# hormone-receptor interaction: molecular aspects

edited by GERALD S. LEVEY

# HORMONE - RECEPTOR INTERACTION

## MOLECULAR ASPECTS

Edited by

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## PREFACE

In recent years there has been an exponential increase in our fundamental knowledge of hormone receptors. Traditionally a "receptor" has been difficult to define in pharmacologic terms. In the context of the various hormones detailed in this volume a receptor can be defined as a chemical structure which provides highly specific binding sites for hormones in their target tissues and which, as a consequence of this binding, have the ability to initiate the subsequent biochemical reactions necessary to produce the end physiologic response to the hormone. In general, this volume is concerned with three broad classes of hormones: polypeptides, steroids, and tyrosine derivatives. The polypeptide hormones as typified by glucagon, insulin, growth hormone, and small peptides such as the pituitary releasing factors, have receptors located at the superficial cell surface on the plasma membrane. Conversely, steroid hormones such as estrogens, progesterone, and cortisol have receptors in the interior of the cell located in the cytoplasm or nucleus. Tyrosine derivatives present a mixed pattern with catecholamine receptors residing on the plasma membrane and thyroid hormone receptors located in the nucleus. Two other hormones acetylcholine and the fatty acid derivatives, prostaglandins, also appear to interact at a superficial cell site. When critically considering these various receptors, regardless of their cellular location, some general principles pertain as defined by Cuatrecasas (P. Cuatrecasas, Adv. Cyclic Nucl. Res. 5:79 (1975)). One, the hormone interaction with the receptor should conform to known steric and structural specificity. Two, the binding sites should be finite in number and therefore, saturable. Three, hormone binding should have tissue specificity consistent with biological specificity. Four, hormone binding should be of high affinity and consistent with its physiologic concentrations. Five, the binding of hormone to its receptor should be reversible. The guidelines serve to provide a basis by which we can intelligently consider the physiologic implications and eventual applicability of these hormone-receptor interactions.

The chapters presented in this volume represent a broad spectrum of current research in the area of hormone receptors by many of the leading investigators in this field and provide a current "State of the Art." It is hoped that this material will furnish the basic framework upon which the reader can build for his own future reading and understanding of this rapidly advancing and fundamentally important area of molecular biology. If this is accomplished the efforts of the many contributors to this volume would have been successful.

*Gerald S. Levey*
*Miami, Florida*

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## CONTENTS

# 1

# INSULIN RECEPTORS IN DISEASE STATES

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## I. INTRODUCTION

With the discovery of methods to measure accurately plasma insulin concentration [1], it soon became apparent that in a variety of conditions the circulating insulin level did not always accurately reflect the physiologic state of the animal.

Thus, in some states, such as obesity, acromegaly, and Cushing's syndrome, circulating insulin levels were elevated, despite normal or elevated blood glucose levels, suggesting peripheral resistance to insulin action. Further, when these animals were given exogenous insulin, blood glucose fell less quickly and less dramatically than in normal animals. Conversely, states such as hypophysectomy and adrenalectomy appeared to be associated with increased insulin sensitivity with low circulating insulin levels and a brisk response to exogenous insulin. Although these states of insulin "resistance" and "sensitivity" were well described, the pathophysiology of most of these disorders was poorly understood. The ability to study directly and quantitatively the interaction of insulin with its membrane receptor has added a new dimension to our understanding of these disorders. These studies have demonstrated that the receptor is not a static structure, but rather may be altered by metabolic, hormonal, and perhaps other factors. The purpose of this chapter will be to review the nature of the insulin receptor and the insulin-receptor interaction in various disease states. A brief summary of the basic features of the insulin receptor will provide the necessary framework on which to place these findings.

