

REVIEW ARTICLE

Interactions of Polypeptide Hormones with Cell Membrane Specific Receptors: Studies with Insulin and Glucagon*

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In living organisms, many effects are initiated at the cell surface by interaction between a substance and a specific recognition site on the cell plasma membrane. This interaction leads to a widespread variety of phenomena such as cell transformation and proliferation, e.g., mitogenic effect of plant lectins upon lymphocytes; cell destruction, e.g., cytotoxic effect of staphylococcal leucocidin upon polymorphonuclear leucocytes; neurohumoral transmission, e.g., acetylcholine – receptor interaction; and modification of various cellular processes, e.g., polypeptide hormone-receptor interaction and catecholamine-receptor interaction. In every instance, these interactions consist of binding of the substance to some component of the plasma membrane usually referred to as “receptor” or “receptor site”.

The first step in the action of a polypeptide hormone is binding to specific receptor sites on the plasma membrane of the cell (for reviews of this topic, see 1–6). Pending complete physicochemical characterization of “pure” receptor structures, the definition of receptor is essentially operational and rests on the

functional characteristics of hormone binding [4]. These usually include the following: 1. The receptor sites bind the corresponding hormone with a high degree of specificity and affinity. 2. The specific binding sites are finite in number. 3. They are located, or predominate, on the cell plasma membrane. 4. Hormone binding is rapid and reversible. 5. Hormone binding to these sites can be related, directly or indirectly, to the biological effects of the hormone.

In this lecture, and as depicted in Figure 1, the term “receptor” will be used to designate *only* that component of the cell membrane which selectively recognizes a particular hormone in a specific binding reaction. The events occurring beyond hormone binding which eventually lead to the hormonal response will not be considered part of the receptor itself [4]. These events involve effector system(s) which generate(s) a variety of cellular processes through a “second messenger”. In the case of glucagon, the effector is the plasma membrane – bound enzyme adenylate cyclase and the second messenger is 3', 5' – cyclic adenosine monophosphate (cAMP) [7]. The messenger(s) of insulin action, on the other hand, is (are) largely unknown. Therefore, the term “receptor” is

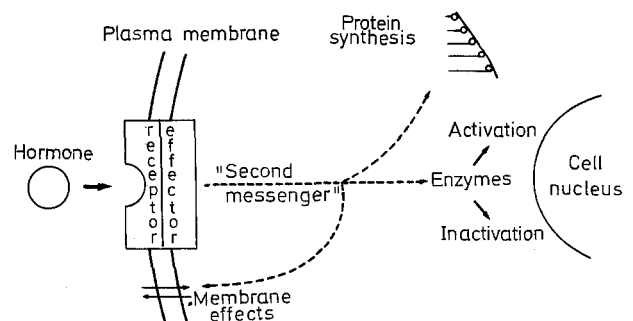


Fig. 1. General scheme of polypeptide hormone action (from 4, modified)

* Dedicated to Claude and Laurent

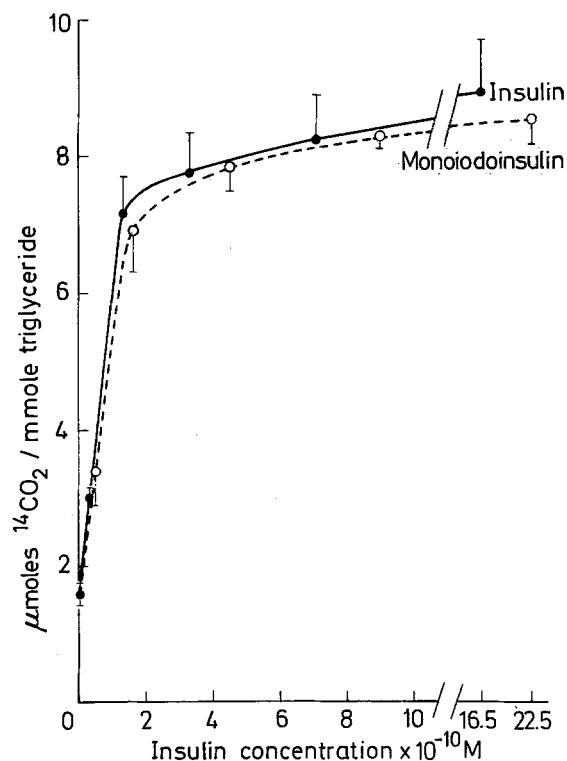


Fig. 2. Biological activity of monoiodoinsulin. Mono ^{127}I -insulin was compared to native insulin in its ability to stimulate glucose oxidation in isolated rat fat cells. The concentration of insulin in the monoiodohormone preparation was measured by spectral analysis and by radioimmunoassay. Virtually no deiodination occurred during exposure to the cells. See 11 for experimental conditions (from 11, with permission)

used as synonymous with hormone discriminator or specific binding site for the hormone. This definition of receptor may appear somewhat restrictive since the classical definition of receptor usually includes not only the recognition function but also the effector function(s), if not all the subsequent cell processes resulting from the hormone interaction with its target cell. However, such functional definition of the receptor, with particular emphasis on hormone recognition, is consonant with several orders of facts: 1. The major and minimal property of the receptor is indeed its specificity for a given hormone. This fundamental property is retained when the receptor has been freed from the cell (either "solubilized" or released into the medium) although it may no longer produce a biological effect [6]. 2. It has often proven difficult to establish a direct correlation between the binding and the biological effect(s) of the hormone. This difficulty rests on several factors. The simple models usually employed to relate hormone binding and hormone action may not be appropriate under every circumstance [4, 6]. Furthermore, such comparison is often difficult in practical terms because the optimal experi-

mental conditions to measure binding frequently differ from those to measure biological effect(s). 3. Finally, hormone receptors may be present in cells which, at a certain stage of organ development or function, do not respond to this particular hormone, or which do not seem to be a major target for this hormone, possibly owing to a temporary ignorance of the cell responsiveness to the hormone [6, 8, 9].

Following this definition of receptor, hormone action on a target cell (Fig. 1) consists of: 1. Steps that involve binding of the hormone to the receptor and formation of the hormone-receptor complex [HR]. 2. Events that occur beyond the receptor. These are initiated by a "coupling" process between [HR] and the effector(s) which results, through the formation of a second messenger, in the modification of a variety of processes, e.g., stimulation of membrane transport systems, enzyme activation or inactivation and modifications of protein synthesis. The cell responsiveness to the hormone integrates all of these events. Therefore, it is clear that studies of hormone binding to a receptor explore a necessary step, but not a sufficient condition, for hormone action.

The studies presented in this review will focus on the very first step, binding of insulin and glucagon to their specific receptors. The following aspects will be discussed: 1. Methodology. 2. Properties of hormone binding. 3. Studies of pathological alterations of hormone binding. 4. Use of receptors in studies of structure-function relationships, and 5. Morphological approach to the study of cell surface insulin receptors.

Methodological Aspects

During the past six years, both new techniques and adaptations of previously described approaches have led to extensive studies of polypeptide hormone binding to receptors (see 1, 4 and 8). The basic methods and techniques for the *direct* study of hormone-receptor interactions are similar to those used in other competitive binding assays. They have largely benefited from the work of Berson and Yalow [10] on the interactions between polypeptide hormones and their specific antibodies. They require isotopically labelled hormone, a suitable receptor preparation, and an appropriate means for separating the hormone-receptor complex from the hormone.

Radiolabelled Hormones

The preparation, isolation and characterization of monoiodoinsulin have been described elsewhere [11, 12]. Monoiodoinsulin was virtually indistinguishable from native insulin in its capacity to stimulate glucose

oxidation by isolated rat fat cells (Fig. 2). Since this early study, it has been amply confirmed that monoiodoinsulin retains most, if not all, of the biological activity of unlabelled insulin (for review of this topic, see 8)¹. Accordingly, the mono ¹²⁵I-insulin obtained after iodination conditions that yield mostly, or predominantly, monoiodohormone, and isolated by chromatography of the iodination mixture on DEAE-cellulose, retains the affinity of unlabelled insulin in binding to receptor sites in rat liver plasma membranes [8, 12]. The specific activity of mono ¹²⁵I-insulin (380 μ Ci/ μ g or 2,300 Ci/mmmole) is high enough to permit the use of low concentrations (i.e., near the physiological values in vivo) of ¹²⁵I-insulin in studies of insulin-receptor interactions in vitro. Unlike insulin, whose biological activity is impaired when iodine incorporation into the molecule exceeds lg-atom/mole, iodoglucagons containing 1 to 4 g-atoms of iodine/mole are more potent than native glucagon in their ability to stimulate adenylate cyclase [13, 14] and to bind to the glucagon receptors [14, 15] in rat liver membranes. Mono ¹²⁵I-glucagon (650 μ Ci/ μ g) prepared in a manner similar [16] to that of mono ¹²⁵I-insulin was indeed about 3.8-fold more potent than native glucagon in stimulating adenylate cyclase in rat liver plasma membranes (Fig. 3). This is only partly accounted for by a higher binding affinity of the monoiodoglucagon for the glucagon receptor (14; author's unpublished observations).

