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THE MOBILE RECEPTOR HYPOTHESIS AND "COOPERATIVITY" OF HORMONE BINDING

APPLICATION TO INSULIN

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SUMMARY

The mobile receptor hypothesis has been proposed to describe the process by which hormone receptor binding initiates a biological response; it states that receptors, which can diffuse independently in the plane of the membrane, reversibly associate with effectors to regulate their activity. The affinity for effector is greater when the receptor is occupied by hormone.

A mathematical expression of the mobile receptor hypothesis is used to show that: (1) The predicted kinetics of hormone receptor binding may be indistinguishable from "negative cooperativity." (2) Receptor occupancy and biological response may be coupled in a non-linear fashion.

By choosing specific parameters, most of the existing data on insulin binding and biological responses can be explained in terms of the mobile receptor hypothesis. Thus, the following are easily explained: (1) A single homogeneous receptor may appear kinetically to be composed of two classes (of high and low affinity) of receptors. (2) Occupancy of the apparent class of high affinity receptors is related linearly to the biological response. (3) The same receptor in different tissues may appear to have different affinity. (4) The binding of different biologically active insulin analogues may exhibit different degrees of "cooperativity." These considerations may also be pertinent to interpretations of other hormone-receptor systems and of various ligand-macromolecule interactions.

INTRODUCTION

The equilibrium and kinetic properties of receptor binding of several hormones deviates from those of a simple bimolecular reaction [1–8]. Also, receptor occupancy by some hormones is not linearly related to the biological response. This is true even for receptors very closely coupled to a biochemical reaction, such as the activation of adenylate cyclase by vasopressin [9] or by glucagon [10]. As described recently by Boeynaems and Dumont [11], such findings can be explained quite simply in terms of the mobile receptor hypothesis, which has been proposed as a special mechanism by

which hormone receptor occupancy generates biological signals [12–16]. The first part of the present report derives the kinetic properties of hormone binding and receptor-effector coupling which can be predicted from the mobile receptor hypothesis. In the second part it is shown that most of the properties of insulin binding can be coherently explained in terms of this hypothesis.

THEORETICAL CONSIDERATIONS

The mobile receptor model. For the purpose of this report, the mobile receptor hypothesis can be stated as follows: Hormone receptors and effectors diffuse independently within the plane of the cell membrane. The receptor may associate reversibly with the effector to regulate its activity. The affinity of the hormone-receptor complex for effector is greater than the affinity of the unoccupied receptor for effector. The interactions are described by the following equations:

$$H + R \overset{K_1}{\rightleftarrows} HR \tag{1}$$

$$HR + E \overset{K_2}{\rightleftarrows} HRE \tag{2}$$

$$R + E \overset{K_3}{\rightleftarrows} RE \tag{3}$$

where K is the apparent macroscopic equilibrium (association) constant, H the hormone, R the receptor and E the effector. The mobile receptor hypotheses requires that $K_2 > K_3$*. The equation for the association of hormone with the receptor-effector complex is

$$H + RE \overset{K_4}{\rightleftarrows} HRE \tag{4}$$

Since

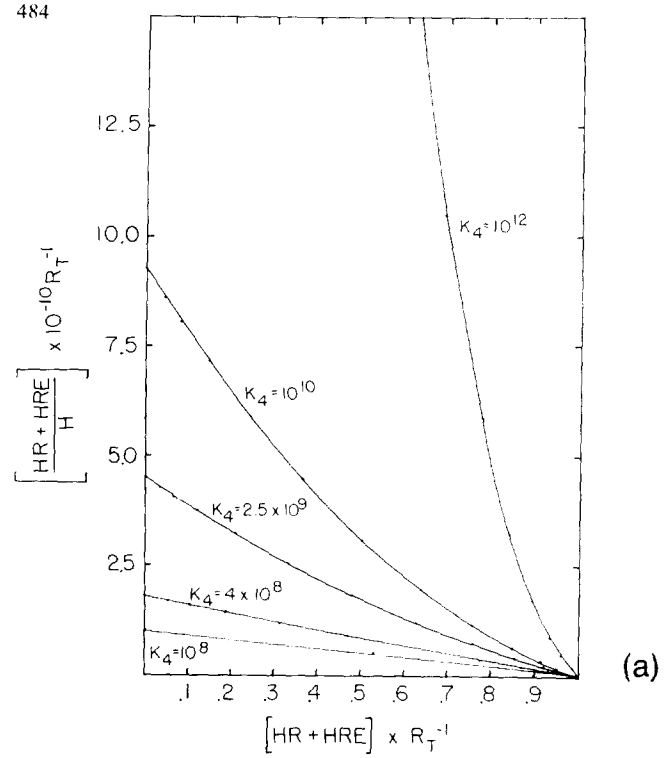
$$K_4 = \frac{HRE}{H \cdot RE} = \frac{HR}{H \cdot R} \cdot \frac{HRE}{HR \cdot E} \cdot \frac{R \cdot E}{RE} = K_1 \cdot K_2 / K_3$$

the above inequality implies that $K_4 > K_1$.