## II. HISTORICAL ASPECTS

Over the past 25 years, evidence has come from a variety of direct and indirect sources that the first step in the action of insulin, as well as other polypeptide hormones, is binding to specific receptor sites on the plasma membrane of the cell. As early as 1949, Levine and his coworkers [2] suggested that the primary step in insulin action was at the membrane level, since insulin stimulated the transport of sugar across the plasma membrane. In the same year Stadie et al. [3] found that rat hemidiaphragms, incubated briefly with insulin and washed, showed a persistent effect of insulin on glycogen synthesis. This persistent hormone effect could have been due to either persistence of the hormone in or on the tissue, or initiation of secondary reactions whose subsequent course was independent of the presence of hormone. It was not until 1966 that Pastan et al. [4] showed that the persistent insulin effect was due to a persistence of hormone on the cell surface, since it was possible to reverse this effect by washing tissue with a solution containing anti-insulin antibodies.

A similar conclusion was reached by Kono [5] who showed that trypsin treatment of intact fat cells resulted in a loss of the cell's ability to respond to insulin without loss of cellular integrity, without altering the glucose transport system itself, and without impairing cellular response to other hormones. This suggested that trypsin destroyed a peptide component on the cell surface, presumably the receptor, which was necessary for insulin action.

A third indirect approach to this question was introduced by Schimmer et al. [6] for ACTH and by Cuatrecasas [7] for insulin. In these studies, the hormone was covalently linked to a large inert polymer such as cellulose or Sepharose and found to be biologically active, suggesting again that the hormone need not enter the cell to be active. Recent studies of insulin-Sepharose [8], however, have demonstrated that the hormone may leak or be released from its complex during incubation, and therefore the interpretation and validity of the original experiments have been questioned [9,10].

Direct studies of the interaction of insulin with its receptor were attempted as early as 1952 [11]. These studies were hindered, however, because of the small amount of insulin bound [12], the question of the biological activity of the labeled insulin [13], and especially, the uncertainty as to the biological significance or specificity of the binding process [14]. In 1969, two methods were introduced which allowed direct studies of the hormone-receptor interaction: (a) methods for labeling polypeptide hormones at high specific activity while preserving their biological properties were developed [15,16], and (b) the use of analogs to define specificity was begun [15,17]. These methods have now been employed for a whole variety of hormones and target tissues (for complete reviews see Refs. 18 and 19).

## III. CHARACTERIZATION OF THE INSULIN RECEPTOR

Insulin receptors have now been demonstrated in a large number of tissue preparations from a wide variety of animal species (Table 1) [20], and studied in detail in several of these [21-38]. Since the insulin receptor has not been isolated in pure form, in all studies it is necessary to define the insulin receptor by its binding characteristics. These include a high affinity for insulin, rapid and reversible binding, and saturability. The essence of the functional definition, however, is the hormonal and biological specificity of the receptor. Thus, a single type of receptor site binds only a single type of hormone and binding of the hormone to this site can be correlated with the biological activity of the hormone. In the case of the insulin receptor, this implies that there is no competition for the receptor by substances unrelated to insulin, and, more specifically, that the relative potency of a variety of hormone analogs to compete for the binding site is in direct proportion to their bioactivity (Fig. 1) [21,23,26,38-40]. This feature is in marked contrast to the specificity demonstrated by antibody binding sites, which are usually not related to bioactivity [21]. Furthermore, this appears to be an intrinsic feature of the receptor, since insulin receptors in all tissues and species thus far studied maintain the same specificity [38,41].

**TABLE 1**

**Tissues and Species in Which Insulin Receptors Have Been Demonstrated by Direct Studies[a]**

| Species | Tissues |
|---|---|
| Man | Liver |
| Monkey | Adipose |
| Rat | Skeletal muscle |
| Mouse | Myocardium |
| Guinea pig | Lung |
| Rabbit | Kidney |
| Sheep | Brain |
| Pigeon | Adrenal gland |
| Turkey | Mammary gland |
| Frog | Ovary |
| | Testis |
| | Uterus |
| | Placenta |
| | Spleen |
| | Circulating monocytes |
| | Thymic lymphocytes |
| | Erythrocytes (nucleated) |
| | Fibroblasts |

[a]Compiled from Refs. 18-20 and 38.