Receptor Preparations

Highly or partially purified plasma membranes from the liver [11, 12, 17–28], adipocyte [24, 27, 29] and myocardium [30] were used in the studies presented here. There is a distinct value in using the most purified preparation of plasma membrane available in binding studies. The specific binding of the hormone is higher in purified plasma membrane preparations than in more crude cell fractions: serial purification of the liver plasma membrane, for example, resulted in a 50 to 60-fold increase in the concentration of insulin receptors per mg of membrane protein [4, 17]. The nonspecific binding (see below) represents a lower proportion of hormone total binding in purified plasma membrane fractions. Moreover, the use of purified plasma membranes avoids, or minimizes, insulin binding by other cellular organelles [31, 32] which may be of different affinity or concerned with different functions as compared to the hormone receptor on the plasma membrane of the cell. This point is of special

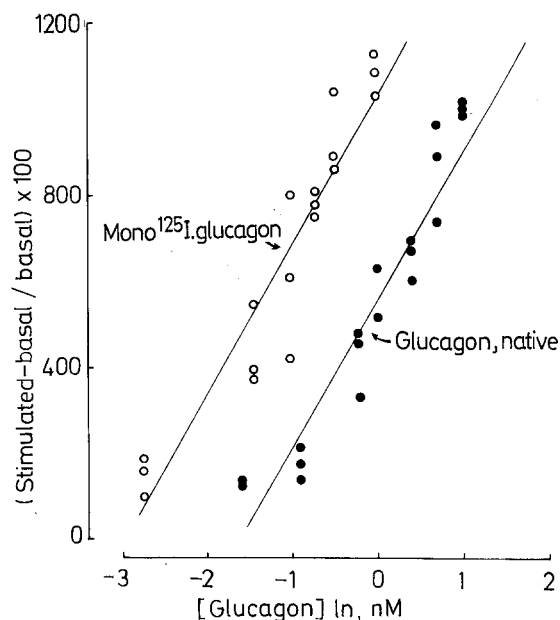


Fig. 3. Enhanced biological potency of monoiodoglucagon in rat liver plasma membranes. Adenylate cyclase activity was measured by radioimmunoassay of the cAMP produced from ATP (see 21). The data are expressed as the percent increase above the basal level and plotted against the concentration of glucagon on a natural logarithmic scale. The concentration of glucagon in the monoiodohormone preparation was measured by radioimmunoassay with the use of ¹³¹I-glucagon as tracer. Monoiodoglucagon was indistinguishable from native glucagon in its ability to react with antiglucagon antibody (data from N. Grenier-Brossette and P. Freychet, unpublished)

importance in comparing the hormone-binding capacities and affinities of membrane preparations obtained from animals in different physiological or pathological states. Although membrane preparations are of more convenient use than isolated cells in many respects, they have not always proven suitable in direct comparative studies of hormone binding and hormone effect. Thus, whereas glucagon bound to its receptor and stimulated adenylate cyclase in highly or partially purified plasma membranes of liver [22, 24, 27] and fat cells [24, 27, 33], insulin failed to alter significantly the basal and the glucagon-stimulated adenylate cyclase activity in partially or highly purified plasma membranes of the liver [21].

Isolated cells may possess a major advantage over cell membrane preparations since they often permit measurement of both the binding of insulin and a biological effect of the hormone [18, 34–42]. Isolated rat liver cells, obtained by enzymatic digestion of the liver, were mostly used in the studies reviewed here [43]. Both peripheral blood mononuclear cells and cultured human lymphocytes have also been employed as a source of insulin receptors (see 9). It has recently been shown that monocytes rather than lym-

¹ See also recent and detailed studies on monoiodoinsulin by Sodoyez, J. C., Sodoyez-Goffaux, F., Goff, M. M., Zimmerman, A. E. and Arquilla, E. R. in *J. biol. Chem.* **250**, 4268–4277 (1975)

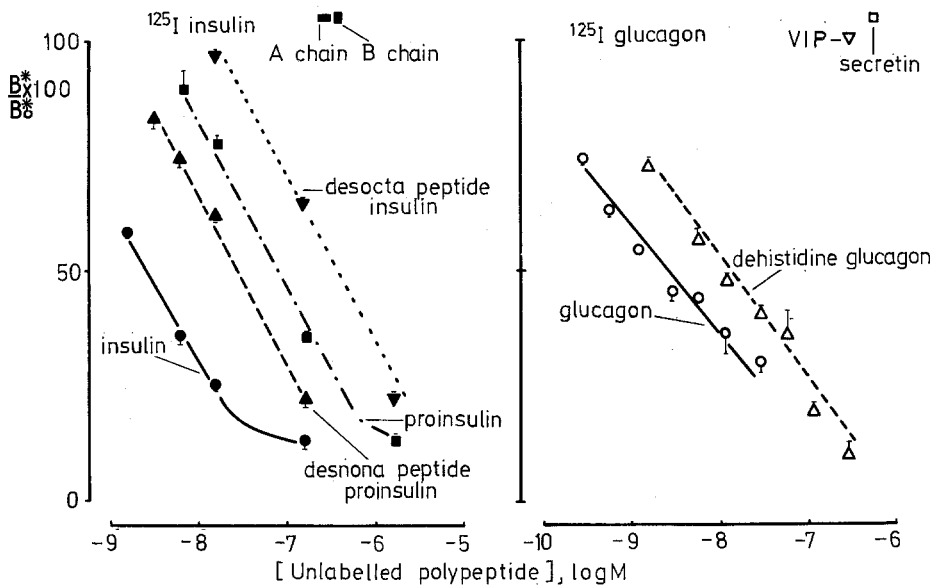


Fig. 4. Specificity and sensitivity of ^{125}I -insulin binding and ^{125}I -glucagon binding in isolated rat liver cells. Binding is expressed as percent of initial binding (B_o^*) of ^{125}I -hormone, i.e., the binding of ^{125}I -hormone in the absence of unlabelled polypeptide, and is plotted against the concentration of unlabelled polypeptide on a logarithmic scale. See 43 for experimental conditions (from 43, with permission)

Table 1. Specificity of ^{125}I -insulin binding

Insulin or derivative	Liver membranes	Fat cells
Porcine, bovine, human	100	100
Guinea pig	1.5	2.8
Chicken	180	190
Fish (bonito)	53	50
Proinsulin (pork)	3.3	2.1
Proinsulin (rat)	5.5	n. d.
Desocta. insulin	1.9	1.5
DAA. insulin	1.7	1.4
DesGly-DesPhe-insulin	0.4	2.0
Suberoylinsulin	1.5	2.6
Diaminosuberoylinsulin	5.9	4.4
Diaminosuberoylinsulin, reduced-reoxidized	5.2	n. d.

The potency of each insulin or derivative was compared to the potency of porcine insulin (100%) in the receptor-binding assay (rat liver membranes) and in the *in vitro* bioassay (isolated rat fat cells).

Desocta. Insulin: Desoctapeptide (B23–30) insulin.

DAA. Insulin: Desalanine (B30)-Desasparagine (A21) insulin.

DesGly-DesPhe-Insulin: Desglycine (A1) – Desphenylalanine (B1) insulin.

Suberoylinsulin and diaminosuberoylinsulin: intramolecularly crosslinked (A1–B29) insulins (see 23).

n. d.: not determined.

(Data from 12, 17, 23, 25 and 47).

phocytes are the insulin-binding cells in preparations of human peripheral blood mononuclear leukocytes [44].

Several problems have thus far hampered the purification and physico-chemical characterization of insulin and glucagon receptors (see [3, 4] and [8]). The techniques employed to isolate receptorbound hormone have been reviewed in detail elsewhere [4, 8].

The Properties of Hormone Binding

Specificity

As pointed out in the introduction, the major property (and minimal requirement) of the receptor is its specificity for a given hormone. This implies that the binding of ^{125}I -hormone can be inhibited only by the corresponding unlabelled hormone. Furthermore, since very high hormone concentrations (as compared to the physiological values), e.g., concentrations above 20 nM (i.e., insulin at 120 ng/ml or 3000 $\mu\text{U}/\text{ml}$ and glucagon at 70 ng/ml) may compete with the adsorption of ^{125}I -hormone to various surfaces including biological materials [45, 46], the binding of ^{125}I -hormone must exhibit sensitivity to low concentrations of the corresponding unlabelled hormone (Fig. 4). The residual binding of labelled hormone at high concentration of unlabelled hormone is considered as

“nonspecific” [4, 8, 12, 17, 34, 43]. This nonspecific component accounts for the linear increase in total binding that occurs at high hormone concentrations [4]. The nonspecific binding is usually subtracted from the total to give specific binding. The proportion of nonspecific binding varies with the concentration of unlabelled hormone arbitrarily assigned (usually $\geq 1 \mu\text{M}$) to determine nonspecific binding and with the experimental conditions (temperature, quality of labelled hormone, type and concentration of receptor preparation). For example, whereas the nonspecific binding of ^{125}I -insulin and ^{125}I -glucagon accounts for only 2% to 5% of total binding in purified liver plasma membranes, it may represent a higher proportion (e.g., 10% to 20%) of total binding when less purified cell fractions or whole cells are used [12, 29, 30, 34–37, 43].

