By contrast, labeling of the subunit is more intense if NaB^3H_4 treatment follows galactose oxidase treatment, a procedure which preferentially labels terminal galactose residues (3). Tryptic peptide mapping has shown that the two subunits are distinct (7),

and that the β subunit is not simply a proteolytic degradation fragment of the α subunit.

In non-reduced gels of the labeled receptor at least six different species can be identified. In cultured human lymphocytes these have apparent molecular weights of 520K, 350K, 220K, 180K, 115K, and 85K (7). Upon reduction, the 115K and 85K behave as the α and β subunits, that is, they exhibit an apparent increase in molecular weight. This suggests that both may contain intrachain disulfide bonds which allow unfolding upon reduction. The other bands appear to result from disulfide linked oligomers of α and/or β subunits, as well as a 210K molecular weight precursor form of the receptor discussed below (Table 1). At present the favored view of the insulin receptor in its native state is an $\alpha_2\beta_2$ oligomer (5,8), however, this stiochiometry is unproven due to the difficulties in determining molecular weight accurately in the absence of reduction and possible anomalous behavior due to its glycoprotein nature. Also the significance of the multiple forms of receptor remains to be determined.

These two subunits of the insulin receptor appear to subserve different functions. The insulin binding site has been localized to the α (135K) subunit by both photoaffinity insulin derivatives (9,10) and affinity labeling with bifunctional chemical cross-linking agents (5,11). These agents also label lightly the β subunit, but with less than 10% of the efficiency of the α subunit. On non-reduced SDS gels, the predominant species of affinity labeled receptor has an apparent molecular weight of 350K, although some labeling of other species occurs.

The β subunit of the insulin receptor, on the other hand, is the more likely candidate to be the effector portion of the molecule. This subunit spans the membrane and can be labeled by cell surface techniques on intact cells, as well as in inverted membrane vessicles (12). The α subunit does not label when inverted membrane vessicles are iodinated, and thus far, there is no evidence that this subunit is exposed on the inner surface of the cell membrane. The β subunit also undergoes autophosphorylation (see below), a reaction which is believed to be important in the mechanism of insulin action.

BIOSYNTHESIS AND TURNOVER OF THE INSULIN RECEPTOR

Estimates of the turnover rate of the insulin receptor have been made using a variety of techniques. Loss of insulin binding in cells treated with inhibitors of protein synthesis or glycosylation suggested that the turnover rate of the receptor was of the order of 12-24 hours (13,14). Using heavy isotope labeling, Lane and co-workers (15) found similar rates in 3T3-L1 cells. All of these methods, however, depend on measurement of insu-

TABLE 1. The structure of the insulin receptor in human lymphocytes

Unreduced Form	Bands After Reduction*	Tentative Identification
520K	210K>95K>135K	Partially cleaved pro-receptor and $\alpha_x\beta_y$ oligomers
350K	135K, 95K	$\alpha_x\beta_y$ Oligomer
220K	135K	α-α Dimer
180K	135K, 95K	α-β Heterodimer
115K	135K	Free α subunit
85K	95K	Free β subunit

*The apparent change in molecular weight and lack of perfect additivity may be due to differences in subunit mobility after reduction and differences in apparent molecular weight in 7.5% gels (after reduction) versus 5% gels (prior to reduction).

lin binding activity. We have developed a technique to study insulin receptor turnover taking advantage of biosynthetic labeling and immunoprecipitation. Using this technique, we have studied both receptor synthesis (16) and degradation (4).

The biosynthesis of the insulin receptor is complex. With ^{3}H-mannose labeling the earliest identifiable precursor which is recognized by the anti-receptor has a molecular weight of 190,000 (16). This precursor appears within 15 minutes and contains core glycosylation, i.e., mannose and N-acetylglucosamine. With continued incubation the radioactivity gradually is found in a glycoprotein of 210K. This appears to be a fully glycosylated precursor, since it also labels with ^{3}H-fucose and ^{3}H-galactose. Almost coincident with the appearance of the 210K protein, the α and β subunits are found, and both the 210K protein and individual subunits reach a maximum 6-8 hours after a pulse-chase labeling. On peptide mapping the 210K protein contains peptides of both the α and β subunits (7,16). Thus, the 210K protein appears to be a pro-receptor containing both the α and β subunits. Exactly where it is cleaved to produce the final receptor is uncertain, but some 210K protein is detected on surface labeling, suggesting that cleavage may not occur until the pro-receptor reaches the plasma membrane (Figure 4).

The degradation of the pre-labeled receptor can be followed using the same pulse-chase techniques. In cultured human lymphocytes, the half-time for degradation of both the α and β subunits of the insulin receptor is 10-11 hours under normal growth conditions (4). This is quite consistent with the data obtained by other

methods. The half-life of the 210K pro-receptor is somewhat shorter ($t_{1/2}$ = 7-8 hr).