Recently, Cuatrecasas and Hollenberg [42] have suggested that all of these criteria are not reliable and that nonreceptor materials, such as silicates, may demonstrate saturability, specificity, high affinity and reversibility. While the appearance of the insulin-nonreceptor interaction may on superficial inspection appear to fulfill these criteria, on a reasonably careful examination there are obvious major differences. Thus, 100-fold more unlabeled insulin is required to displace [$^{125}$I]insulin from talc than from its liver plasma membrane receptor. Furthermore, this binding shows nothing in the way of biological specificity [42], with marked inhibition of binding by proinsulin and desoctapeptide insulin which are only 5% and 1% as biologically active as insulin, as well as marked inhibition by growth hormone 1-39 peptide and reduced and carboxymethylated insulin which are biologically inactive with respect to insulin-like activity.

The quantitative aspects of the insulin-receptor interaction are complex and the subject of some controversy [25]. Of the investigators who have studied this problem only Cuatrecasas and his coworkers [30,31,43] have reported a single class of high affinity ($K \sim 10^{10}\ M^{-1}$) binding sites in both liver and adipose tissue. Other investigators studying these and other tissues have noted lower affinities for the binding sites [28,29,32] and often curvilinear Scatchard

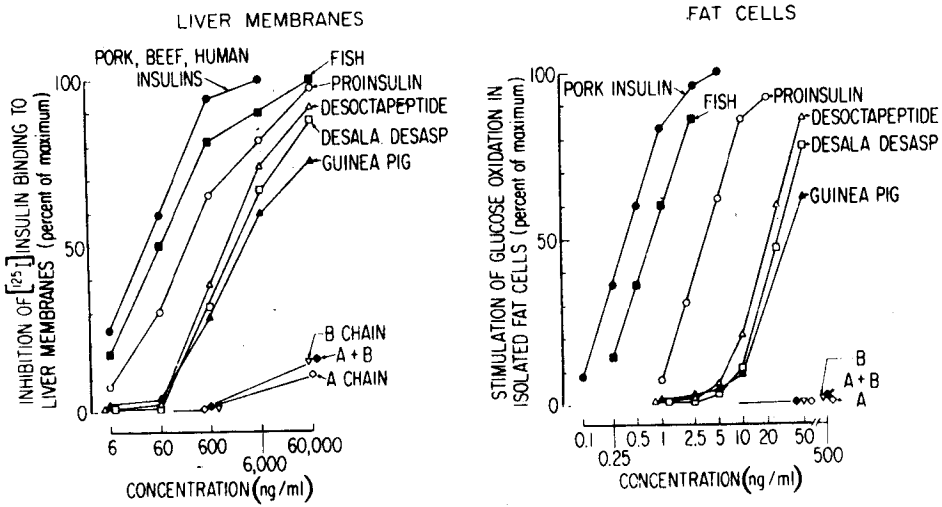


**FIG. 1** Effect of insulin and insulin analogs on [$^{125}$I]insulin binding to liver membranes and on glucose oxidation in fat cells. Left: The inhibition of [$^{125}$I]insulin (porcine) binding to liver membranes expressed as percent of maximum is plotted as a function of the concentration of unlabeled peptide. Right: The stimulation of glucose oxidation in isolated fat cells expressed as percent of maximum is plotted as a function of the concentration of unlabeled peptide. (From Freychet et al. [21]).

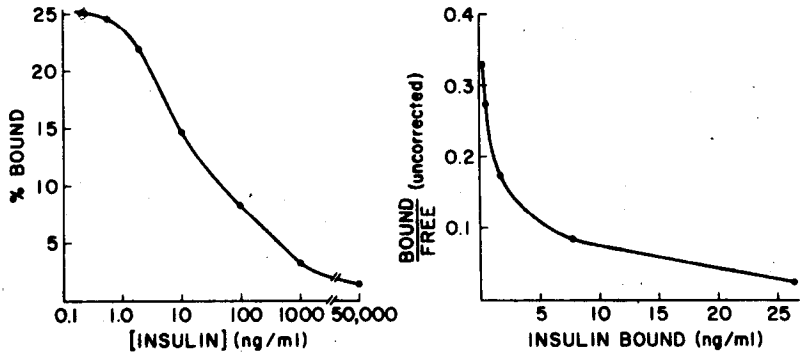


**FIG. 2** [$^{125}$I]insulin binding to liver plasma membranes. Left: Liver membranes (0.6 mg/ml) were incubated with [$^{125}$I]insulin (100 pM) at 30°C for 60 min. Percent of total [$^{125}$I]insulin bound to liver membranes as a function of total insulin concentration. Right: Scatchard plot of the data on the left. (From Kahn et al. [25].)