It is therefore clear that there must be at least two species of "binding sites" of different affinity for hormone even though there is only a single and homogeneous receptor. The relative concentrations of these binding sites will vary depending on the fractional receptor occupancy. Although it can be seen intuitively that such a system will mimic cooperative behavior, a more quantitative evaluation follows.

Non-linear Scatchard plots. If three of the four equilibrium constants are known, it is possible to determine the relative concentrations of the different species of

* The interaction of receptor with effector depends on the local concentration of receptor and effector in the membrane and not on their concentrations in solution. Diluting membrane would decrease the concentration of receptor and effector, without changing their local concentrations. Therefore, it is meaningful to normalize the equilibrium constants and association rate constants of reactions between membrane-bound species by dividing them by the receptor concentration, which is proportional to the ratio of the membrane volume, in which the reaction occurs, and the total volume of solution.



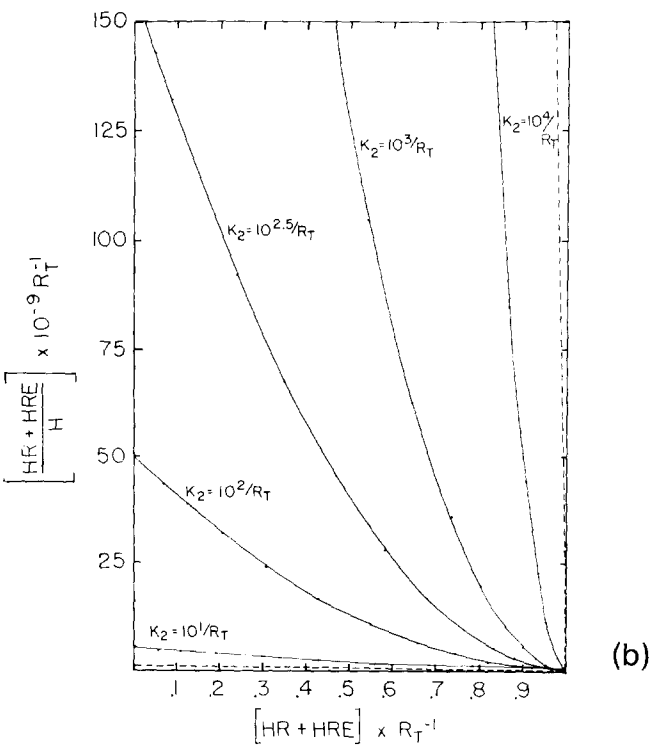


Fig. 1. See opposite page for legend.

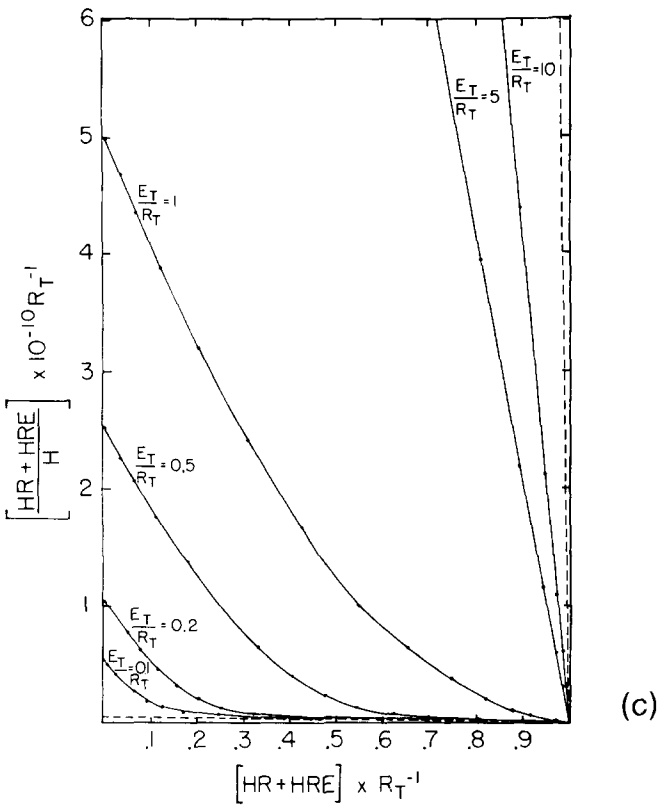


Fig. 1. Simulated Scatchard plots of ligand-receptor interactions for hypothetical ligands which bind to receptors in accord with the mobile receptor hypothesis. Calculations were made using Eqn. A11 of Appendix A and indicated values for the equilibrium constants, effector concentrations and receptor concentrations. (a) Effect of increasing K_4. $K_1 = 10^8$, $E_T = R_T = (K_2K_3)^{-\frac{1}{2}}$. (b) Effect of increasing K_2 and K_3. $K_1 = 5 \cdot 10^8$, $K_4 = 5 \cdot 10^{13}$, $E_T = R_T$. The two broken lines have slopes of $-K_1$ and $-K_4$. (c) Effect of spare receptors and spare effectors. $K_1 = 5 \cdot 10^8$, $K_2 = 10^2 \cdot R_T^{-1}$, $K_3 = 10^{-3} \cdot R_T^{-1}$, $K_4 = 5 \cdot 10^{13}$. Broken lines have slopes of $-K_1$ and $-K_4$.