The biological specificity of insulin and glucagon receptors was further established with the study of natural analogues and chemically modified derivatives of the hormone. Thus, proinsulin and proinsulin-like molecules competed with the binding of ^{125}I -insulin in liver plasma membranes [12, 17] and cells (Fig. 4, left) in direct proportion to their biological potency in vitro (Table 1). This quantitative relationship has thus far been consistently observed with a variety of insulin (and proinsulin) analogues, insulins from various animal species and insulin derivatives obtained by chemical modifications of the insulin molecule [12, 17, 25, 43]. The same high degree of biological specificity was observed with insulin binding in a variety of cell membrane or intact cell systems [4, 30, 47]. With glucagon (Fig. 4, right), the deshistidine derivative was only about 10% as potent as native glucagon in competing with the binding of ^{125}I -glucagon [43, 48] whereas secretin and Vasoactive Intestinal Polypeptide (VIP) did not affect the binding of ^{125}I -glucagon [24, 33, 43, 48]. Insulin and glucagon did not affect the binding of each other (to the extent that there was no cross contamination). In both insulin and glucagon-binding systems, other polypeptide and protein hormones were without effect [17, 43]. It could be expected that antihormone antibodies might not exhibit such a high degree of biological specificity as that demonstrated by hormone receptors. Anti-insulin antibodies were indeed much less discriminative of the hormone biologically active structure than were receptor sites [17].

Kinetic and Quantitative Aspects

The specific binding of ^{125}I -insulin and ^{125}I -glucagon is rapid and temperature dependent [43, 49]. In isolated liver cells, maximal binding was reached by 10 minutes at 37°C and by 20 to 30 minutes at 30°C with

both ^{125}I -hormones. The initial rate of binding was slower at 20°C . With both hormones, the binding was less stable at 37°C than at 30°C . The level of binding reached at steady-state was diversely affected by the temperature: a lesser proportion of glucagon was bound at 20°C than at 30°C , whereas about 3 times as much insulin was bound at 20°C as observed at 30°C [43]. A similarly higher binding of insulin at lower temperatures has also been observed in other isolated cell systems [50, 51] as well as in membrane preparations of liver [49] and heart muscle [30]. The lower and unstable binding of insulin at higher temperatures can be explained, at least in part, by accelerated degradation of hormone [18] and receptor [49].

Insulin and glucagon-receptor complexes can be dissociated by dilution of the reacting species, addition of homologous unlabelled hormone in excess, or decrease in pH [4, 9, 12, 34, 43, 52, 53]. With insulin and in many of the binding systems studied, including intact cells and cell membranes, the rate of dissociation was affected not only by the temperature but also by the concentration of insulin in the medium. It has thus been shown that the rate of dissociation of the insulin-receptor complex is accelerated as the occupancy of receptors by the hormone increases [9, 52, 53]. This observation has been interpreted as reflecting insulin-induced negative cooperativity between the receptor sites [9, 52, 53]. Therefore, the rate of dissociation of ^{125}I -insulin from the receptor may vary depending on the temperature, the degree of receptor occupancy at steady-state and the experimental design employed to dissociate the ^{125}I -hormone-receptor complex. For example, as shown in Fig. 5 B, ^{125}I -insulin dissociated from liver cells by dilution alone with an apparent half-time of about 30 minutes at 20°C [54]. Faster dissociation rates have been observed at higher temperature or with the addition of unlabelled insulin, both in isolated cell and in cell membrane systems [12, 34, 37, 49, 50, 52, 53]. Moreover, the dissociation usually does not follow simple first order kinetics [49] and this complicates further the quantitative analysis of the data. The ^{125}I -glucagon-receptor complex was also observed to dissociate in isolated liver cells [43]. In liver plasma membranes, guanyl nucleotides markedly increase and accelerate the dissociation of receptor-bound glucagon [55].

In addition to the interaction of insulin and glucagon with their receptors, two additional reactions occur which influence the quantitative study of hormone-receptor interaction, i.e., degradation (or inactivation) of hormone and degradation (or disappearance) of receptors. In liver plasma membranes, the insulin receptor site and the insulin-degrading site differ by several criteria [18]. In like manner, glucagon

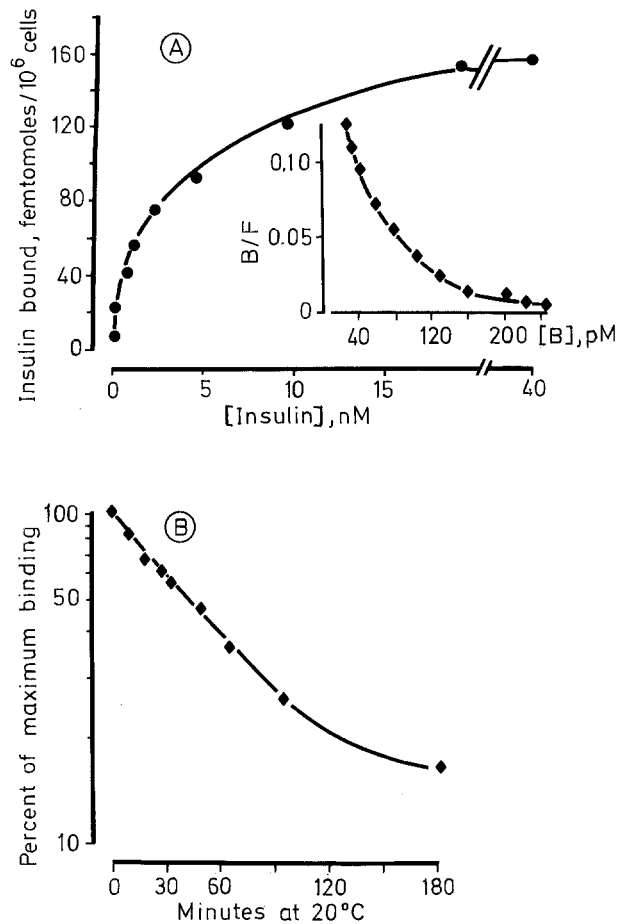


Fig. 5 A and B. (A) Insulin binding to isolated rat liver cells at steady-state. Cells were exposed for 30 min at 30°C to increasing concentrations of insulin (0.1 nM ^{125}I -insulin and increasing concentrations of unlabelled insulin). Binding is expressed as amounts of hormone specifically bound and plotted against the concentration of total insulin in the incubation medium. See 54 for experimental conditions. Inset: Scatchard plot of the binding data (see text). (B) Time course of dissociation of ^{125}I -insulin in isolated rat liver cells at 20°C. Cells ($1.5 \times 10^6/\text{ml}$) were incubated in two steps: 1 – ^{125}I -insulin at 0.1 nM was allowed to bind to the cells for 30 min at 30°C. The percent of labelled hormone specifically bound was measured and is referred to as maximum binding (100%). 2 – Cells were collected by centrifugation, washed twice with cold buffer and resuspended in insulin-free medium (time 0 on figure) at 20°C. Cell-bound radioactivity was measured at the indicated times and was corrected for the nonspecific binding determined throughout in a simultaneous experiment (from 54, with permission)

is rapidly inactivated by liver plasma membranes [56]. The insulin-degrading system in isolated liver cells closely resembles that observed in purified plasma membranes of the liver [57, 58]; like hormone interaction with its receptor, hormone degradation appears to occur primarily at the plasma membrane of the target cell [57–59].

