Insulin Binding to Its Receptor: Is the Receptor More Important Than the Hormone?

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Our understanding of the importance of target cell mechanisms has grown substantially over the last two decades with the emergence of second messengers, protein kinases and phosphatases, receptors, and other participants in the action of catecholamines, insulin, and other peptide hormones. In their pioneer work during the late 1940s and early 50s, Levine and his colleagues heralded some of the concepts of today.[1] First, they focused attention on the plasma membrane as an important site for insulin action at a time when the plasma membrane was receiving little attention. We now know that the components of the plasma membrane play many essential roles in the action of insulin and all other water-soluble hormones. Second, they speculated that the chemical nature of the hormone molecule (e.g., peptides versus steroids) might play a decisive role in its interaction with target cells at a time when the prevailing theories were either too general (i.e., all hormones were considered to have a single mechanism) or too narrow (i.e., each hormone was unique). We now know that the water-soluble hormones and the lipid-soluble hormones each have a pattern of action that is typical for all members of that group (Figure 1). The present article, which focuses on the role of cell surface receptors for insulin, rests squarely on the early work of these prophets.

INSULIN RECEPTORS

Insulin is an intercellular messenger that carries a signal from the secretory cells in the pancreatic islets to target cells throughout the body, especially liver, muscle, and fat. For the system to work, a complex series of biochemical events are needed, especially in the secretory cells and the target cells. A failure in the system may occur at any step, yet traditionally we have always placed blame on the hormone—if blood glucose was too high, it was because there was a deficiency of insulin, and if blood glucose was too low, it was because insulin was excessive. This paradigm prevailed as long as we measured plasma glucose but not insulin.

Measurements of insulin in pancreas and plasma[2–4] showed that only a minority (10–20%) of diabetic patients are insulin deficient, i.e., have type I diabetes, who are absolutely insulin requiring. Most patients with diabetes are not absolutely insulin requiring (i.e., have type II diabetes), and many have normal or supernormal amounts of biological intact insulin being delivered from their β-cells to their target cells (Table 1), but often the responses of the target cells are reduced (Table 2).

To examine defects in the target cell, we turned our attention to the first step, the binding of hormone to its specific receptors. Although hormones and receptors were both well conceptualized by the first decade of this century, progress with the hormones proceeded much more rapidly for many reasons but especially because the hormones were more accessible. The natural advantages of the hormone over the receptor (from the point of view of investigation) are presented in outline in Table 3.

Early workers in the field generally thought that all hormones (and other messenger molecules) entered cells freely where they bound directly to the enzymes that carried out the biochemical steps typical for that hormone.[5] In general, each hormone in its action at the target cell was considered to be unique. Occasional scholars tried to generalize but they tended to include all hormones. We now know that the lipid-soluble hormones [steroids; 1,25$(OH)_2$ vitamin D; and the iodothyronines] do enter the cell easily, interact with receptors inside the cell, and have the nucleus as a major obligatory site of action. In our early studies,[6] we showed that peptide hormones (e.g. TSH, insulin, ACTH), which represent 80% or more of all hormones, have their receptors on the outer surface of the plasma membrane of the target cell, and the first step is a rapid equilibration between hormone in the medium and a fixed number of sites on the cell surface. (In those days the cell surface of vertebrate cells was a largely underdeveloped, sparsely populated neighborhood.) Some of the important conclusions of these studies,[6] heralded by earlier studies of Dr. Levine, are as follows: (1) peptide hormones have receptors on the cell surface, (2) peptide hormones as a group differ from the steroid hormones, and (3)