hormone, receptor, and effector at equilibrium (Appendix A). In the case where $K_2 = K_3$, hormone binding conforms to the classical hyperbolic binding isotherm and the Scatchard plot (HR+HRE)/H vs. (HR+HRE) is linear. However, if $K_2 > K_3$ the Scatchard plot is concave up, the degree of curvature depending upon the ratio, K_2/K_3 (Fig. 1A). For given values of the equilibrium constants, the Scatchard plot will be bounded by lines having slopes $-K_1$ and $-K_4$ (Fig. 1B). The shape of the curve will be determined by the concentrations, R_T and E_T. If $R_T = E_T$ and is much less than $1/K_2$, the Scatchard plot will approach a straight line with a slope of $-K_1$. If $R_T = E_T$ and is much greater than $1/K_3$, the Scatchard plot will approach a straight line with a slope of $-K_4$. At intermediate values the Scatchard plot will be curved. Similar results are obtained by varying the ratio, R_T/E_T (Fig. 1C).

Hill plots and negative cooperativity. Hill plots determined by Eqns. 1–4 may be identical to those resulting from true negatively cooperative interactions (Fig. 2). It is instructive to examine such plots in further detail. The Hill plot is bounded by two

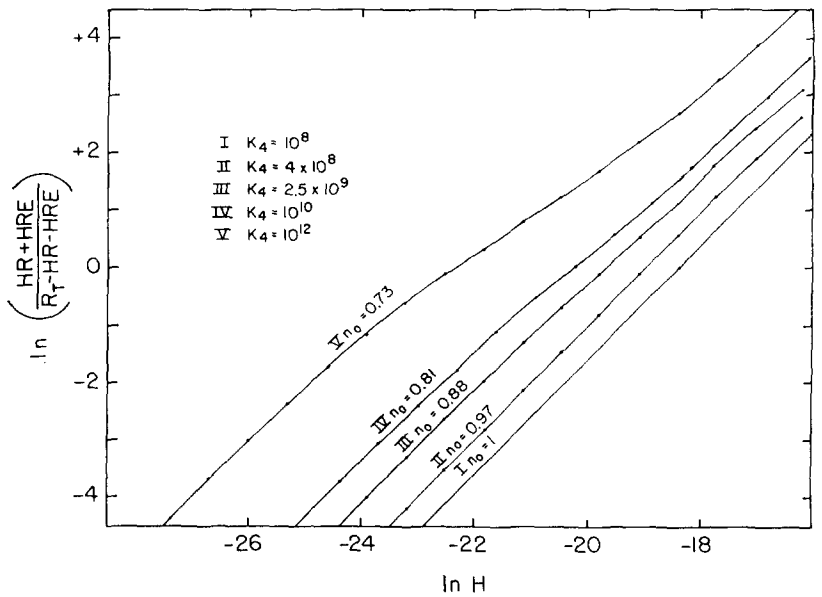


Fig. 2. Simulated Hill plots of hypothetical ligands binding to receptors in accord with the mobile receptor hypothesis. The same parameters used to obtain the curves in Fig. 1A were used to obtain these Hill plots. n_0 is the Hill coefficient when the value of the ordinate is zero.

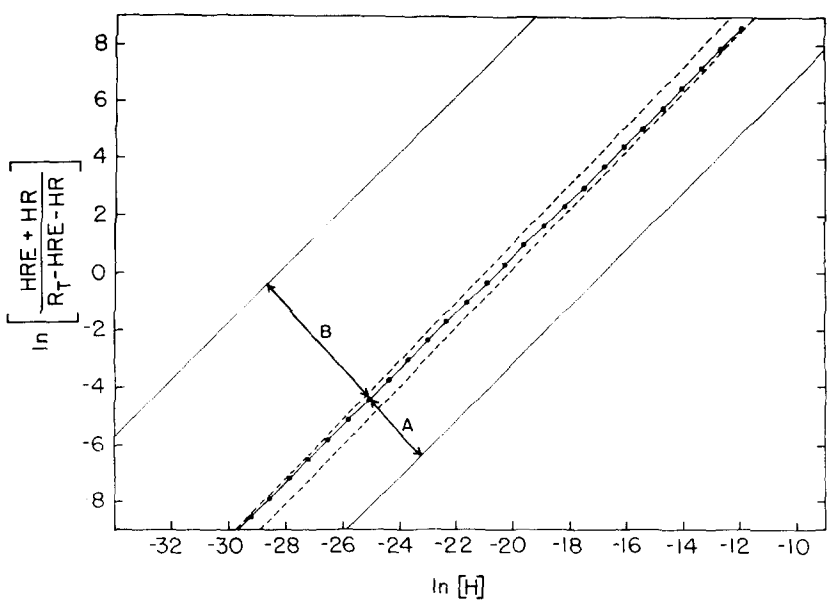


Fig. 3. Theoretical considerations from Hill plots. The Hill plot was calculated for a hypothetical ligand which interacts with receptor according to the mobile receptor hypothesis where $K_1 = 5 \cdot 10^8$, $K_2 = 10^2 \cdot R_T^{-1}$, $K_3 = 10^{-3} \cdot R_T^{-1}$, $R_T = E_T$. Broken lines are asymptotes of unit slope. Light lines have unit slope and cross the abscissa at ln K_1 and ln K_4. The distance A is proportional to the free energy of stabilization due to the presence of E. The distance B is proportional to the increment in free energy of stabilization which would result if the receptor sites were saturated with E.