Down-regulation of insulin receptors can be produced by culturing the lymphocytes with high concentration of insulin (17). The mechanism of down-regulation can be studied using these techniques. With 10^{-6}M insulin, there is a 3-fold increase in the degradation rate of the α and β subunits of the receptor and a decrease in the half-time to about 3 hours (Table 2). There is little change in the $t_{1/2}$ of the pro-receptor and no apparent change in the overall synthesis rate. This increase in degradation rate results in about a 75% reduction in receptor number in these cells. The exact mechanism of this increased degradation in uncertain, however, down-regulation is blocked by inhibitors of protein synthesis (17). Some of the lost receptors may also be shed into the external mileau.

TABLE 2. Turnover of insulin receptors on cultured lymphocytes

	Normal Growth Conditions	Down-Regulation
Receptor Half-life, $T_{1/2}$	~ 10 hrs	~ 3 hrs
Degradation Rate, K_D	0.065 hr^{-1}	0.231 hr^{-1}
Synthesis Rate, K_S	1300 hr^{-1}	1300 hr^{-1}
Receptor Concentration, R	20,000/cell	5600/cell

INSULIN RECEPTOR PHOSPHORYLATION

Considerable evidence has been accumulated to show that phosphorylation and dephosphorylation of proteins may play an important role in insulin action. Phosphorylation has also been shown to occur for some receptors, such as the EGF receptor (18), and this leads us to investigate the possibility that the insulin receptor might undergo phosphorylation.

Using both cultured human lymphocytes and rat hepatoma cells, the intracellular ATP was first labeled by incubation of the cells in ^{32}P-orthophosphate. The insulin receptor was then isolated from the cells by sequential solubilization, lectin chromatography, immunoprecipitation, and gel electrophoresis. In both cell types, in the basal state, ^{32}P was incorporated into the β subunit of the receptor, indicating receptor phosphorylation (19). Insulin treatment of the cells for 15 minutes resulted in a 5 to 10-fold increase in labeling of the receptor (Figure 3, right), with no demonstrable change in the phosphate labeling of other membrane glycoproteins. This insulin effect began within 1 minute and was maximal by 30 minutes. Dose-response curves revealed a ED_{50} for insulin of 5 x 10^{-9}M, and the analogue specificity for this effect was identical to that for insulin binding to its receptor (Figure 5). In the intact cell these appear to be multiple sites of phosphorylation. In the basal state there is predominantly phosphoserine with a trace of

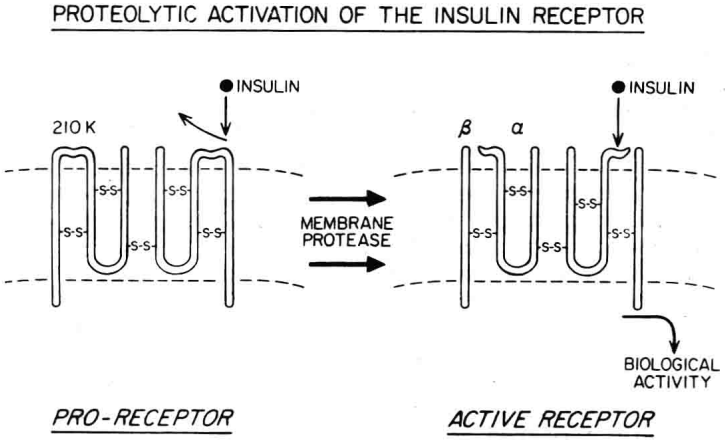

Figure 4. Model for insulin receptor precursor and its cleavage to yield the insulin receptor subunits. This cleavage is portrayed as a membrane event, but it may well occur before the receptor is inserted into the plasma membrane. It is also not yet known if this results in an activation of the receptor, or the pro-receptor is also active.

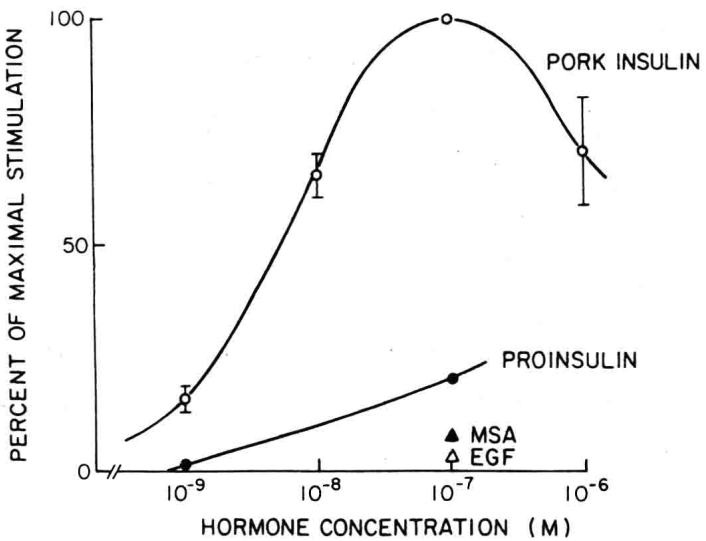

Figure 5. Dose response curves for insulin receptor phosphorylation in hepatoma cells. MSA refers to the insulin-like growth factor, multiplication stimulating activity. EGF is epidermal growth factor.