When the binding data are corrected for hormone and receptor degradation, or, when degradation is negligible under appropriate experimental conditions

(low temperature, low concentration of membranes or cells), hormone binding at steady-state can be considered as approximating equilibrium conditions. In isolated rat liver cells [43, 54], the insulin-specific binding sites appeared to be saturable with hormone at about 20 nM, i. e., 120 ng/ml or 3000 $\mu\text{U}/\text{ml}$ (Fig. 5 A). The specific binding of glucagon in the same cells was found to be saturable over a similar range of apparent hormone concentrations [43]. From the data shown in Figure 5 A, it can be calculated that the apparent maximal binding capacity (at 30°C) is about 80 000–90 000 insulin molecules per liver cell of a 120–150 g rat. With insulin at concentrations near the basal values found in hepatic portal blood after an overnight fast (i. e., 1.2 to 2 ng/ml or 30–50 $\mu\text{U}/\text{ml}$), the number of hormone molecules bound per cell (at 30°C) ranges from 6000 to 10 000, i. e., about 7% to 12% of the apparent maximal occupancy. The values calculated for glucagon from the data obtained under the same experimental conditions are similar, although slightly higher than for insulin [43]. It should be kept in mind that, because of the complexity of the binding reaction and the restrictions (hormone and receptor degradation) stated above, these figures represent at best approximations. They compare favourably with those found in other insulin-binding isolated cell systems, e. g., about 50 000 insulin molecules bound per isolated rat fat cell at 37°C [37]. The amount of hormone bound to isolated liver cells also compares favourably with the amount bound to purified liver plasma membranes. From morphological considerations and assuming that, due to invaginations and villous processes, the plasma membrane surface of the liver cell is increased 5-fold over a smooth spherical model [60], one can estimate that 1 mg of liver plasma membrane protein corresponds roughly to about 2×10^7 liver cells. The apparent maximal binding capacity (at 30°C) of this number of rat liver cells is 3 to 3.5 pmoles (calculated from the data shown in Fig. 5 A), which does not differ greatly from the maximal binding capacity (at 30°C) of 1 mg of rat liver plasma membrane protein, i. e., about 2.2 (range 1.6–2.7) pmoles [12, 20, 49]. Another study [58], comparing insulin binding to intact cells and cell plasma membranes of the rat liver in a more systematic way and at 20°C, has also revealed a similar amount of binding in the two types of preparation with a slightly higher binding in whole cells when insulin concentration exceeds 100 ng/ml (2500 $\mu\text{U}/\text{ml}$). The latter finding may reflect some intracellular penetration of the ^{125}I -insulin at very high hormone concentrations [58].

When the binding data, expressed as the ratio of Bound (B) to Free (F) insulin, are plotted against the concentration of bound insulin by the method of Scatchard, the resulting plot is not linear but cur-

vilinear upward (Fig. 5 A, inset). This apparent heterogeneity of binding, which is observed even after subtraction of the nonspecific component, has been found in many insulin-binding systems including both intact cell and cell membrane preparations (for reviews of this topic, see 4 and 9). It has also been observed in a number of binding systems for some other polypeptide hormones including glucagon ([52, 61], author's unpublished results). These findings are compatible with either discrete receptor populations of varying affinity or, as mentioned above in discussing the dissociation data, with negative cooperativity between the binding sites of a single receptor population [9, 52]. In the latter case, owing to insulin-induced site-site interactions of the negative type, the insulin receptor is switched progressively from a high affinity-slow dissociating state to a low affinity-fast dissociating state as fractional saturation of receptors by hormone increases [9, 53]. In considering the potential physiological relevance of these results, it has been pointed out [4, 9, 62] that hormone-induced negative cooperativity between receptors of a single population provides a system which acts as a "buffer" against high concentrations of the hormone, while being exquisitely sensitive to low concentrations. A similar "buffering" effect at the level of hormone-receptor interaction would also be obtained if the cell were equipped with discrete receptor populations of varying affinity (a model which cannot be totally ruled out with the experimental evidence presently available), but at the expense of the synthesis of several receptor populations.

Alterations of Hormone Binding in Pathological States

Over the past three years there has been an increasing body of evidence to suggest that polypeptide hormone (and more particularly insulin) receptors are not static structures, but may fluctuate by changing their affinity for the hormone and/or their concentration on target cells [4-6, 19, 29, 30, 38, 42, 63-71]. As discussed above, insulin-induced negative cooperativity provides one possible mechanism by which the cell may adjust its sensitivity to varying concentrations of the hormone in vivo. A change in the total receptor concentration [Ro] is another mechanism by which the hormone-receptor interaction can be altered and, as will be discussed later, the sensitivity of the cell to the hormone may be modulated.

Studies in the Obese Hyperglycemic Mouse

The search for a defective insulin receptor has been conducted mainly in animal models with genetic obesity. Among these, the obese hyperglycemic (ob/ob)

mouse has been studied in detail because it was hypothesized that some alteration in the insulin-receptor interaction might explain the loss of sensitivity to exogenous insulin (or insulin-"resistance") which has been observed in this syndrome both in vivo and in vitro [72]. The specific binding of insulin is markedly impaired in purified plasma membranes of liver [19, 30, 63, 69, 71], fat cell [29] and heart muscle [30] in the ob/ob mouse. Thus, when liver and heart muscle membranes were prepared from ob/ob mice and from their nonobese littermates and incubated with varying concentrations of insulin, membranes of the obese bound only 20% to 30% as much insulin as did membranes of the nonobese mice over the entire range of insulin concentrations tested (Fig. 6). Similarly, fat cell membranes of ob/ob mice bound only about 20% as much insulin as those of thin mice [29].

A decrease in receptor concentration rather than an alteration in receptor affinity in membranes of the ob/ob mouse is strongly suggested by the fact that the binding of insulin is defective over a wide range of hormone concentrations, including those which occupy most, if not all, of the specific sites (Fig. 6). This was further demonstrated by subjecting steady-state binding data to Scatchard analysis (Fig. 7). The resulting curves were identically shaped and parallel, indicating that the affinity of the receptors for insulin was unaltered in membranes of the ob/ob mouse. The decrease in receptor concentration [Ro] (obtained from the intercept of the plot at the abscissa as $B/F \rightarrow 0$) can fully account for the decrease in insulin binding observed in membranes of the ob/ob mouse over the whole range of site occupancy by the hormone (Fig. 7). These results are similar to those reported for liver [69, 71] and fat cell [29] membranes of the ob/ob mouse. Control studies have made it unlikely that the defect could only reflect the direct occupation of receptors by the high circulating levels of insulin in the obese hyperglycemic syndrome. These studies also excluded an artificial selection of cell membranes to account for the decreased binding [19, 30, 63, 69]. Finally, the defect is not due to an increased insulin-degrading activity in membranes of the ob/ob mouse [30, 63, 69]; on the contrary, liver membranes from ob/ob mice degraded insulin less actively than those from thin animals [63].

The decreased insulin receptor concentration in plasma membranes of the ob/ob mouse is not a mere reflection of the "dilution" of receptors as a consequence of increased cell size, at least in the liver. Despite their increased size, isolated liver cells from the obese hyperglycemic mouse bound 50% to 60% less insulin than those from their thin littermates; the defect was of the same magnitude as that observed in the plasma membrane (about 70%) when expressed

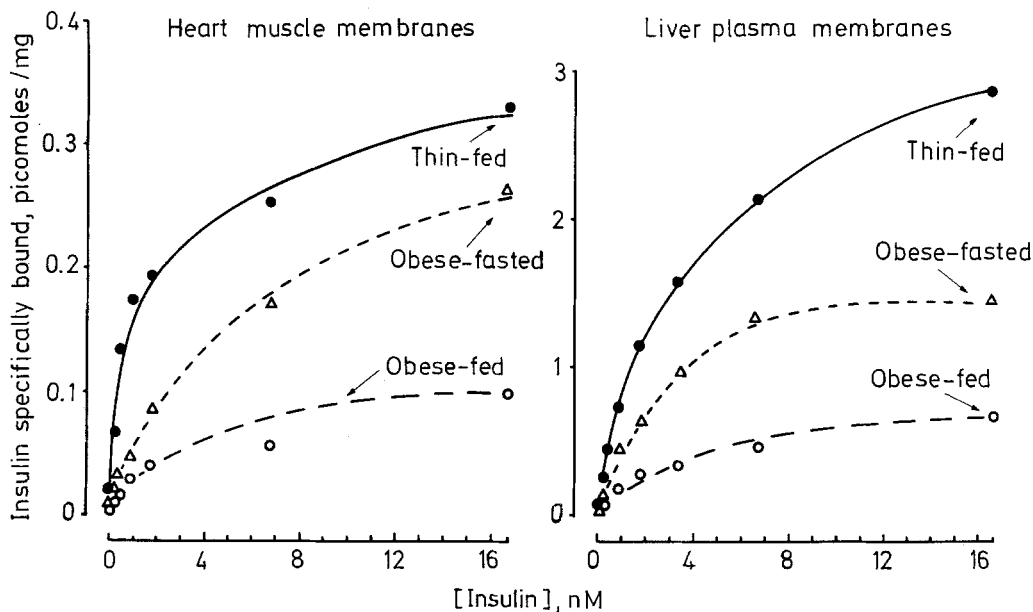


Fig. 6. Insulin binding at steady-state to heart muscle and liver membranes prepared from ob/ob mice fed ad libitum or fasted for 40 h and from their thin littermates (fed ad libitum). Binding is expressed as amounts of hormone specifically bound per mg of membrane protein and is plotted against the concentration of total insulin (^{125}I -labelled plus unlabelled) in the incubation medium. See 30 for experimental conditions and attendant metabolic parameters (from 30, with permission)

