A Receptor State Space Model of the Insulin Signalling System in Glucose Transport

CATHERYN W. GRAY
School of Mathematics and Statistics
UNSW Australia, Sydney, New South Wales, Australia

ADELLE C.F. COSTER
School of Mathematics and Statistics
UNSW Australia, Sydney, New South Wales, Australia

[Received on November 16, 2014]

Insulin is a potent peptide hormone that regulates glucose levels in the blood. Insulin-sensitive cells respond to insulin stimulation with the translocation of glucose transporter 4 (GLUT4) to the plasma membrane (PM), enabling the clearance of glucose from the blood. Defects in this process can give rise to insulin resistance and ultimately diabetes.

One widely cited model of insulin signalling leading to glucose transport is that of Sedaghat, Sherman and Quon (Sedaghat, Sherman and Quon, 2002. Am. J. Physiol. Endocrinol. Metab. 283, E1084–E1101). Consisting of twenty deterministic ordinary differential equations (ODEs), it is the most comprehensive model of insulin signalling to date. However, the model possesses some major limitations, including the non-conservation of key components.

In the current work, we detail mathematical and sensitivity analyses of the Sedaghat model. Based on the results of these analyses, we propose a reduced state space model of the insulin receptor subsystem. This reduced model maintains the input-output relation of the original model but is computationally more efficient, analytically tractable and resolves some of the limitations of the Sedaghat model.

**Keywords**: insulin signaling, insulin receptor, ODE model, local sensitivity analysis

1. Introduction

Insulin plays a pivotal role in glucose metabolism and whole-body glucose homeostasis. Ordinarily, glucose enters the cell by facilitated diffusion involving a glucose transporter (GLUT) molecule (Manolescu, Witkowska, Kinnaird, Cessford & Cheeseman (2007)). GLUT4 is the insulin-sensitive glucose transporter, and is found in fat cells (adipocytes) and skeletal muscle cells. In the basal state less than 5% of the cell’s GLUT4 complement is present at the PM (Slot, Geuze, Gigengack, James & Lienhard (1991)); however, in response to insulin stimulation, GLUT4 translocates to the PM where it facilitates the entry of glucose into the cell. This results in the lowering of blood glucose levels.

In mammals, insulin is synthesized and stored in secretory granules in the β-cells of the pancreas. Even in the fasting state, the β-cells release small quantities of insulin into the blood in a pulsatile fashion (Landersdorfer & Jusko (2008); Pedersen, Bertram & Sherman (2005); Schmitz, Rungby, Edge & Juhl (2008)), however, the amplitude of the insulin pulse increases dramatically within a few minutes of a rise in blood-sugar levels. Once secreted, the insulin is carried through the blood stream until it is either degraded by the liver or encounters its cognate receptor.

The insulin receptor (IR) is a trans-membrane protein composed of two identical half-receptors. Each half-receptor consists of an extracellular α-subunit and a PM-spanning β-subunit (De Meyts...
Inhibition of the constitutively active β-subunit is relieved when insulin binds to the α-subunit, allowing the activated IR to phosphorylate a number of signalling intermediaries, such as IRS-1. Activated IR are down-regulated by internalisation, degradation and recycling processes within the cell (Standaert & Pollet (1984)). The insulin is degraded in cellular lysosomes and the IR itself is either degraded or recycled to the PM.

Numerous substrates of IR have been identified (Saltiel & Kahn (2001)). At least six eponymous substrates (IRS-1–6) are known (Taniguchi, Emanuelli & Kahn (2006)), but of these, the IRS-1 isoform plays a central role in the metabolic action of insulin. After phosphorylation by the activated IR, IRS-1 recruits phosphatidylinositol 3-kinase (PI3K) to the PM, resulting in the forward propagation of the insulin signal.

PI3K is a scaffold protein of major importance in a number of signalling pathways (Lindmo & Stenmark (2006)). In the insulin signalling pathway, PI3K catalyzes the formation of the secondary messenger phosphatidylinositol 3,4,5-trisphosphate (PIP₃) (Fröjdö, Vidal & Pirola (2009)). PIP₃ functions as a docking site at the PM for the protein kinase Akt, which mediates most of the metabolic effects of insulin: the activation of only a small percentage of the Akt2 pool in insulin-responsive cells results in maximal translocation of GLUT4 to the PM (Ng, Ramm, Lopez & James (2008); Rowland, Fazakerley & James (2011); Tan, Ng, Meoli, Kumar, Khoo, Fazakerley, Junutula, Vali, James & Stockli (2012)).

