Up- and Down-regulation of Insulin Receptors

KINETIC MODELS*

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A theoretical model for insulin receptor synthesis and degradation in differentiating 3T3-L1 adipocytes is described. This three-step irreversible ordered sequence model explains the up- and down-regulation of receptors in terms of the level of insulin concentration. Kinetic expressions were derived for the model. Numerical solutions for these equations, based on data reported by Reed and Lane (14), and Hanley et al. (15) were used for computer-generated curves illustrating insulin-dependent receptor synthesis and degradation. Results show that this model provides the best fit to the reported data and lend support to the suggestion that the free recycled receptor may differ from the newly synthesized receptor. A possible role for the recycled receptor in signal modulation is suggested.

The binding of insulin to target cell surface membranes has come under considerable scrutiny in recent years (1-10). Inulin is known to modulate a large number of cellular processes, and the evidence strongly suggests that its effects are triggered exclusively by its interaction with surface protein receptors (1-19). Insulin action at the cellular level must involve several biochemical events: (i) the cellular recognition sites or receptors, (ii) the signal generated by insulin-receptor interaction that mediates the hormonal effects, (iii) the nature of the chemical modification of the enzyme or transport system affected, and (iv) receptor-mediated endocytosis and internalization effects on redistribution of surface receptors and modulation of the rate of receptor synthesis and/or degradation (18, 19). Several studies have indicated that the kinetics of binding differs markedly from the kinetics of insulin degradation; this suggests that separate cellular compartments are involved (20-24).

The chronic exposure of cells to insulin in vitro causes a decrease, or down-regulation (3, 17, 25, 26) in the level of cell surface insulin receptors. This correlation between ambient insulin concentration and surface insulin receptor number extends to other cell types studied in culture or in vitro (3, 17-19, 25, 26). Kosmokos and Roth (27) and Van Obberghen et al. (28) recently proposed that increased receptor degradation may be responsible for such down-regulation.

Evidence such as recovery from down-regulation upon the removal of insulin (3, 15), differentiation-induced increases in surface receptor levels of thymocytes (29) and 3T3-L1 preadipocytes (18, 19, 30-32), and drug-induced increases (33, 34) suggests that cells also have the capacity to up-regulate the number of insulin receptors. Ronnett et al. (19) have shown that the rate constants for inactivation of the cell surface and total cellular insulin receptors were identical in the up-regulated state or in the down-regulated state and that the rate-limiting step in the receptor inactivation pathway occurs at the cell surface.

The heavy isotope "density shift" technique (35-37) has been used to assess the effect on the rate of synthesis and degradation of the insulin receptor (14-19). It has been suggested that insulin-induced receptor down-regulation involves the translocation of insulin receptors from the cell surface to an intracellular location (18, 19).

In this communication, we describe kinetic models for the regulation of insulin receptors that include the redistribution of cell surface receptors to intracellular compartments and modulation of receptor synthesis, and we examine the possible ramifications of receptor recycling in a number of cellular processes.

KINETIC MODELS

Two-step Irreversible Ordered Sequence Model (Reed and Lane) (14)—In heavy isotope labeling studies of receptor synthesis, Reed and Lane (14) have proposed a kinetic model (Fig. 1A) which attempts to explain (i) to what extent, if any, an altered rate of receptor down-regulation contributes to the increase of insulin receptors in differentiating 3T3-L1 preadipocytes, and (ii) whether this down-regulation is influenced by both receptor synthesis and degradation rates, which maintain an unchanging level of receptor.

This kinetic model (Fig. 1A) is based on a two-step irreversible ordered sequence, where [A] is the amino acid concentration added to cells for the synthesis of receptors; [R]f is the total concentration of receptors, and [D] is the concentration of the degradation product. The rate expression for this model yields \( \frac{d[R]}{dt} = k_e - k_d[R]_f \), where \( k_e \) is a constant receptor synthesis rate, \( k_d \) is a first order rate constant for degradation of [R], the complex. Then,

\[
[R]_f = \frac{k_e}{k_d} \left[ 1 - e^{-k_d t} \right] + R_0 e^{-k_d t}
\]

where \( [R]_f = [R]_0 + [R]_0 [R]_0 \) is the free receptor concentration at \( t = 0 \), and \( [R]_f \) is the receptor-insulin complex concentration.