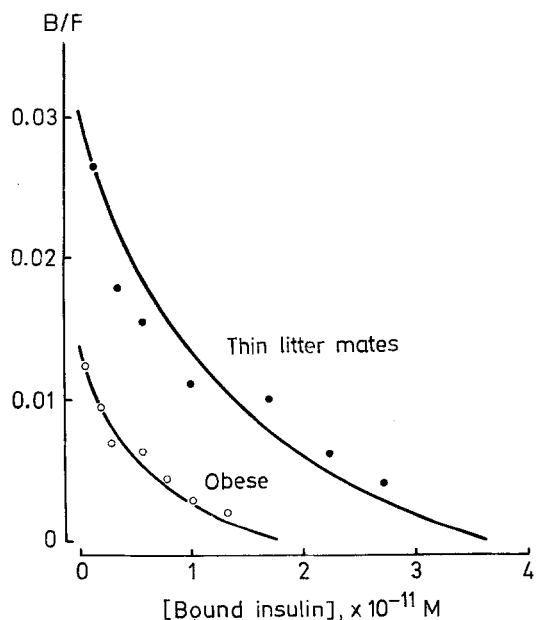


Fig. 7. Scatchard plot of the binding data in heart muscle membranes at steady-state for the ob/ob (obese) and thin mice. The B (Bound)/F (Free) ratio of ^{125}I -insulin is plotted against the concentration of total bound insulin. Within simultaneous experiments, ^{125}I -insulin at 0.06 nM was incubated with membranes of the thin (0.10–0.19 mg protein/ml) and of the obese (0.14–0.17 mg protein/ml) for 22 h at 5°C. The data have been corrected for the non specific binding and have been normalized to binding to 0.1 mg of membrane protein/ml (^{125}I -insulin binding was a linear function of membrane protein concentration over the range of membrane concentrations used in these studies, see 30). Each point is the mean of two repeated experiments carried out with two different membrane preparations

per unit of cell surface area [63]. Thymic lymphocytes of the ob/ob mouse, whose mean cell size is the same as that of cells from normal mice, also showed a 50% decrease in insulin binding [38]. The defect in insulin binding was also present in membranes prepared with similar yield and degree of purification from organs of similar weight in both types of mice, such as heart muscle [30]. All of these observations suggest that the defective insulin binding in the ob/ob mouse reflects not only a decreased concentration of receptors on the cell surface but also a decreased number of receptors per cell. It has been, however, much more difficult to measure insulin binding reliably and conclusively in isolated fat cells of the ob/ob mouse because of their fragility.

This defect involves insulin binding predominantly. In contrast to insulin binding, growth hormone and catecholamine binding were not found to be altered in liver membranes of the ob/ob mouse [63]. Glucagon binding was decreased by only about 20% to 30% in liver membranes of the obese mouse, in sharp contrast to the 70% to 80% decrease in insulin binding [63, 73]. It has been suggested that the defect in insulin receptors in the ob/ob mouse may be simply a reflection of a more general alteration in membrane glycoproteins [74]. It should be pointed out, however, that the specific activity of 5'-nucleotidase, another membrane glycoprotein [75], was similar in membranes of obese and non obese mice [19, 30, 63, 68] which were also indistinguishable in their protein subunit patterns [63]. Moreover, fasting obese mice for

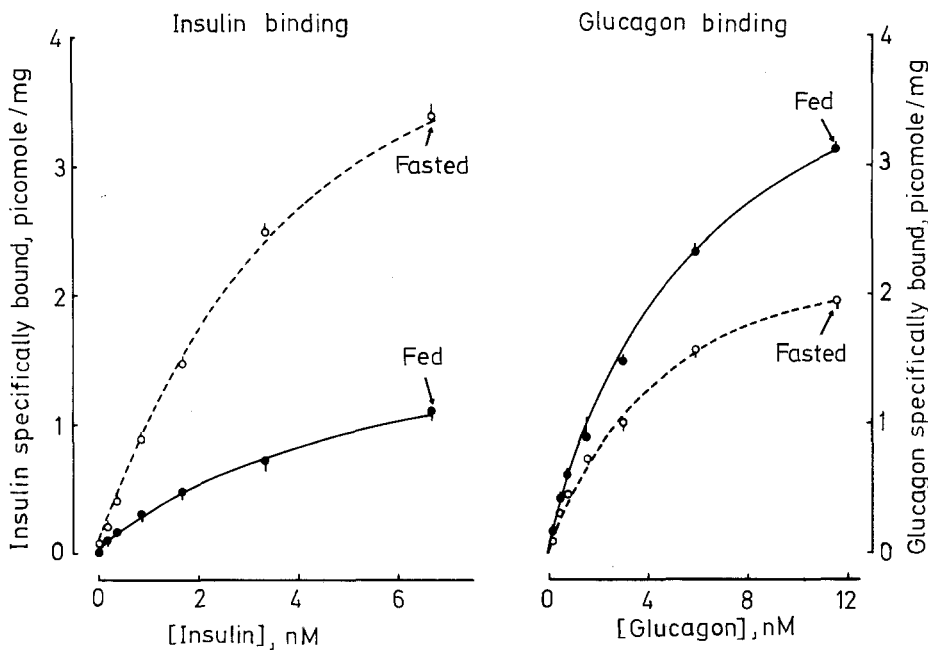


Fig. 8. Effect of fasting on the binding of insulin and glucagon in liver plasma membranes of *ob/ob* mice. Insulin and glucagon binding was measured in the same membrane preparations of fed and 12-day fasted obese mice. Amounts of hormone specifically bound are plotted as a function of total hormone concentration. The binding of ^{125}I -insulin and ^{125}I -glucagon (0.03 nM in the absence and in the presence of increasing concentrations of homologous unlabelled hormone) was measured in membranes of the fed and fasted mice (0.15 mg protein/ml) after 60 min at 30°C. Each point is the mean \pm one-half of the range of duplicate determinations in one experiment. Similar results were obtained in two other experiments (data from Y. Le Marchand, E. G. Loten, F. Assimacopoulos-Jeannet, M. E. Forgue, P. Freychet and B. Jeanrenaud, to be published)

3 days caused very little increase, if any, in the binding of wheat germ agglutinin to liver (whether expressed per mg of membrane protein or per whole liver), whereas insulin binding was increased about 3 and 2-fold per mg of membrane protein and per total liver, respectively [74]. These observations suggest that the insulin-binding defect in the *ob/ob* mouse is rather selective, although its biochemical substratum remains to be elucidated.

To evaluate the dynamics of the binding defect and to test the possibility of reversal under conditions where the obese hyperglycemic syndrome is improved, *ob/ob* mice were food restricted. Insulin binding increased by 2 to 3-fold in cardiac muscle and liver membranes from obese mice that had been fasted for 40 hrs (Fig. 6). Fasting *ob/ob* mice for shorter (24 h) periods also resulted in a 2-fold increase in insulin binding [69]. Fasting had opposite effects on insulin and glucagon binding in liver membranes of *ob/ob* mice: after a prolonged (12 days) fast, the binding of glucagon was decreased by 30% to 40% whereas, in the same membranes, the binding of insulin was increased 3 to 4-fold (Fig. 8). In fasting studies of relatively short duration (24 to 40 h), the decrease in plasma insulin concentrations was the most prominent change as compared with body weight and blood glucose [30, 69]. When the food intake of the obese mice

was restricted until their body weights were restored to normal, the binding of insulin was also improved but not fully restored to normal in liver membranes [69] and only slightly increased, if any, in fat cell membranes [29]. These food restricted *ob/ob* mice still retain an abnormally high proportion of fat tissue, which may account, at least in part, for the failure of full restoration of the insulin receptors and for the persistence of moderately elevated plasma insulin levels, in spite of the body weight reduction to "normal" [6, 69].

In an attempt to separate the variables of diet, weight reduction, change in blood glucose and fall in endogenous plasma insulin, *ob/ob* mice were treated with streptozotocin. Insulin binding increased markedly in the treated animals. When subgroups of treated animals that were selected according to the decrease in endogenous hyperinsulinaemia were compared, the higher degree of increase in insulin binding was observed in those mice with the lower concentrations of plasma insulin [73]. This inverse relationship between the concentration of circulating insulin and the apparent concentration of insulin receptors has also been observed in some other, but not all [76], animal models of obesity [68–70]. Interestingly, the decrease in glucagon binding that, in contrast to insulin binding, followed a prolonged fast in *ob/ob* mice

(Fig. 8) was associated with a significant increase in the plasma glucagon level [73], suggesting that an inverse relationship between the concentration of the hormone and that of its own receptor may also exist in the case of glucagon [73, 76, 77].