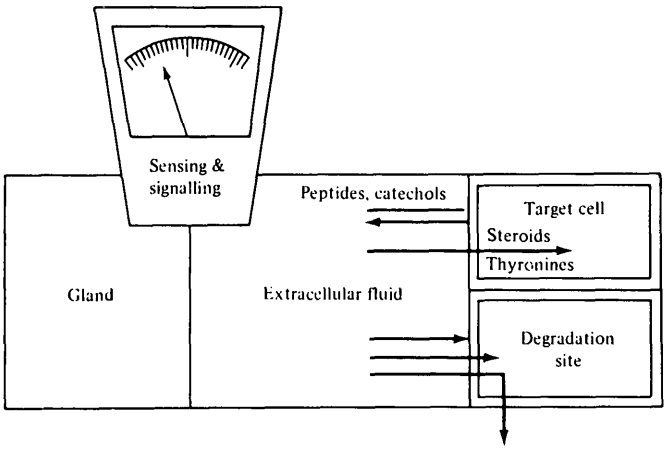

FIG. 1. Two classes of hormones at the target cell. The lipid-soluble hormones, including 1,25(OH)$_2$ vitamin D, readily traverse the plasma membrane of the cell, have their receptors intracellularly, and the nucleus is their major site of action. The vast majority of the hormones (>80%) are water soluble, including insulin, other peptide hormones, and the catecholamines. Their primary interaction with the target cell is reversible binding to a limited number of receptors on the cell surface.

information from one hormone within the group can be extrapolated to others within the group.[1,6]

The receptor provides two essential functions: (1) recognition of the active hormone from among all other substances (often the hormone represents one in 10^5–10^9 of the molecules to which the cells is exposed); recognition is manifest by binding of hormone to its receptor; (2) the combination of hormone with receptor must initiate a series of events at the target cell that results ultimately in a biologically relevant action (e.g., for insulin, glucose storage or utilization).

We set out to provide a specific quantitative method by which that first step, binding of hormone to receptors on the cell surface, could be measured directly. In 1969, a new method was introduced by which ACTH and angiotensin binding to their specific receptors could be measured directly;[7–10] this approach has been applied widely to measurements of many other hormones as well as numerous other messenger ligands that have their receptors on the cell surface. Interestingly, the cell surface now appears to be a common, if not favorite, site for receptors.

Direct measurements of hormone binding to receptors, especially studies with the insulin receptor, showed that (1) receptors are highly regulated in vivo, responding to signals from inside and outside the cell[11,12] (Table 4) and (2) receptors are frequently involved in disease states[13–15] (Table 5).

TABLE 1
Characteristics of type I and type II diabetes

	Fraction of total	Endogenous plasma insulin (typically)
Type I	20%	Deficient
Type II	80%	Normal or elevated

TABLE 2
Definition of target cell defect

1. Blood glucose elevated
2. Plasma insulin by RIA is normal or elevated
3. Biological properties of the circulating insulin are normal
4. Responsiveness to exogenous insulin is subnormal
5. No defect in the delivery of insulin to the target cell (i.e., circulating anti-insulin antibodies are low or absent)

While initially we worked hard to establish the idea that receptors are as important as the hormone, I shall raise the possibility here that the receptor may, in fact, be more important than the hormone.

INFORMATION TRANSFER (TABLE 6)

For a messenger molecule (ligand) to be active, it must be recognized by a component of the cell (receptor) and the combination of the two must activate biochemical processes in the cell that lead to the important biological event. Typically, in vivo, both ligand and receptor are needed. However, it is worthwhile (for therapeutic and other purposes) to consider whether the capacity for cell activation is actually within the ligand, within the receptor, or whether both contribute directly. In the case of insulin (as well as TSH and possibly all peptide hormones) the receptor appears to have the program for activation; the hormone, in binding to receptor, acts to get the receptor to express its program. This conclusion is based on observations that antibodies directed against receptors can bind to receptor[16] and generate the full program of metabolic responses characteristic of insulin action,[17,18] including events at the membrane (transport), early intracellular events (activation and inactivation of enzymes), and delayed effects (enzyme biosynthesis). That the receptor in the absence of hormone has the program for cell activation adds a wide range of therapeutic possibilities in addition to the major traditional approach of trying to deliver insulin in better ways.