The Reed and Lane model explains down-regulation in terms of variation in \( k_d \) as a function of insulin concentration.
Insulin Receptor Regulation

... the predicted levels of free and bound receptor (d[R]/dt, d[R]/dt; Appendix, Equation 1) were solved and the concentrations of free and bound receptor plotted as a function of time (An Olis Graphics (Athens, GA) subroutine, FPZBASIC, was used in plotting the functions; Olis Model No. 3600).

Two methods were used to solve the differential equations (38). In the first, the equations were simplified by the assumptions $k_{-2} < k_3$ and $k_{-2} < k_3$ and then solved algebraically using Equations 2 and 3. Letting $[RI] = concentration$ of heavy receptor-insulin complex and $[RI] = concentration$ of light receptor-insulin complex, and using the following reported values, $k_3 = 0.0075 / h \cdot 10^6$ cells, $k_5 = 0.103 / h \cdot 10^6$ cells, $k_1 = 0.001 / h \cdot 10^6$ cells, $[RI]_o = 0.053 mol / 10^6$ cells, and $k_{-2} = 0$. Plots of $[R]$ and $[RI]$ were then generated to see the effect of varying the rate constants.

Using Equation 1 (44), the receptor concentration versus time was also plotted and compared with that obtained from the proposed system.

In the second method, the three differential equations were solved simultaneously using the fourth order Runge-Kutta formula (39). Here $\Delta t$, the step-size time increment, was set at 0.05 h and the error obtained using $\Delta t/2$ was found to be 1.10 $\times 10^{-4}$. Values and initial conditions were as follows: $k_3 = 0$; $[RI]_o = 0.053$; $k_{-2} = 0.10$ to 0.1; $k_5 = 0.5$ to 1.5; $k_1 = 0.103$. Plots of $[R]$, $[RI]$, and $[RI] + [R]$ were then generated for various values of $k_3$ at given values of $k_{-2}$. In the case of receptor recycling degradation, $k_3 = 0$, varying $k_{-2}$.

RESULTS

A comparison of the kinetic models for heavy and light receptor synthesis and degradation in differentiating 3T3-L1 adipocytes is shown in Fig. 2. The model proposed (Fig. 1B) appears to give a better fit to the experimental data taken from Fig. 6 of Reed and Lane (14), as shown in the kinetic curves of Fig. 2. The dip in the curves marking the total insulin-bound receptor, where $[RI] = [RI]_o + [RI]$ (Equations 13 and 14, Appendix) is indicative of insulin-dependent binding to receptor, and only a limited number of sites are available at the initial time, $t = 0$. The first term of the equation considers the insulin independence of initial receptor binding and characterized the marked dip and the time lag period for heavy receptor synthesis. The second term represents light receptor degradation (see the Appendix).

The insulin-dependent kinetics of heavy and light receptor synthesis and degradation are shown in Fig. 1 of the Appendix. As the insulin concentration is decreased, both the dipping of these curves and the time lag period become more pronounced. This figure, therefore, represents the kinetic model described in case (i) and is based on Equations 2 and 3.

The up- and down-regulation kinetics of receptor synthesis is shown in Fig. 3. In up-regulation, $[R]_T$ increases as the concentration of insulin decreases while $[RI]_T$ decreases. $[R]_T = [RI]_T + [R]$ (see Appendix, Equation 12). In down-regulation, $[R]_T$ decreases as the concentration of insulin increases, and $[RI]_T$ increases as well. The kinetic model shown here is again case (i), with no visible evidence of receptor degradation and a constant level of free receptor concentration.

Fig. 4 shows the insulin-dependent kinetics of heavy and

and there is some evidence of receptor degradation. Recycling is not as efficient as in case (i).