plots [24,25,34-36,38], suggesting heterogeneity of binding sites or negative cooperativity (Fig. 2). Recently, De Meyts et al. (44) have partially clarified this problem by demonstrating that the insulin receptor sites do not behave independently (a necessary assumption for simple bimolecular equilibrium analysis [45]); rather, the insulin receptors interact in a way referred to as negative cooperativity. This means that binding of a hormone molecule to the receptor lowers the affinity of other receptors (and perhaps for the occupied receptor itself) for hormone. Thus, the apparent affinity decreases with increasing fractional occupancy of receptors. This decreasing affinity with increasing occupancy has been experimentally demonstrated by showing that the rate of dissociation of labeled hormone from receptors is increased when other receptors are occupied by unlabeled hormone. This, like the biological specificity, appears to be a fundamental property of the insulin receptor and has now been demonstrated in insulin receptors from a number of species and a variety of tissues (38,41,44,46). In addition, this property provides a sensitive measure for occupied receptors (occupancy of as few as 5-10% of receptors causing a significant increase in dissociation rate), which is useful in studying receptor populations in disease states (see Sec. VIIA.).

## IV. HORMONE AND RECEPTOR DEGRADATION

In addition to the interaction of insulin with its receptor, two additional reactions occur which may influence a quantitative study of the insulin-receptor

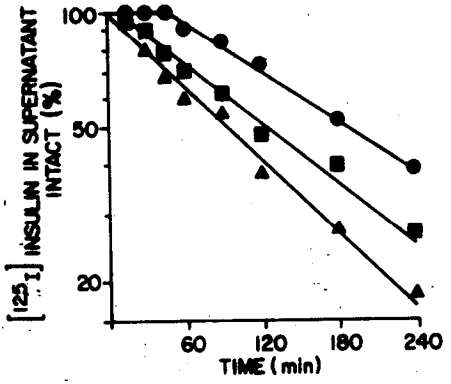


**FIG. 3** Effect of membrane concentration on time course of insulin degradation. [$^{125}$I]insulin (20 pM) was incubated with liver membranes at 30°C in 60 mM sodium. Membrane concentrations were 0.2 (●), 0.4 (■), and 0.8 (▲) mg/ml. Aliquots of supernatant of the incubation tubes were taken at the indicated time points and intact [$^{125}$I]insulin was measured by its ability to rebind to a fresh aliquot of membranes. (From Kahn et al. [25].)

interaction in disease states: hormone degradation and receptor degradation. Insulin degradation (Fig. 3) appears to be a separate process from insulin binding to receptors with major differences in pH and ionic strength optima, temperature, and specificity for insulin analogs [22,23]. In most comparative studies it is important to obtain some measure of degradation, since an increase in degradation would result in less intact insulin being available for binding to receptor and thus an apparent decrease in insulin receptors.

Receptor "degradation" is a less well studied reaction [25,36,47]. Like hormone degradation, this reaction is a function of time, temperature, ionic strength, and membrane concentration (Fig. 4). Under conditions of high ionic strength, the insulin receptor half-life may be as short as 30 min [25]. The chemical nature of the "degradation" is not yet defined, but in at least one case appears to be related to release of the receptors into the incubation medium [47].

## V. CONSIDERATIONS OF RECEPTOR PREPARATIONS FOR COMPARATIVE STUDIES

The insulin receptor interaction has been studied in a wide variety of receptor preparations, including intact cells, particulate fractions of cells, and solubilized