Other Studies

This inverse relationship has also been documented by *in vitro* studies demonstrating a time-dependent loss of insulin receptors in human lymphocytes cultured in the presence of high insulin concentrations [78]. The data presently available both *in vivo* and *in vitro* suggest that the circulating insulin level may exert negative influence(s) on the insulin receptor concentration. Not every variety or degree of hyperinsulinemia, however, has been found to be accompanied by the same fall in insulin receptor concentrations. Thus, in a preliminary study on a small number of acromegalic patients, insulin binding to peripheral blood mononuclear cells did not appear to be altered, although most of these subjects were mildly hyperinsulinaemic [67]. Conversely, insulin binding was found to be decreased in mononuclear blood cells from a small number of nonobese, non ketotic diabetics without overt hyperinsulinism [66]. The possibility exists that the regulation of insulin receptors may be diversely affected by varying physiological or pathological circumstances. The inverse relationship that has often been observed between the presence and degree of hyperinsulinaemia and the magnitude of the insulin-binding defect in most animal and human obesities does suggest that the level of insulin may play a significant, if not unique, role in altering insulin receptor concentrations. It is however likely that more than a single variable is involved in receptor regulation [70].

The mechanism(s) by which insulin may affect insulin receptor concentrations is largely unknown. It has been suggested that the insulin-induced reduction of receptor concentrations *in vitro* may result from a proteolytic activity of insulin unrelated to the specific hormone-receptor interaction [79]. This may explain, at least in part, some of the data observed *in vitro* at very high (≥ 1000 nM) insulin concentrations. However, in view of the rather high degree of specificity of the insulin-binding defect and of its reversal in the face of insulin concentrations commonly found in physiological or pathological circumstances, more specific mechanisms may be involved, e.g., accelerated loss (degradation or release from the cell) of receptors and – or – alteration in the rate of receptor synthesis.

Possible Consequences of Receptor Defect on the Cell Responsiveness to Hormone

At this point, one has to consider how and to what extent the defective hormone binding may affect the biological response to the hormone. Most of the current experimental evidence with polypeptide hormones support the occupancy theory, i.e., the observed biological effect (E) is some function (f) of the concentration at equilibrium of hormone-receptor complex [HR], or

$$E = f[\text{HR}] \quad (1)$$

although the exact quantitative relationship between (E) and [HR] is often difficult to establish from experimental data. From the law of mass action,

$$[\text{HR}] = K [\text{H}] [\text{R}] \quad (2)$$

where (K), [H] and [R] represent the affinity, and the concentrations at equilibrium of free hormone and free (unoccupied) receptor, respectively. Substituting equation (2) into (1)

$$E = f(K) [\text{H}] [\text{R}].$$

That is, the biological effect is some function of the affinity and of the concentrations of free hormone and free receptor. Therefore, changes in (K) or [R] may affect the biological effect. It should, however, be kept in mind that step(s) subsequent to hormone binding may also affect the insulin responsiveness of the target cell. It has already been pointed out that, due to negative cooperativity, varying affinity may affect the biological response. Let us now consider the possible consequences of a decrease in receptor concentration.

It has been shown that lipogenesis and glucose metabolism in fat cells are maximally stimulated by insulin when only a small fraction of the total receptors is occupied [35, 41]. This does not necessarily imply that some of the receptors (“spare” or “reserve” receptors) are inactive but simply that, beyond a certain level of receptor occupancy, some step(s) subsequent to the hormone-receptor interaction become(s) saturated or rate-limiting in the response [6]. Moreover, since not all the biological effects of insulin occur at the same hormone concentrations [80], the proportion of spare receptors is not fixed and can vary with different responses that are being measured. Regardless of this varying proportion of spare receptors, it is apparent that any decrease in receptor concentration will affect the hormone dose-response curve. Although this remains to be demonstrated directly in pathological states with cells that are deficient in re-

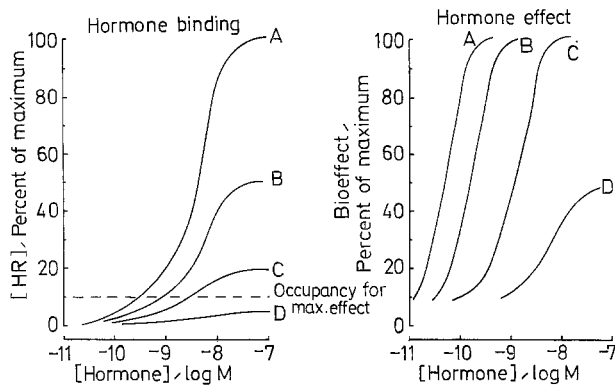


Fig. 9. Decreased receptor concentration: consequences on hormone binding and hormone effect (see text). (Theoretical curves constructed from information in 6, 35, 82 and 84)

ceptors, evidence for such a rationale stems from earlier pharmacological studies [81] and from results reported in fat cells treated with trypsin [35, 82]. In the theoretical examples shown in Figure 9, it is assumed that the biological effect is maximal when 10% of the receptors are occupied. Curves A represent the normal (all the receptors are present) binding curve (Fig. 9, left) and the corresponding biological dose-response (Fig. 9, right). If the receptor concentration is decreased, e.g., due to a reduction in the total number of receptors on the cell by a disease process as in B, C and D, occupancy of the same number of receptors to exert the same biological effect as in A will now require higher concentrations of the hormone (Fig. 9, left) and hence result in shifts of the dose-response curves to the right (Fig. 9, right), i.e., the cell sensitivity to hormone is decreased. The hormone, however, is still capable of exerting maximal stimulation at appropriate concentrations, provided that sufficient receptors remain to achieve the [HR] required for maximal hormone effect; this is the case with examples B and C. If the decrease in receptor concentration exceeded the capacity of those receptors which are spare for the biological response that is being measured, then not only the cell sensitivity to hormone but also the hormone maximal effect would be depressed, as shown in D (Fig. 9). In the *ob/ob* mouse, the decrease in insulin-binding is of a degree similar to that of case C. Therefore, it could be expected that maximal stimulation of endogenous lipogenesis occurs in the liver and adipose tissue of this animal [83], despite the binding defect. This, however, occurs at insulin concentrations that are 20 to 50-fold higher than normal. It should be remembered again that, since the relative proportion of spare receptors may vary with the particular biological response that is measured, the same decrease in receptor concentration may be limiting for the expression of the maximal hormone effect for one

type of response without altering the maximal effect for another type of response. Finally, it is possible (although still speculative) that the number of receptors per unit of surface area is at least as important as the total number per cell for generation of the biological effect [84]. Morphological data [85, 86], which suggest that insulin receptors tend to occur in clusters, may give some substance to this hypothesis [62].

Although it is apparent from these theoretical considerations that any decrease in receptor number should result in a decrease in cell responsiveness to the hormone, the extent to which a defective binding is actually responsible for a decreased sensitivity to insulin in the *ob/ob* mouse is presently unknown. It should be kept in mind that hormone binding to receptor is not a sufficient condition for the expression of hormone action and that changes in events subsequent to insulin binding may also be rate-limiting and therefore affect the tissue responsiveness to insulin. Thus, in starved (12-day fasted) and streptozotocin-treated *ob/ob* mice, insulin effect remained nil or poor in the liver and adipose tissue, despite the attendant amelioration of insulin binding to its receptor [73]. Many more studies are required to evaluate the actual implication of the binding defect in the alteration of tissue responsiveness to insulin in the obese hyperglycemic syndrome. As more information becomes available about the chain of events at very early stages of the syndrome and regarding the primary abnormality involved, it will also be possible to define more precisely the occurrence of the binding defect.

The Use of Receptors in Studies of Structure-Activity Relationships for the Hormone

Insulin and Insulin Analogues and Derivatives

In studies comparing *in vitro* insulin activity to the hormone structure, at least five functional properties of the hormone can be considered: 1) The binding affinity for the receptor; 2) The ability to induce site-site interactions between receptors (negative cooperativity); 3) The ability to react with the degrading site(s); 4) The biological potency and efficacy; and 5) the immunological reactivity. Whereas the last two properties are measured by *in vitro* bioassays and immunoassays, the first three can be explored directly by investigating interactions between insulin and intact cell or cell membrane systems. As pointed out above, there has always been a close parallel between the varying binding affinities and the varying biological potencies for all of the insulins that have thus far been examined ([12, 17, 23, 25, 47], see also Table 1). In contrast, *in vivo* bioassays have often revealed

Table 2. Activity of proinsulin-like and insulin-like peptides (percent of native insulin)

	In vitro		In vivo ^a
	Binding affinity ^b	Bioactivity ^c	
<i>Proinsulin-like</i>			
Proinsulin	3.3	2.1	18
Split proinsulin	3.7	2.8	20
Desdipeptide proinsulin	14	19	58
Desnonapeptide proinsulin	14.3	20	62
<i>Insulin-like</i>			
Diarginine insulin	88.9	73	62
Monoarginine insulin	79.1	70	66

^a Data from R. E. Chance, *Diabetes* 21 (Suppl. 2), 461–467 (1972).