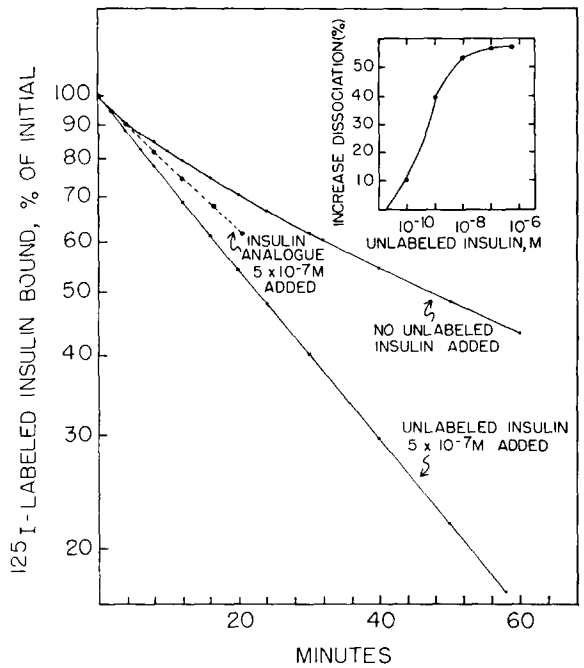


Fig. 4. Simulated time course predicted by the mobile receptor hypothesis of the dissociation of labeled insulin bound to fat cells and the effect of adding unlabeled insulin or an unlabeled insulin analogue which alters the affinity of receptor for effector less than insulin. The constants for the interaction of labeled insulin with fat cells are chosen to be: the rate constant for the interaction of labeled insulin with free receptor, $k_1 = 5 \cdot 10^5\ M^{-1} \cdot s^{-1}$, $k_{-1} = 4 \cdot 10^{-3}\ s^{-1}$; the rate constants for the interaction of the labeled insulin-receptor complex with effector (see footnote page 2), $k_2 = 2 \cdot 10^{-1} \cdot R_T^{-1}\ M^{-1} \cdot s^{-1}$, $k_{-2} = 5 \cdot 10^{-4}\ s^{-1}$; the rate constants for the interaction of free receptor with effector (see footnote page 2), $k_3 = 8 \cdot 10^{-5} \cdot R_T^{-1}\ M^{-1} \cdot s^{-1}$, $k_{-3} = 5 \cdot 10^{-3}\ s^{-1}$; the rate constants for the interaction of labeled insulin with the receptor-effector complex, $k_4 = 5 \cdot 10^6\ M^{-1} \cdot s^{-1}$, $k_{-4} = 1.6 \cdot 10^{-6}\ s^{-1}$; the concentration of receptor, $R_T = 2 \cdot 10^{-15}$ M; the concentration of effector, $E_T = 10^{-16}$ M. The rate constants for the interaction of unlabeled insulin are the same as for labeled insulin. The rate constants for the interaction of a hypothetical insulin analogue are the same except that $k_2 = 5 \cdot 10^{-3} \cdot R_T^{-1}\ M^{-1} \cdot s^{-1}$, $k_4 = 5 \cdot 10^5\ M^{-1} \cdot s^{-1}$, and $k_{-4} = 6.4 \cdot 10^{-6}\ s^{-1}$. The time course was calculated by solving Eqns. B1–B9 of Appendix B numerically using IBM SSP subroutine HPCG. Initial concentrations of various species of receptor and effector are the equilibrium concentrations resulting from the presence of $5 \cdot 10^{-11}$ M labeled insulin, calculated using Eqn. A11 of Appendix A. The initial free labeled insulin concentration is set at zero. Insert. Effect of increasing concentrations of native insulin on the fraction of bound labeled insulin which has dissociated after 20 min, calculated as above.

lines (l_1 and l_2) of unit slope which intersect the zero abscissa at $-\log K_1$ and $-\log K_4$, respectively (Fig. 3). The free energy of stabilization of hormone binding due to the presence of effector at any point on the Hill plot is proportional to the distance between that point and l_1 [17]. Similarly, the distance between any point on the Hill plot and line l_2 reflects the change in free energy of stabilization that would result by increasing the actual concentration of effector to infinity, i.e. if all the binding sites occupied by hormone were also bound to effector.