Case (iii), a three-step irreversible ordered sequence for heavy receptor synthesis: $AA \rightarrow R \rightarrow RI \rightarrow D$, $k_3 = 0$, $k_{-2} = 0$, and $[RI]_o = 0$ at $t = 0$. The free receptor concentration is maintained at a higher level than in case (i).

Computational Procedure—Using a NorthStar computer system with a floating point BASIC, the two rate equations for free and bound receptor (d[R]/dt, d[R]/dt; Appendix, Equation 1) were solved and the concentrations of free and bound receptor plotted as a function of time (An Olis Graphics (Athens, GA) subroutine, FPZBASIC, was used in plotting the functions; Olis Model No. 3600).

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Fig. 4 shows the insulin-dependent kinetics of heavy and
three-step irreversible ordered sequence model (Appendix Equations 2-14). The global goodness of fit of the R² value was 0.9983 and the correlation coefficient of each data point to the fitted curve was 0.9998 (Appendix Equation 13). The plots were generated by the numerical solution of the two differential equations of Appendix Equations 2 for [RI]₁. For heavy receptor synthesis, [RI]₁ = [RI]₁₀ pmol/h/10⁶ cells, kₗ₁ = 0.0075 pmol/h/10⁶ cells, and [RI]₁₀ = 0.053 pmol/10⁶ cells. In all cases, k₋₁ = 0.1/h/10⁶ cells.

light receptor synthesis and degradation, but in this case the kinetic plots were generated by the numerical solution of the two differential equations for [RI]₁. Here the insulin dependence of light receptor degradation, [RI]₁ = [RI]₁₀ - [RI]₁₀, is evident as in the kinetic model described in case (ii). The free receptor concentration is no longer maintained at a steady state (see also Appendix Fig. 2). Under these conditions, it is evident that the recycling of receptor is minimal, so that both insulin and receptor undergo continued degradation.

The kinetic model of case (iii), in which the total free receptor concentration is maintained at a high level, is best described by Fig. 3 of the Appendix, in which the free receptor recycling appears to modulate the receptor lifetime and insulin transport through the membrane. This model provides the best fit to the Lane and Reed data and has the added advantage of describing up- and down-regulation without changing kₘ, the first order rate constant for the degradation of insulin receptor.

**DISCUSSION**

The three-step irreversible ordered sequence model for the regulation of insulin receptor explains the up- and down-regulation of receptor in terms of insulin concentration, thus eliminating some of the difficulties inherent in the two-step model (14).

In order to maintain the level of free insulin receptor in a steady state, this kinetic model describes a reciprocal relationship between the total number of receptors and the insulin concentration, a relationship known as ligand-induced down-regulation of insulin binding, which may be formulated as

\[
\frac{[RI]₀}{[RI]₁₀} = 1 + \frac{1}{kₘ + k₋₁/kₚ₁}
\]

The free receptor generated from recycling the RI complex may differ from the receptor newly synthesized from amino acids. It is possible, therefore, that the recycled receptor may have the option of binding or not binding to insulin, thus signaling transport activation and modulating receptor synthesis. Such a conclusion would be consistent with the insu-
lin-dependent degradation of light and heavy receptor shown in Fig. 4 (and Fig. 2 of the Appendix).

While a theoretical kinetic model (see Appendix Fig. 4) such as this suggests the possibility that the recycled receptor may play a role in the modulation of further transport activation, it remains to be determined in further studies whether there is any difference in the biological activity of light and heavy receptors which would provide experimental evidence to support such a conclusion.

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Insulin Receptor Regulation

Two methods were used to solve the differential equations of (11) above. Assuming that \( k_{21} \ll k_{12} \), and that \( k_{21} = 0 \), the two equations (12) were solved algebraically using the relations shown in the figure.