^b Receptor-binding assay in rat liver membranes (from 12).

^c In-vitro bioassay in isolated rat fat cells (from 12).

higher relative activities than in vitro [23, 47, 87]. In the case of proinsulin and proinsulin-like molecules, it is likely that such discrepancy (Table 2) can be accounted for by the slower degradation rate of proinsulin that appears to be responsible, at least in part, for its longer half-life and prolonged activity in vivo when compared to insulin.

Receptor-binding studies with insulin analogues and chemically modified insulins have indicated that a decreased affinity for the receptor is the common mechanism by which a modification or a specific alteration of the insulin native structure may alter its biological potency. The only apparent exception to the close relationship between the binding affinity and the biological potency has been the finding that hagfish insulin possesses a relative binding affinity which exceeds its relative biological potency [88]. Since all of the insulin analogues and derivatives under study have exhibited the same maximal activity (efficacy) as native porcine insulin [17, 23, 47], they can be considered as full agonists of lower potency. This also applies to insulin from various animal species, with the exception of chicken (or turkey) insulin, which behaves as a "superagonist" when compared to porcine insulin. Again, a higher binding affinity for the insulin receptor can fully account [25] for the enhanced biological potency that this avian insulin exhibits in the rat (Table 1). A more stable hormone-receptor complex appears to be involved in the higher binding affinity of chicken insulin [89], possibly in relation to the presence of a histidine residue in position A 8 [90].

Studies of modified insulins have generally revealed that any substantial change in the three-dimensional structure of the hormone molecule results in a marked loss of biological activity [91]. Receptor-binding studies have confirmed and extended these find-

ings. In the A chain, the invariant residues A1 Gly, A5 Gln, A19 Tyr and A21 Asp, which are separate in sequence, are brought together by the molecular folding in the three-dimensional structure. They constitute a region that extends on the surface of the molecule and is very likely to be involved in the binding of insulin to the receptor [12, 17, 23, 47, 90]. Residues B 23–B 26 (also involved in the dimerisation of insulin) are part of this receptor-binding region [17, 47, 90]. Deletion of residues B 23–B 26 and – or – A 21 also causes a loss in the ability of insulin to induce negative cooperativity [92].

Common or Discrete Receptor Sites for Hormones that Possess Structural Homologies

Another application of structure-function studies with the use of receptors is to investigate whether hormones that possess structural homologies, but have different potencies or exert different types of effect, react with the same or with separate receptors. Proinsulin, for example, was found to bind, through the insulin moiety, only to insulin receptors in liver membranes [12]. Because guinea pig insulin differs markedly in structure from other mammalian insulins and has very low biological potency, the possibility was examined that guinea pig might have a receptor specific for its own insulin, i.e., a site which would have an affinity for the guinea pig insulin equal to or higher than its affinity for other insulins. This was not found to be the case. In fact, guinea pig insulin was only 1% to 5% as potent as porcine insulin in competing with the binding of both porcine and guinea pig ¹²⁵I-insulins, in guinea pig as well as in rat liver membranes (Fig. 10). These and other comparative studies suggest that the insulin receptor has remained remarkably constant despite evolutionary changes occurring in the insulin structure [93].

In studies of glucagon and structurally related intestinal peptides such as secretin and VIP, it was observed that glucagon on one hand, secretin and VIP on the other hand, had clearly separate receptors in liver and adipose tissue [24, 27, 33]. A fraction of gut "glucagon", the immunoreactive glucagon-like material from the intestine, reacted with some, but not all, of the glucagon receptors in liver [22, 24] and adipose tissue [24].

Radioreceptorassays of Insulin and Glucagon

¹²⁵I-labelled hormone and standardized receptor preparations can be used to characterize and measure the unlabelled hormone. The principle and technique of such "radioreceptorassay" are similar to those of other competitive binding assays and, more particu-

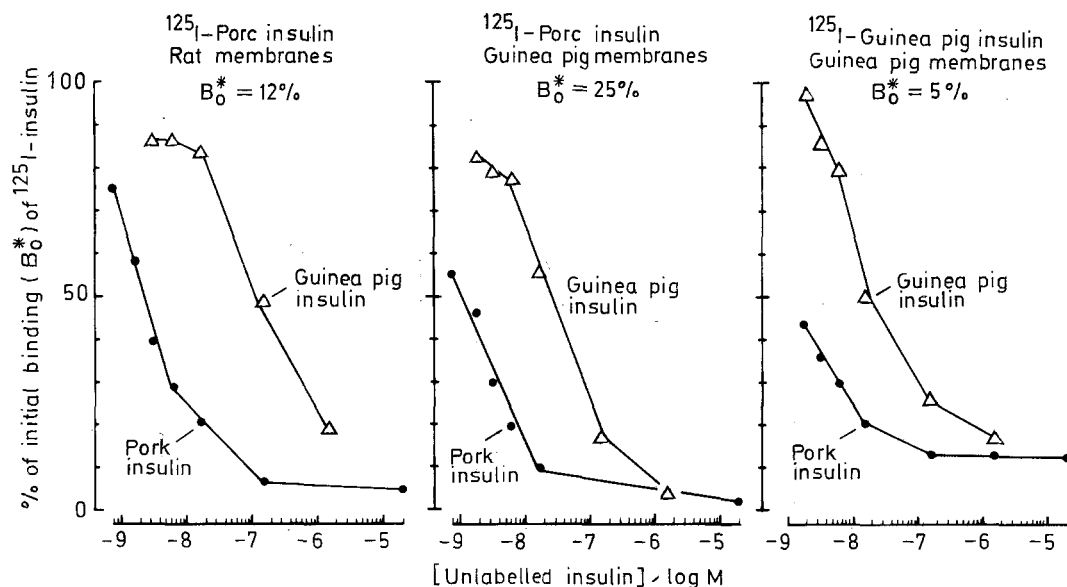


Fig. 10. Competitive binding of guinea pig and pork insulins in rat and guinea pig liver plasma membranes. The data are expressed as in Fig. 4. Initial binding (B_0^*), expressed as percent of total radioactivity, is indicated for each binding system. Incubations were carried out for 60 minutes at 30°C with ^{125}I -insulins at 0.2 nM in the absence and in the presence of unlabelled hormone at the concentrations indicated and with membrane protein at 0.10 mg/ml (guinea pig) and 0.15 mg/ml (rat). The higher insulin-binding capacity of guinea pig membranes (author's unpublished observations) is reflected by the higher binding of ^{125}I -pork insulin to guinea pig membranes ($B_0^* = 25\%$) than to rat membranes ($B_0^* = 12\%$). Guinea pig insulin was a gift of S.P. Wood and T.L. Blundell

larly, radioimmunoassays. However, in place of the antibody which recognizes the antigenic determinants of the molecule, the receptor recognizes the biologically active structure of the hormone (Table 1). This is of particular interest in the case of proinsulin and proinsulin-like molecules whose immunological reactivity is usually higher than their actual biological potency, at least with most of the anti-insulin sera used in conventional radioimmunoassays. The sensitivity of insulin and glucagon radioreceptorassays has been improved by technical modifications of the original receptor-binding assays. Thus, by combining the effects of incubation at 4°C and the preincubation of liver membranes with unlabelled insulin, the sensitivity has been increased by a factor of 3 to 5 [8] over that of the usual one-step binding assay. This sensitized radioreceptorassay can measure unlabelled insulin at 0.5 ng/ml incubation medium (i.e., about $12.5\ \mu\text{U/ml}$ or 0.1 nM). This is similar to the sensitivity of the radioreceptorassay using human cultured lymphocytes [87]. The sensitivity of the glucagon radioreceptorassay can be also improved by preincubating the unlabelled hormone with the receptor preparation prior to the addition of labelled glucagon. With this technique it is possible to measure reliably unlabelled glucagon at 0.2 ng/ml incubation medium (Fig. 11); similar results have recently been reported by Holst [15]. Radioreceptorassays for insulin and glucagon complement the conventional radioimmunoassays