Kinetics of hormone-receptor dissociation. The rate of dissociation of hormone-receptor complexes for several hormones is more rapid in the presence of increasing

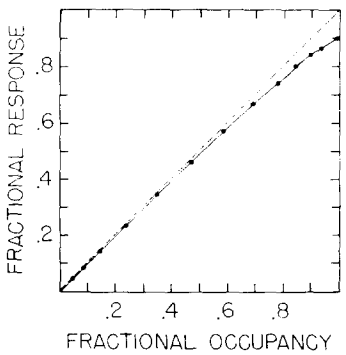


Fig. 5. Relation between hormone-receptor binding and biological response for a hypothetica ligand as predicted by the mobile receptor hypothesis. For a ligand where $K_2 = 10^2 \cdot R_T^{-1}$, and $K_3 = 10^{-2} \cdot R_T^{-1}$, and $R_T = E_T$ fractional receptor occupancy, $(HR + HRE)/R_T$, and fractional response, $(RE + HRE - RE_0)/(E_T - RE_0)$ where RE_0 (basal response) is the value of RE in the absence of hormone, were calculated at different hormone concentrations using Eqn. A11 of Appendix A.

concentrations of a competitive, unlabeled ligand [1–5]. Such results have been interpreted as reflecting negative cooperativity between receptors [1]. However, this phenomenon can be entirely explained by the mobile receptor hypothesis without the need to invoke negative cooperativity. If the only kinetically significant intermediates are those appearing in Eqns. 1–4, the dissociation of bound, labeled hormone in the presence of an unlabeled competive ligand is described by a system of first-order ordinary differential equations (Appendix B); these have been solved numerically (Fig. 4). Clearly, dissociation is more rapid in the presence of unlabeled ligand. These results may be appreciated better if it is considered that hR (unlabeled ligand bound to receptor) competes with HR for the stabilizing effect of E.

Non-linear coupling between receptor occupancy and biological response. The mobile receptor hypothesis predicts that receptor occupancy will be linearly related to biological response* only if the concentration of free effector is much greater than $1/K_2$. Fig. 5 presents an example where this condition prevails at low fractional receptor occupancy but not near saturation. Experimental data of a similar type have been obtained for vasopressin binding and adenylate cyclase activation [9].

APPLICATIONS TO INSULIN BINDING AND DISCUSSION

The mobile receptor hypothesis can explain many quantitative aspects of insulin binding and its relation to biological responses which in the past have been difficult to interpret and reconcile. The eight rate constants (See footnote page 483) and the relative receptor and effector concentrations specified in the legend to Fig. 4 fit quite well the published, experimental data.

Correlation of binding with biological response, the problem of "multiple" and "spare" receptors.

Isolated fat cells appear to possess a class of high affinity binding sites. There

* Biological response is assumed to be proportional to the sum of RE and HRE, after basal activity is subtracted.

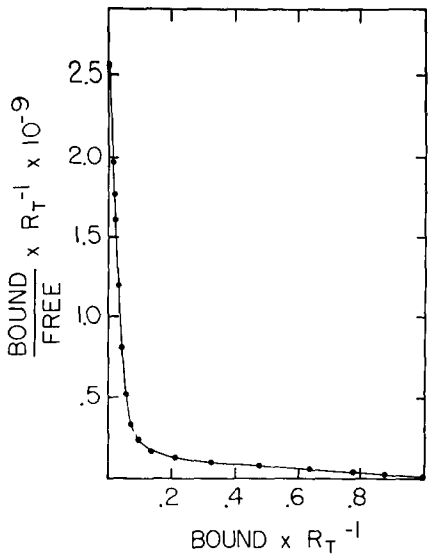


Fig. 6. Simulated Scatchard plot of insulin binding to a single homogeneous class of receptors of fat cells as predicted by the mobile receptor hypothesis. The equilibrium constants for binding are: for the binding of insulin with free receptor, $K_1 = 1.25 \cdot 10^8\ M^{-1}$, for the binding of insulin receptor complex with effector (see footnote p. 483), $K_2 = 4 \cdot 10^2 \cdot R_T^{-1}\ M^{-1}$; for the binding of free receptor with effector (see footnote p. 483), $K_3 = 1.6 \cdot 10^{-2} \cdot R_T^{-1}\ M^{-1}$; for the binding of insulin with the receptor effector complex, $K_4 = 3.125 \cdot 10^{12}\ M^{-1}$. These were derived from the rate constants given in the legend to Fig. 4. The concentration of receptor and effector are the same as in the legend to Fig. 4. The method of calculating the Scatchard plot using these parameters is described in Appendix A.

is excellent correlation between the occupancy of these high affinity binding sites by iodoinsulin and the biological response to insulin as measured by glucose transport and inhibition of lipolysis [18]. Saturation of this apparent high affinity binding site results in a maximal biological response, suggesting there are no "spare" receptors. On the other hand, there are indications that fat cells also possess another or "second" class of insulin binding sites. Although these sites are of lower affinity, they are more numerous than the high affinity sites [19]. Recently, Gliemann et al. [8] have presented convincing kinetic evidence that all insulin binding sites are equivalent and are involved in eliciting a biological response, but only a small fraction of these sites need to be occupied to produce a maximal biological response.