TABLE 3
Comparison of hormones and cell surface receptors

	Hormone	Cell surface receptor
Highly concentrated in a localized site Extirpation Purification	Yes	No
Soluble in simple solvents	Yes	No
Simple structure	Yes	No
Bioeffect when introduced in vivo or in vitro	Yes	No
Present in blood	Yes	No
Name of its own	Yes	No

TABLE 4
Biologically relevant regulators of the insulin receptor

1. Insulin	6. Exercise	9. Cell program
2. Other hormones	7. Diet	Differentiation; maturation
3. pH; other ions	Calories,	Growth; cell cycle
4. Ketone bodies	composition	Tumor; transformation
5. Drugs	Fiber	Viral infection
	8. Eating	

Receptor affinity and concentration are both affected by insulin (homologous effect). The two best-studied hormones that affect insulin sensitivity are growth hormone, which largely affects receptor concentration, and glucocorticoids, which largely affect receptor affinity, at least in experimental animals. The insulin receptor is very sensitive to pH, even within the range observed in vivo, and to a lesser extent to other common ions. Ketone bodies, especially β-OH-butyrate, have effects under some conditions. Both the sulfonylureas and biguanides can increase receptor concentration. (This is not to be construed as a recommendation for their use in patients.) Exercise increases insulin binding to receptors. Insulin binding is very sensitive to diet with high calories, high carbohydrates, and high fat-reducing receptor concentration. Dietary fiber, both soluble and insoluble, increases insulin binding to receptor. Eating causes a shift in the insulin binding curve. There may also be diurnal changes in insulin binding independent of eating and exercise. Any major change in cell program can also alter insulin binding, typically by altering receptor concentration.

EVOLUTION

Insulin is a highly conserved protein but differences do exist among insulins of different species, and the biopotencies differ up to 100-fold (e.g., chicken versus guinea pig insulin) or even 1000-fold (chicken insulin versus some of the insulin-like growth factors or somatomedins). On the other hand, the receptor for insulin among all vertebrates appears to be much more highly conserved.[19,20] Thus insulin receptors in all vertebrates studied have about the same affinity for insulin, and all insulin receptors including those in the guinea pig prefer chicken insulin > pork = beef = human > fish > guinea pig > insulin-like growth factors (Figure 2). (In fact, the guinea pig compensates for its low affinity insulin by having unusually high concentrations of both circulating insulin and cell surface receptors.) If we assume that more important functions are more rigidly constrained in evolution, then the receptor may be considered more important than the hormone.

TABLE 5
Diseases associated with cell surface receptors

	Year	Receptor
Diabetes	1972	Insulin
Graves' disease	1973	TSH
Myasthenia gravis	1973	Acetylcholine
Familial hypercholesterolemia	1974	LDL
Malaria	1978	Duffy blood group on RBC
Allergic disorders	1980	Catecholamine

TABLE 6
Information transfer

I. Hormone + receptor ⇆ hormone-receptor complex → activation of cell processes
II. Where is the message? H? R? or HR?
III. Classes
  A. Ligand has the message
  Receptor acts only to concentrate, process, and/or translocate the ligand to intracellular site. Experimentally, an element of ligand (in the absence of receptor) can activate the relevant biological event.
    1. Toxins, e.g., cholera, diphtheria (enzyme)
    2. Low density lipoproteins (cholesterol)
    3. Viruses (nucleic acid)
  B. Receptor has the message
  Receptor has the full program for activation of the cell and, experimentally, receptor in the absence of specific natural ligand can produce full effect. Ligands' only function is to get the receptor to express its program.
    1. Polypeptide hormones (insulin, TSH)
    2. Acetylcholine (nicotinic) receptors
    3. IgE receptors
  C. Receptor and ligand
  Both receptor and ligand together contribute information to cell activation.
    1. Steroid hormones (progesterone)
    2. Egg and sperm

BASAL ACTIVITY?

A hormone can probably do nothing biologically in the absence of receptor. Does the receptor have basal activity in the absence of hormone? Hormones generally act to increase or accelerate processes that are already present in the target cell. Is the receptor, in the absence of hormone, helping to generate some of this basal activity? Along the same line, does the receptor evolutionarily or ontogenetically antedate the hormone?