GLUT4 translocation—the endpoint of the system—is the least well characterised portion of the pathway. GLUT4 trafficking involves at least six steps, all of which are potential targets for regulation by insulin: retention within the cell under basal conditions; release from retention in response to insulin stimulation; movement along microtubules toward the PM; tethering, docking and fusion events at the PM; endocytosis from the PM; and the sorting and redistribution of internalised GLUT4 (Brewer, Habtemichael, Romenskaia, Mastick & Coster (2014); Brewer, Romenskaia, Kanow & Mastick (2011); Stockli, Fazakerley & James (2011)). To date, the regulation of these processes remains only partially understood.

Comprehensive mathematical models of the insulin signalling pathway are rare (Chew, Shia, Lee, Majid, Chua, Sarmidi & Aziz (2009)). The pathway is complex due to the large number of chemical species involved; feed-forward and feedback mechanisms within the cell; redundancy and complementarity between isoforms of the major signalling proteins; and cross-talk with other signalling pathways. One model that captures some of this complexity is that of Sedaghat, Sherman and Quon (2002).

The Sedaghat, Sherman and Quon model (Sedaghat model) is a mathematical model of the insulin signalling pathway in adipocytes. Despite the lapse of more than ten years since its publication, the Sedaghat model is still the most comprehensive model in the literature and is regularly cited. To date, the model been used as the basis of a mathematical study of multi-drug treatment of diabetes (Luni & Doyle (2011)) and as the insulin signalling module in several multi-scale models of glucose metabolism and regulation (Chew et al. (2009); Liu, Hsin & Tang (2009); Magrofuoco, Elvassore & Doyle (2012)). Recently, it has been extended to incorporate the mitogenic action of insulin via the regulation of the transcription factor FOXO (Bates, Liang & Shingleton (2013); Smith & Shanley (2013)) and as one component of a systematic model of hepatic insulin signalling (Huang, Wu, Du, Liu & Chan (2014)). However, the Sedaghat model has some important limitations: key biochemical components in the network are not conserved and the differential equations of the insulin receptor (IR) subsystem are stiff.

In the current work, mathematical and sensitivity analyses of the Sedaghat model are presented. The behaviour of the Sedaghat model over a range of insulin inputs is investigated and sensitive and insensitive parameters in the model identified. Based on the results of this analysis, a reduced state space model of the IR subsystem (the Receptor State Space model) is proposed. Whilst preserving the
input-output relation of the original Sedaghat model, the Receptor State Space model is analytically tractable, computationally efficient and overcomes some of the limitations of the Sedaghat model.

2. Methods

2.1 Structure of the Sedaghat, Sherman and Quon Model

The Sedaghat model is composed of twenty deterministic ODEs involving the twenty-one most significant chemical species in the insulin signalling pathway. Most of the ODEs in the model are derived from the law of mass action, resulting in a set of equations that are first or second degree polynomials in the state variables. The model has been extended by the addition of a number of feedback loops, but the current work focuses on the simpler feed-forward model.

A schematic diagram of the Sedaghat model is shown in Figure 1. State variables are represented by the nodes in the diagram; chemical reactions by edges labelled with the appropriate rate constants and other factors that modulate these reactions. Nodes lying on the left side of the diagram represent chemical species that are located at the PM. For the purposes of this study, the model is divided into two subsystems: the IR subsystem and the downstream subsystem, with the subsystem division occurring immediately above IRS-1. Differential equations, initial conditions and parameter values of the IR subsystem are listed in Table 1.

Insulin signalling commences when extracellular insulin ($x_1$) binds to free IR present at the PM ($x_2$). Singly-bound receptors ($x_3$) then undergo autophosphorylation to a new, phosphorylated state ($x_5$). Following this, the IR either binds a second insulin molecule ($x_4$) or dissociates from the first, becoming once again a free IR. The binding of a second insulin molecule does not affect the phosphorylation state of the receptor, but dissociation causes almost immediate dephosphorylation. The signal is propagated down the cascade via the sum of the variables $x_4$ and $x_5$.