This apparent paradox is easily explained by the mobile receptor hypothesis. A simulated Scatchard plot of insulin binding to fat cells has an appearance that suggests two classes of receptor (Fig. 6). The high affinity class has an affinity of about $5 \cdot 10^{10}\ M^{-1}$, and the low affinity class of about $10^8\ M^{-1}$. These values are similar to those obtained experimentally [19]. However, it is clear from the formulations derived in Fig. 6 that the existence of these two classes is only apparent, and that all receptors are in fact equivalent. Furthermore, when considered according to the formulations predicted from the mobile receptor hypothesis, there is an approximately linear relationship between the biological response (see footnote page 7) and the occupancy of the apparent high affinity binding sites (Fig. 7). (Note similarity of the curves in Fig. 7 to Fig. 4 in ref. 18.)

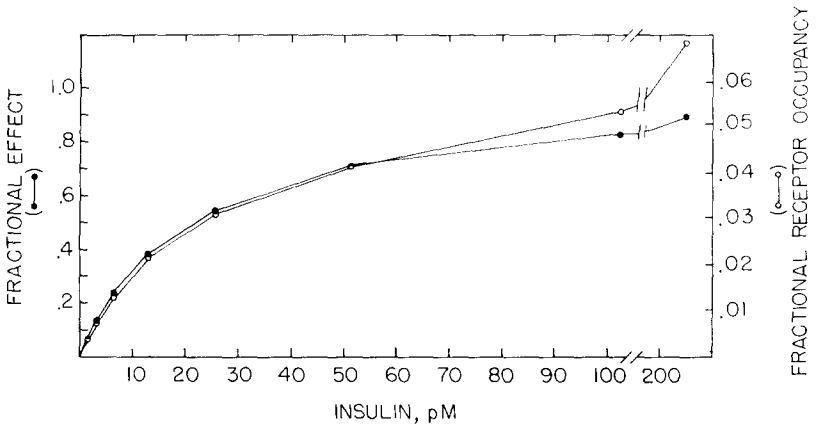


Fig. 7. Simulated correlation between insulin binding and biological response predicted by the mobile receptor hypothesis. The constants for insulin binding are the same as for Fig. 6. Fractional occupancy and fractional response were calculated as described in the legend to Fig. 5.

Insulin binding and activity in thymocytes and monocytes

Certain effects of insulin such as stimulation of glucose transport and inhibition of lipolysis have an ED_{50} near 10^{-10} M, referred to here as high affinity responses. Other effects, such as stimulation of amino isobutyrate uptake [20, 21], thymidine incorporation [21] and induction of tyrosine aminotransferase synthesis [22] occur only with much higher concentrations of insulin (i.e. low affinity responses). Thymocytes and fibroblasts appear to have only low affinity responses, and the affinity of these cells for insulin binding is also correspondingly lower than for fat cells [20, 21]. One possible explanation for this phenomenon is that the insulin receptors of fibroblasts and thymocytes are different from that of fat cells and liver. However, since the relative affinity of different insulin analogs is the same for the fibroblast receptor as for the receptors of fat and liver, and since the affinity for binding of these analogues correlates well with their ED_{50} [23], the possibility is suggested that the receptors are essentially the same in these various tissues.

Another possible explanation for these findings is provided by the mobile receptor hypothesis. The many diverse effects of insulin may result from interactions between insulin-receptor complexes and various types of effectors. For example, equivalent insulin-receptor complexes could separately bind and thus perturb adenylate cyclase, glucose carriers or the amino acid carriers in the cell membrane. It is possible that while the affinity of the receptor for effectors such as adenylate cyclase or glucose carriers could be markedly altered by insulin occupancy, the affinity of the receptor for amino acid carriers may only be altered slightly by the presence of the hormone. In cells which lack effectors whose affinity for receptor is greatly modified by the binding of insulin (i.e. sensitive adenylate cyclase or sensitive glucose transport system), the apparent affinity of insulin binding to the receptor would be less than in cells which had such effectors. This would not result from differences in the receptor, but from differences in the effector resulting in less stabilization of insulin binding. In such cases, it would therefore be expected that the apparent affinity of binding and the biological potency of insulin would be decreased to a similar degree (Fig. 8).

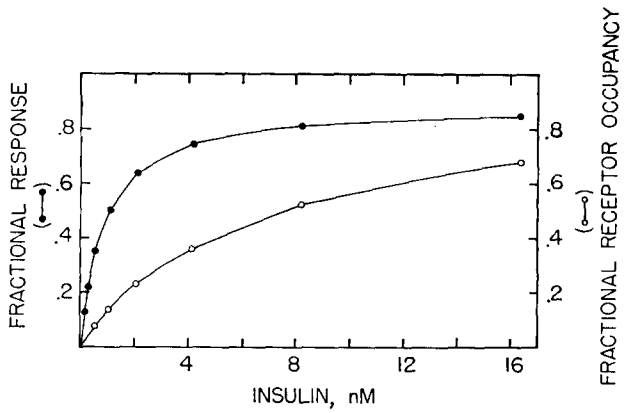


Fig. 8. Simulated correlation of insulin binding and biological response in tissue with "low affinity" receptors, showing that the decreased apparent affinity may result from a difference in effector. The constants used to calculate these plots were the same as those used in Fig. 7 except that $K_2 = 10 \cdot R_T^{-1}$ M^{-1} and $K_3 = 8 \cdot 10^{-2} \cdot R_T^{-1}$ M^{-1}. There was a corresponding change in K_4.