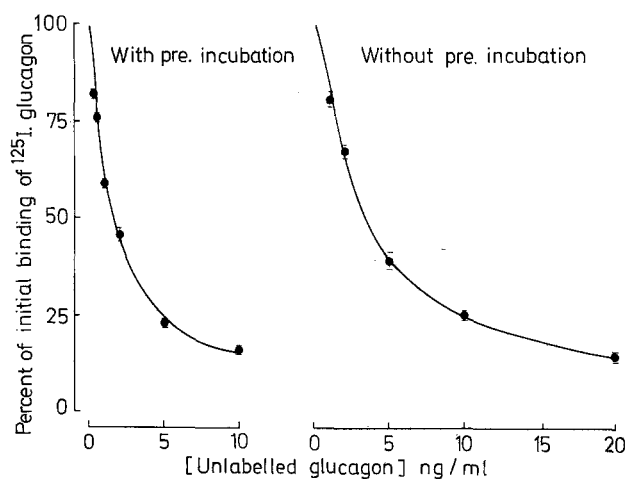


Fig. 11. Radioreceptorassay of glucagon: standard curve of inhibition of ^{125}I -glucagon binding by unlabelled glucagon. Data are expressed as in Fig. 4. To the right: ^{125}I -glucagon at 0.02 nM was incubated with partially purified liver plasma membranes (0.2 mg protein/ml) for 60 min. at 30°C in the absence and in the presence of unlabelled glucagon at increasing concentrations. This binding assay is sensitive to 1 ng glucagon/ml incubation medium. To the left: membranes were preincubated with unlabelled glucagon for 30 min at 30°C prior to the addition of ^{125}I -glucagon for a subsequent incubation of 2 h at 22°C . This binding assay is sensitive to 0.2 ng glucagon/ml incubation medium. Incubations were carried out in Krebs Ringer phosphate buffer, pH 7.5, containing 2.5% bovine serum albumin (Fraction V), and 2000 KIU/ml Trasylol, 100 $\mu\text{g/ml}$ bacitracin as inhibitors of glucagon degradation. Each point is the mean \pm S.E.M. of three separate experiments (unpublished data from N. Grenier-Brossette and P. Freychet)

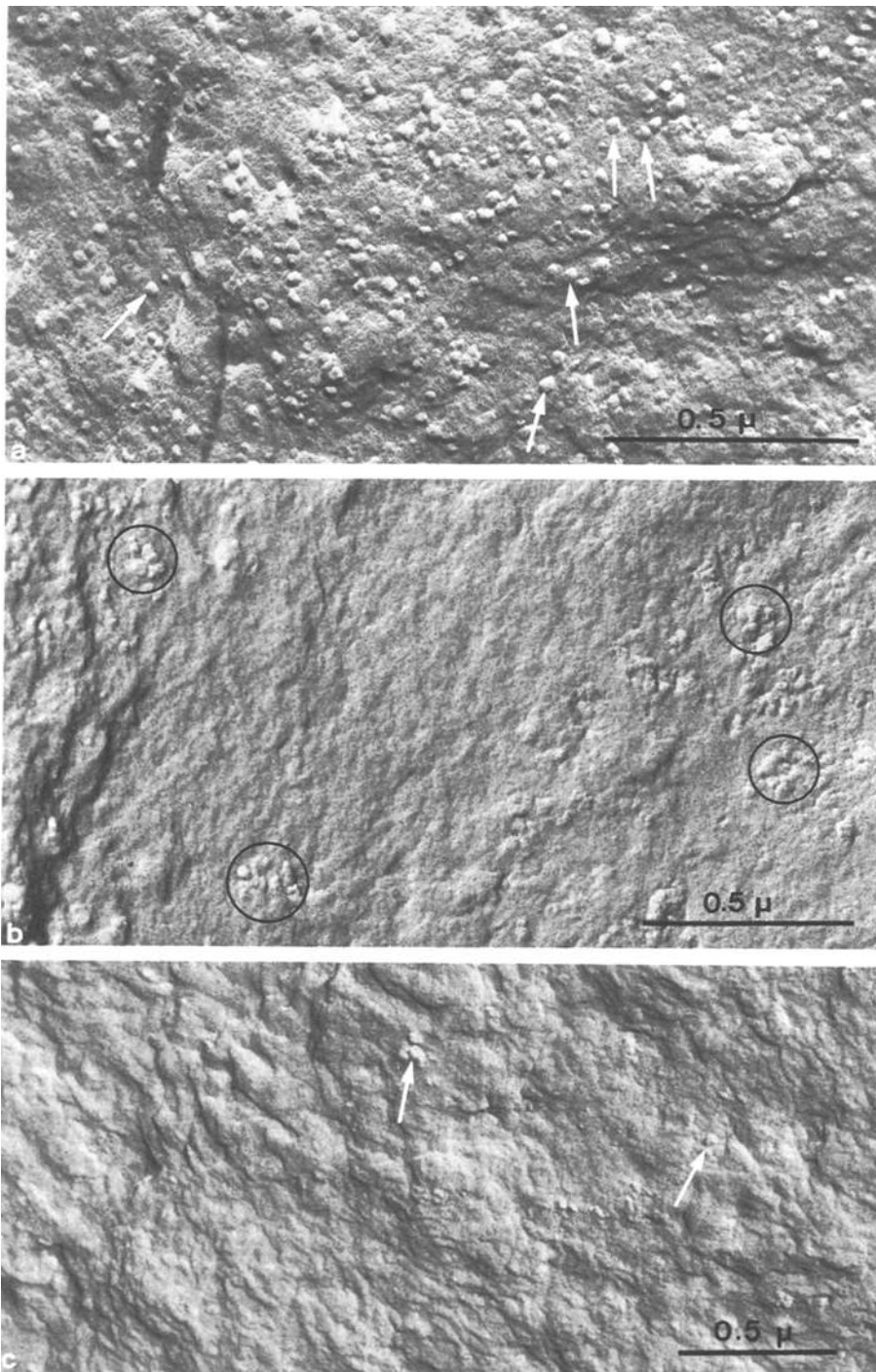


Fig. 12 a–c. Surface replicas (shadow-casting) of purified liver plasma membranes exposed to insulin-dextran-ferritin without: (a) and (b), or with prior exposure to native insulin: (c). (a): Predominantly diffuse distribution of ferritin molecules (arrows); (b): predominantly clustered distribution of ferritin molecules (circles); (c): membrane surface free of ferritin, except in a few areas indicated by the arrows (from Orci et al., 85, with permission)

and bioassays and offer a unique advantage in that they are based on affinity for a biospecific receptor rather than on immunospecificity. They also allow rapid and specific screening of a variety of natural and synthetic hormone and hormone-like molecules.

A Morphological Approach to the Study of Cell Surface Insulin Receptors

Insulin linked to ferritin has been used to visualize insulin-binding sites in plasma membranes of liver [85] and fat cells [86]. In our technique, a low molecular weight soluble dextran (T 10) was used both as a link and a spacer between insulin and ferritin [94]. This insulin-dextran-ferritin (IDF) complex was active in the insulin radioreceptorassay and radioimmunoassay, and appeared to retain the qualitative characteristics of native insulin. However, probably because it contains more than one insulin molecule per ferritin molecule, the IDF complex was only about 17% as potent as could have been expected from its insulin content determined by radioimmunoassay [94]. With this preparation, it has been possible to visualize insulin binding to liver plasma membranes when both thin sections (conventional electron microscopy) and larger areas of the membrane (freeze-etching) are examined [85]. When even larger areas of the plasma membrane were studied in shadow-casting preparations, ferritin-labelled and unlabelled areas were observed. In labelled areas, the distribution pattern was either diffuse (Fig. 12 a) or clustered (Fig. 12 b). Such visualization was largely prevented in membranes that had been pretreated with native insulin (Fig. 12 c). It is presently unknown whether this bimodal pattern of distribution reflects the actual distribution of insulin receptor sites or is due, at least in part, to the preparative procedures employed. The number of visible binding sites (about 90 IDF/ μm^2 and 12 patches/ μm^2 in the diffuse and the clustered patterns, respectively) compares favourably [95] with that calculated from measurements of insulin binding to isolated rat liver cells (25–60 sites/ μm^2).

In conclusion, the binding of insulin and glucagon to sites that possess the specificity and affinity of biologically important receptors has been demonstrated in intact cells and in cell plasma membranes. These studies have extended in two directions: 1) Measurements of the hormone-receptor interaction in pathological states. A binding defect, characterized by a decreased concentration of insulin receptors in cell membranes, has been demonstrated in the obese hyperglycemic mouse and in some other obese states with hyperinsulinism. This defect appears to evolve from environmental rather than genetic influences

and may be secondary, at least in part, to the endogenous hyperinsulinaemia. 2) Studies of structure-activity relationships with the use of biospecific receptors have allowed the delineation of the bioactive region of the insulin molecule and the investigation of the hormone specificity of receptors for peptides that possess structural homologies.

Studies on the relationship between hormone binding to receptor and activation of cell function, and on the role of cooperativity in the hormone-receptor interaction as well as in the receptor-effector “coupling” process will probably lead to a better understanding of the mechanism(s) of hormone action. Relating hormone binding to receptor and hormone action is of special importance in the case of insulin whose mechanism of action is largely unknown. Further studies are required to evaluate the role and importance of a defective binding in tissue sensitivity to the hormone, and the factors which are involved in regulating receptors in normal and pathological states. The use of biospecific radioreceptorassays to detect and measure the various endogenous forms of the hormone is of great physiological and pathological interest. Finally, the morphological studies of cell surface receptors provide a new means by which the structure and function of cell membranes can be explored.

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