The model explicitly includes IR recycling and degradation. Once- and twice-bound receptors are internalized in an identical manner ($x_7$ and $x_8$) and dephosphorylated. Upon dephosphorylation, the receptors return to the intracellular pool ($x_6$), where they are either exocytosed to the PM or degraded. Synthesis of new intracellular receptors is assumed to occur at a constant, but very low, rate. These sinks and sources are noted in Figure 1.

The multiplicative factor PTP, representing the relative activity of protein tyrosine phosphatases in the cell, modulates the receptor dephosphorylation rate of both surface and internalized receptors. It is thought that under certain pathological conditions, protein tyrosine phosphatases contribute to the development of insulin resistance by increasing the dephosphorylation rate of IR, leading to the premature termination of signalling (Goldstein, Li, Ding, Ahmad & Zhang (1998)). However, it is assumed that PTP equals one under normal physiological conditions.

Although the IR subsystem is the focus of the current work, all analyses of this subsystem are presented in the context of the larger model. Consequently, we include a brief description of the downstream portion of the Sedaghat model.

Post receptor signalling of the model is initiated by the activation of IRS-1 ($x_9$ in Figure 1) by once- and twice-bound phosphorylated IR from the IR subsystem. IRS-1 then binds to PI3K ($x_{11}$) forming the IRS-1/PI3K complex ($x_{12}$). The IRS-1/PI3K complex converts phosphatidylinositol 4,5-bisphosphate ($x_{14}$) to PIP$_3$ ($x_{13}$), the next major signalling component in the cascade. PIP$_3$ is also produced by the phosphorylation of phosphatidylinositol 3,4-bisphosphate ($x_{15}$). It is assumed that the synthesis and degradation of these signalling molecules does not occur at an appreciable rate, and hence these processes are not included explicitly in the model.

The GLUT4 translocation machinery is located downstream from PIP$_3$. Even under fasting condi-
FIG. 1: A schematic diagram of the Sedaghat model. Nodes in the diagram represent state variables; edges represent chemical reactions.
### Differential Equations

\[
\begin{align*}
\frac{dx_2}{dt} &= k_{-1} x_3 + k_{-3} \text{(PTP)} x_5 - k_1 x_1 x_2 + k_{-4} x_6 - k_4 x_2 \\
\frac{dx_3}{dt} &= k_1 x_1 x_2 - k_{-1} x_3 - k_3 x_3 \\
\frac{dx_4}{dt} &= k_2 x_1 x_5 - k_{-2} x_4 + k_{-4} x_7 - k_4 x_4 \\
\frac{dx_5}{dt} &= k_3 x_3 + k_{-2} x_4 - k_2 x_1 x_4 - k_{-3} \text{(PTP)} x_4 \\
&\quad + k_{-4} x_7 - k_4 x_4 \\
\frac{dx_6}{dt} &= k_5 - k_{-5} x_6 + k_6 \text{(PTP)} (x_7 + x_8) + k_4 x_2 - k_{-4} x_6 \\
\frac{dx_7}{dt} &= k_4 x_4 - k_{-4} x_7 - k_6 \text{(PTP)} x_7 \\
\frac{dx_8}{dt} &= k_4 x_4 - k_{-4} x_8 - k_6 \text{(PTP)} x_8
\end{align*}
\]

### Initial Conditions

\[
\begin{align*}
x_2(0) &= 9 \times 10^{-12} \text{M} \\
x_3(0) &= 0 \\
x_4(0) &= 0 \\
x_5(0) &= 0 \\
x_6(0) &= 1 \times 10^{-13} \text{ M} \\
x_7(0) &= 0 \\
x_8(0) &= 0
\end{align*}
\]

### Parameter Values

\[
\begin{align*}
k_1 &= 6 \times 10^7 \\
k_{-1} &= 0.20 \\
k_2 &= k_1 \\
k_{-2} &= 100 k_{-1} \\
k_3 &= 2500.00 \\
k_{-3} &= k_{-1} \\
k_4 &= \frac{k_3}{k_{-4}} \\
k_{-4} &= 0.003 \\
k_5 &= 2.1 \times 10^{-3} \\
k_{-5} &= 2.1 \times 10^{-4} \\
k_6 &= \begin{cases} 
10 k_{-5} \text{ M} \text{ min}^{-1} \text{ if } x_6 + x_7 + x_8 > 1 \times 10^{-13}, \\
60 k_{-5} \text{ M} \text{ min}^{-1} \text{ otherwise}
\end{cases} \\
k_{-5} &= 1.67 \times 10^{-18} \\
k_6 &= 0.461 \\
\text{(PTP)} &= 1.0 \\
\text{(IR}_p\text{)} &= 8.97 \times 10^{-13} \text{ M}
\end{align*}
\]
GLUT4 cycles between an intracellular pool ($x_{20}$) and the PM ($x_{21}$); however, the rate of exocytosis is greatly increased when Akt ($x_{16}$) and PKC-ζ ($x_{18}$) are activated by PIP$_3$. As a consequence, the expression of GLUT4 at the PM increases from an initial value of 4% to slightly less than 40% within 10–15 minutes of the start of insulin stimulation.