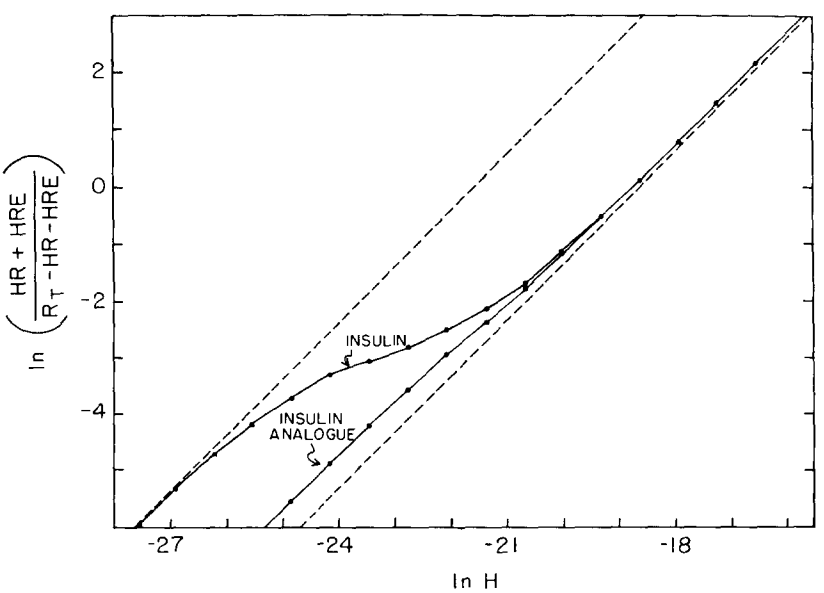


Fig. 9. Simulated Hill plot of the binding of insulin and a hypothetical insulin analogue which alters the affinity of the receptor for effector less than insulin showing that such an analogue would appear to induce less "negative cooperativity". The equilibrium constants for insulin are the same as in Fig. 6. The equilibrium constants for the insulin analogue are derived from the rate constants for the hypothetical insulin analogue in Fig. 4. They are the same as those for insulin except that K_2 is 1/40 that of insulin and there is a corresponding decrease in K_4. Calculations were made on the basis of the mobile receptor hypothesis using the method described in Appendix A.

The mobile receptor hypothesis would also predict that a decrease in S_{50} or ED_{50} could result simply from a decrease in the concentration of effector or receptor, respectively, in the cell membrane.

Biologically active insulin derivatives which do not exhibit "negative cooperativity"

Certain biologically active insulin derivatives appear to have little or no ability to increase the rate of dissociation of cell bound, labeled insulin [1]. To explain this, de Meyts et al. [1] proposed a site on the insulin molecule which induced negative cooperativity in addition to and distinct from those regions necessary for binding and activation of biological responses.

Although alternative explanations based on hormone dimerization considerations have been proposed [2, 6], the mobile receptor hypothesis provides another simple explanation. If the increased affinity of the receptor for the effector as a result of occupancy of the receptor by the insulin derivative is less than that which results from occupancy by native insulin (i.e. K_2 is less for the insulin derivative than for insulin), the insulin derivative will appear to exhibit less negative cooperativity (Fig. 9). Also, the rate of dissociation of cell-bound, labeled insulin would be affected less by the presence of such an insulin derivative, even if differences in the apparent binding affinity of the insulin derivative are considered (Fig. 4).

CONCLUSIONS

While the mobile receptor hypothesis can explain all the currently available quantitative data of insulin binding and the resulting biological responses, it may, of course, not be the correct explanation. At this time there is no direct evidence to either support or refute it. However, it so coherently fits the experimental data with so few assumptions that it must be considered a prime candidate for further testing.

APPENDIX A*

At equilibrium the following equations must be satisfied.

$$K_1 = \frac{HR}{H \cdot R} \tag{A1}$$

$$K_2 = \frac{HRE}{HR \cdot E} \tag{A2}$$

$$K_3 = \frac{RE}{R \cdot E} \tag{A3}$$

$$K_4 = \frac{HRE}{H \cdot RE} \tag{A4}$$

* The calculations for Figs. 1–3 and 5–9 were made using an IBM 370 computer. Details of the FORTRAN program used are deposited with and can be obtained from: Elsevier Scientific Publishing Company, BBA Data Deposition, P. O. Box 1527, Amsterdam, The Netherlands. Reference should be made to BBA/DD/038/77301/000(0000)000.