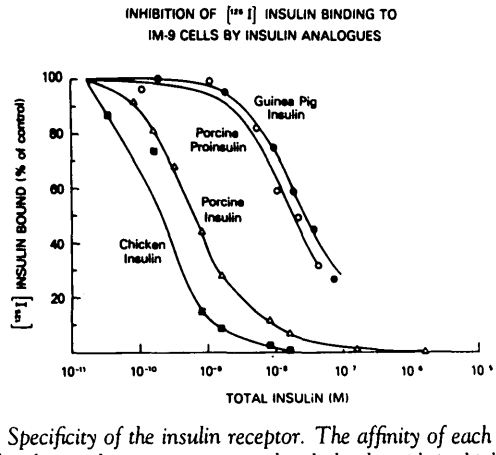

FIG. 2. Specificity of the insulin receptor. The affinity of each species of insulin for the insulin receptor is correlated closely with its biological potency. The insulin receptor is the same on all tissues and in all vertebrate species studied thus far, and is independent of the insulin present in that species (see text).

REGULATION

The strength of the signal to the cell depends not simply on hormone concentration but on how much hormone-receptor complex is present, which depends about equally on hormone concentration, receptor concentration, and the affinity of receptor for hormone. In the regulation of hormone action in vivo the only variable for the hormone is a change in its concentration (affinity of hormone varies between species but not within a single organism). On the other hand, both the concentration of receptor and its affinity for hormone are regulated, and in the regulation of receptor affinity, both the association and dissociation rates can be regulated separately. Thus the receptor, which is a much larger and more complex molecule than the complementary hormone, is capable of a much wider range of regulatory events.

DISEASES

In a large fraction of disorders of glucose metabolism and insulin sensitivity, the target cell dominates (the hormone levels are in a direction opposite to the clinical state)[21,22] (Table 7). In many of these conditions, changes in the target cell receptor appear to play an important role. For example, with moderate insulin resistance (as occurs in thin patients with type II diabetes, obese patients with and without hyperglycemia, or acromegaly) receptor binding of hormone is reduced in concordance with the clinical state;[21–25] this concordance is observed not only for patients as a group but even more strikingly with individual patients. Furthermore, when treatment is effective, the insulin resistance, the hyperinsulinemia, and defects in insulin binding are alleviated.

EXTREME INSULIN RESISTANCE

There are two major categories of patients who have markedly elevated concentrations of biologically active insulin being delivered to the target cells, but the sensitivity of their target cells to stimulation is markedly reduced.[25,26] One category of such patients has antireceptor (auto) antibodies, which act acutely to reduce insulin binding and chronically to reduce the sensitivity of the target cell to insulin at sites beyond binding to receptor. In the second category of patients with extreme insulin resistance at the target cell, antireceptor antibodies are absent and the patients have a defect either at the level of receptor binding of hormone or at some early step beyond hormone binding.[27–32] There are three major groups in this second category of patients with extreme insulin resistance at the target cell; within each group, those with and those without binding defects are phenotypically indistinguishable. In some of these patients, the receptor defect has been detected in fibroblasts or β-lymphocyte cell lines cultured from these patients. In all of the patients with receptor defects, the severity of the receptor defect correlates with severity of the insulin resistance and the response to treatment.

TABLE 7
Involvement of insulin receptors in disorders of glucose tolerance and insulin sensitivity

- I. Target cell dominates (i.e., plasma hormone concentration discordant with clinical state)
  - A. Insulin resistance
    - 1. Moderate resistance
      - a. Clinical
        - (1) Obesity
        - (2) Type II (insulin-independent) diabetes (obese and thin)
        - (3) Acromegaly
      - b. Experimental animals
        - (1) Glucocorticoid excess
        - (2) Growth hormone excess
        - (3) Uremia
    - 2. Extreme resistance
      - a. Immunological (i.e., anti-receptor antibodies)
        - (1) Type B extreme resistance
        - (2) Ataxia telangiectasia
        - (3) IgA or IgE deficiency
        - (4) NZO mouse
      - b. No autoimmunity (? role of genetics)
        - (1) Type A
        - (2) Leprechaunism
        - (3) Lipoatrophic diabetes
  - B. Insulin supersensitivity
    - 1. Anorexia nervosa
    - 2. Glucocorticoid deficiency (in experimental animals)
    - 3. Growth hormone deficiency
- II. Hormone dominates (i.e., plasma hormone concentration concordant with clinical state)
  - A. Insulin deficiency
    - 1. Clinical
      - a. Type I (insulin-dependent) diabetes
      - b. Pancreatic diabetes (e.g., chronic pancreatitis)
    - 2. Experimental animals
      - a. Streptozotocin-induced hypoinsulinemia
      - b. Hypoinsulinemic diabetic Chinese hamster
  - B. Insulin excess
    - 1. Insulinoma
    - 2. Infants of diabetic mothers
    - 3. Other hypoglycemias in the newborn
    - 4. Chronic insulin excess in experimental animals
  - C. Disorders of receptor design (i.e., specificity spillover)
    - 1. Infants of diabetic mothers
    - 2. Non-islet cell tumors with hypoglycemia