The kinetic model, including the proposed model, were based on the following assumptions: total receptor insulin complex \( [R]_2 \), \( [R]_3 \), \( [R]_4 \), \( [R]_5 \) and \( [R]_6 \) were the complex of 

\[
[R]_2 = \frac{[R]_1 [I]}{k_{11} + [I]} + \frac{[R]_3}{k_{33} + [I]} + \frac{[R]_4}{k_{44} + [I]} + \frac{[R]_5}{k_{55} + [I]} + \frac{[R]_6}{k_{66} + [I]}
\]

In addition, for the two-step reproducible ordered sequence model (11), the equations were based on

\[
[R]_2 = \frac{[R]_1 [I]}{k_{11} + [I]} \quad \text{synthesis of heavy receptor}
\]

and \( [R]_3 = (k_{21} + k_{33} + [I])^{-1} \) 

\( [R]_3 = (k_{21} + k_{33} + [I])^{-1} \) 

great receptor degradation.

The algebraic solution of these equations is shown in Figure 1.

In the second method, the three differential equations (Eq. 1, above) were solved simultaneously along the fourth order Runge-Kutta (RK) method.

The three differential equations were simplified into a system by substituting the first equation and replacing \( s(A) \) in equation 2, above, by \( s(A) \). This assumption is based on the fact that, in certain cases, the binding of insulin is not greatly altered by the concentration of \( A \). For 50 nM \( s(A) \), \( k_{r2} = 0.001 \) is roughly five-fold increased in concentration of \( A \), and 30 nM \( s(A) \), \( k_{r2} = 0.001 \) is roughly three-fold increased.

Plots of \( [R]_3 \) and \( [R]_4 \) vs. time are shown in Figures 2 and 3, where the curves generated for carbonic anhydrase II were generated for carbonic anhydrase III. The kinetics of light receptor degradation are shown in Figure 2. Figure 3 describes the sum and regulation of receptor synthesis.

![Image of Figure 1](image-url)

Figure 1. Kinetics of light and heavy receptor synthesis and degradation as differential equations (Eq. 1) were solved simultaneously along the fourth order Runge-Kutta (RK) method.

1. \( k_{r2} = 0.001 \) nM, \( k_{r3} = 0.005 \) nM, \( k_{r4} = 0.002 \) nM, \( k_{r5} = 0.003 \) nM, \( k_{r6} = 0.004 \) nM, \( k_{r7} = 0.005 \) nM, \( k_{r8} = 0.006 \) nM, \( k_{r9} = 0.007 \) nM, \( k_{r10} = 0.008 \) nM, \( k_{r11} = 0.009 \) nM, \( k_{r12} = 0.010 \) nM.

![Image of Figure 2](image-url)

Figure 2. Kinetics of light receptor degradation: \( [R]_1 \) vs. \( [R]_3 \) represents the total number of free receptor plus total receptor light receptor degradation, based on the differential equations as Figure 1, except \( k_{r2} = 0.001 \) nM, \( k_{r3} = 0.002 \) nM, \( k_{r4} = 0.003 \) nM, \( k_{r5} = 0.004 \) nM, \( k_{r6} = 0.005 \) nM, \( k_{r7} = 0.006 \) nM, \( k_{r8} = 0.007 \) nM, \( k_{r9} = 0.008 \) nM, \( k_{r10} = 0.009 \) nM, \( k_{r11} = 0.010 \) nM.

![Image of Figure 3](image-url)

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![Image of Figure 4](image-url)

Figure 4. Proposed kinetic model for the receptor recycling pathway, incorporating situations in which the cell surface receptors and internalized receptors (with and without insulin) bind the receptor recycling enzyme (RTE), which is subsequently internalized through the coated pit in turn ATP. Additionally, ATP may be internalized without being bound. Finally ATP, GTP and GTP are exchanged, recycling receptors. The internalization rate constant is \( k_{r2} \), while \( k_{r3} \) represents the dephosphorylation step. The rate constant for the conversion of ATP to GTP is represented by \( k_{r4} \), while the internalization rate constant of ATP is \( k_{r5} \). GTP is the rate constant for the reversible binding of ATP to cell surface receptors.