$$R_T = R + HR + RE + HRE \tag{A5}$$

$$E_T = E + RE + HRE \tag{A6}$$

Where R_T and E_T represent the total receptor and effector concentrations, respectively. Substituting for R_T in accord with Eqn. A5,

$$\frac{HRE}{R_T} = \frac{HRE}{R + HR + RE + HRE} \tag{A7}$$

Multiplying numberator and denominator by $\frac{H \cdot E}{HRE}$,

$$\frac{HRE}{R_T} = \frac{H \cdot E}{\frac{H \cdot E \cdot R}{HRE} + \frac{H \cdot E \cdot HR}{HRE} + \frac{H \cdot E \cdot RE}{HRE} + H \cdot E}$$

$$= \frac{H \cdot E}{\frac{1}{K_1 K_2} + \frac{H}{K_2} + \frac{E}{K_4} + H \cdot E} \tag{A8}$$

Substituting $E_T - RE - HRE$ for E according to Eqn. A6, and then substituting $HRE/(H \cdot K_4)$ for RE according to Eqn. A4 gives

$$\frac{HRE}{R_T} = \frac{H\left(E_T - \frac{HRE}{H \cdot K_4} - HRE\right)}{\frac{1}{K_1 K_2} + \frac{H}{K_2} + \frac{E_T - \frac{HRE}{H \cdot K_4} - HRE}{K_4} + \left(E_T - \frac{HRE}{H \cdot K_4} - HRE\right) H} \tag{A9}$$

Eqn. A9 can be rearranged to give

$$-\left(1 + \frac{1}{H \cdot K_4}\right)\left(\frac{1}{K_4} + H\right)(HRE)^2 + \left(\frac{1}{K_1 K_2} + \frac{H}{K_2} + \frac{E_T}{K_4} + E_T \cdot H + \frac{R_T}{K_4} + H \cdot R_T\right)$$

$$HRE - H \cdot R_T \cdot E_T = 0 \tag{A10}$$

Eqn. A10 is a quadratic in HRE in standard form and can be solved to give

$$HRE = \frac{-B \pm \sqrt{B^2 - 4AC}}{2A} \tag{A11}$$

where

$$A = -\left(1 + \frac{1}{H \cdot K_4}\right)\left(\frac{1}{K_4} + H\right)$$

$$B = \frac{1}{K_1 K_2} + \frac{H}{K_2} + \frac{E_T}{K_4} + E_T \cdot H + \frac{R_T}{K_4} + R_T \cdot H$$

$$C = -H \cdot R_T \cdot E_T$$

The concentrations of the other species of receptor and effector can be solved by substituting HRE into Eqns. A1–A6.

APPENDIX B

If a competitive ligand, h, can also bind to R according to the following kinetic equations

$$h + R \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} hR$$

$$hR + E \underset{k_{-6}}{\overset{k_6}{\rightleftarrows}} hRE$$

$$h + RE \underset{k_{-7}}{\overset{k_7}{\rightleftarrows}} hRE$$

where k values represent rate constants, and if the reactions described by Eqns. 1–4 (see text) proceed without the formation of other kinetically significant intermediates, the following nine differential equations will describe the time course of the association and dissociation of H, a labeled ligand,

$$\frac{dH}{dt} = k_{-1} \cdot HR + k_{-4} \cdot HRE - (k_1 \cdot H \cdot R + k_4 \cdot H \cdot RE) \tag{B1}$$

$$\frac{dR}{dt} = k_{-1} \cdot HR + k_{-3} \cdot RE + k_{-5} \cdot hR - (k_1 \cdot H \cdot R + k_3 \cdot R \cdot E + k_5 \cdot h \cdot R) \tag{B2}$$

$$\frac{dHR}{dt} = k_1 \cdot H \cdot R + k_{-2} \cdot HRE - (k_{-1} \cdot HR + k_2 \cdot HR \cdot E) \tag{B3}$$

$$\frac{dE}{dt} = k_{-2} \cdot HRE + k_{-6} \cdot hRE + k_{-3} \cdot RE - (k_2 \cdot HR \cdot E + k_6 \cdot hR \cdot E + k_3 \cdot R \cdot E) \tag{B4}$$

$$\frac{dHRE}{dt} = k_2 \cdot HR \cdot E + k_4 \cdot H \cdot RE - (k_{-2} + k_{-4}) \cdot HRE \tag{B5}$$

$$\frac{dRE}{dt} = k_3 \cdot R \cdot E + k_{-4} \cdot HRE + k_{-7} \cdot hRE - (k_{-3} + k_4 \cdot H + k_7 \cdot h)RE \tag{B6}$$

$$\frac{dh}{dt} = k_{-5} \cdot hR + k_{-7} \cdot hRE - (k_5 \cdot R + k_7 \cdot RE) \cdot h \tag{B7}$$

$$\frac{dhR}{dt} = k_5 \cdot h \cdot R + k_{-6} \cdot hRE - (k_{-5} + k_6 \cdot E)hR \tag{B8}$$

$$\frac{dhRE}{dt} = k_6 \cdot hR \cdot E + k_7 \cdot h \cdot RE - (k_{-6} + k_{-7})hRE \tag{B9}$$

If the initial conditions and the values of the rate constants are known, these equations may be solved numerically.

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