HEIGHTENED SENSITIVITY OF INSULIN

In conditions characterized by heightened sensitivity to insulin (e.g., anorexia nervosa), the hormone levels are opposite to the clinical state (both plasma insulin and blood glucose are low normal or subnormal) while receptor binding is concordant (elevated); appropriate treatment restores insulin binding and insulin sensitivity to normal.[33]

DISORDERS OF HORMONE EXCESS

Even in conditions in which hormone secretion represents the major problem, receptors may play a key role. For exam-

ple, in type I diabetes, insulin deficiency dominates but ketone bodies and acidosis may have important effects on the target cell including effects on the receptor. Acidosis acutely lowers the affinity of receptor for hormone and chronically may act to reduce receptor concentration. Likewise, in disorders dominated clinically by insulin excess such as patients with insulinomas or some neonates with hypoglycemia (e.g., infants of diabetic mothers, or those with nesidioblastosis, or Beckwith's syndrome), changes in the receptor often play a major role in how well or how badly the hyperinsulinemia is tolerated.

DISEASES OF RECEPTOR DESIGN[5,21,22]

An individual hormone, in addition to binding to its own receptor, may also bind to receptors of a closely related hormone. When present in excess, the hormone may produce excessive effects through the pathway of its own receptors as well as stimulate effects through the receptors of the related hormone. For example, in infants of diabetic mothers there is hypersecretion of insulin, stimulated by excess substrates from the mother. We speculate that insulin, through its own receptor, produces the excess deposition of both fat and glycogen as well as postnatal hypoglycemia. At the same time, insulin, through the receptors for the insulin-like growth factors, produces excess skeletal growth and macrosomia. We have designated this degeneracy in biological specificity as specificity spillover. Other possible examples are listed in Table 8.

Another example of specificity spillover within the insulin system may be found in patients with non-islet cell tumors who have hypoglycemia without elevated levels of insulin by radioimmunoassay. About one-third to one-half have elevated levels in plasma of an insulin-like growth factor (probably IGF-II), detected by specific radioreceptor assays but which is undetected by the insulin immunoassay. This material, structurally quite similar to insulin, can bind to the insulin receptor and at high concentrations can generate all the metabolic effects of insulin, including hypoglycemia. Interestingly, in all the examples cited, where two or more ligands bind to two or more types of receptors, the biological effect observed is determined by which receptor is occupied and is independent of which ligand is bound. Again, receptor is more important than hormone.

SUMMARY

The development of methods to measure insulin showed that many (if not most) patients with disorders of glucose metabolism have a target cell disorder rather than a secretory defect. Methods to measure the insulin receptor have shown that in patients with target cell disorders the receptor is often involved in a meaningful way. Furthermore, even in diseases where hormone secretion (deficiency or excess) is dominant, the receptor may play an important role in the clinical state of the patients. In the future, development of techniques to assess postreceptor events should uncover defects at these sites as well.

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TABLE 8
Candidates for specificity spillover

Clinical condition	Hormone in excess	Reacts with receptors for	To produce
Nonislet cell tumors with hypoglycemia	IGF-II	Insulin	Hypoglycemia
Infants of diabetic mothers	Insulin	Insulin-like growth factors	Macrosomia, increased skeletal growth
Untreated Addison's disease; "autonomous" overproduction of ACTH	ACTH	α-MSH	Skin darkening
Acromegaly	HGH	Prolactin	Amenorrhea and galactorrhea
Choriocarcinoma	HCG	TSH	Hyperthyroidism
Primary hypothyroidism of childhood (Van Wyck-Grumbach)	TSH	LH, FSH	Precocious puberty
Glucocorticoid excess (of endogenous origin)	Hydrocortisone	Aldosterone	Hypertension

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