2.2 Mathematical Stability Analysis

An eigenvalue analysis of the Sedaghat model was performed by a conventional procedure. A quasi-steady state of the system was found, and the Jacobian of the linearized system at this point was determined. The eigenvalues of the Jacobian were then calculated numerically to determine the stability of this point.

2.3 Parametric Sensitivity Analysis

A local parametric sensitivity analysis (PSA) of the Sedaghat model was carried out by simulating the model over a 60 minute interval in MATLAB (R2014a Mathworks 2014). When GLUT4 is at the PM, glucose can flow down its concentration gradient into the cell. Thus the time integral of GLUT4 expressed at the PM (the glucose transport) was defined as the model output. The percentage change in the glucose transport ($\Delta GT$) was calculated as model parameters were individually perturbed by ±5% and ±10%.

Of a wide variety of possible insulin input profiles, an initial fifteen minute insulin pulse over a total 60 minute simulation was chosen. This pulsatile delivery profile facilitates the study of both the transition from basal to maximal GLUT4 expression and also the relaxation back to the basal state.

In addition, the PSA was performed at four different insulin concentrations. Much of the experimental and theoretical work on the insulin signalling pathway uses insulin concentrations at either the basal or maximal level, usually taken to be 100 or 200 nM. However, plasma insulin concentrations in vivo are generally less than 1 nM (Akirav, Chan, Inouye, Riddell, Matthews & Vranic (2004); Satoh, Nguyen, Trujillo, Imamura, Usui, Scherer & Olefsky (2005)). Consequently, a logarithmic scale of insulin concentrations that spans both the physiological and experimental range (0.1 nM, 1 nM, 10 nM and 100 nM) was used.

For each parameter in the model, a four-tuple representing $\Delta GT$ at the various insulin concentrations was calculated. These four-tuples were then clustered in MATLAB using the Euclidean metric and classified as either sensitive (causing a substantial change in the glucose transport) or insensitive (causing little or no change in the glucose transport).

2.4 Model Reduction

Based on the results of the PSA, a model reduction of the IR subsystem was carried out. The purpose of this model reduction was to preserve the input-output relation of the IR subsystem of the Sedaghat model rather than embody all the complexity of the IR binding subsystem. In addition, it was thought desirable to conserve IR numbers and, if possible, reduce the stiffness of the differential equations in the Sedaghat IR subsystem.

Insensitive states and parameters were removed from the model, and rate constants for the reduced model were calculated by fitting the time course of GLUT4 expression at the PM in the reduced model to that of the full Sedaghat model. Fitting was performed using the simulated annealing algorithm in MATLAB, with a vector of corresponding parameter values from the full model as an initial condition.
3. Results

3.1 Stability Analysis

The Sedaghat model has no fixed points, however it does have a quasi-steady state in the basal condition (that is, at the vector of initial conditions but with zero insulin input). The differential equation given for $x_6$ (intracellular IR) in the Sedaghat model is

$$\frac{dx_6}{dt} = k_5 - k_-5x_6 + k_6(\text{PTP})(x_7 + x_8) + k_4x_2 - k_-4x_6,$$

where $k_5$ is the rate of synthesis and $k_-5$ the rate of degradation of intracellular IR. These parameters are defined in the model as

$$k_-5 = 1.67 \times 10^{-18},$$

and

$$k_5 = \begin{cases} 10k_-5, & \text{if } x_6 + x_7 + x_8 > 1 \times 10^{-13}, \\ 60k_-5, & \text{otherwise.} \end{cases}$$

It can be seen that the rate of synthesis generally substantially exceeds the rate of degradation, although both rates are small, being of the order of MATLAB’s machine precision (Moler (2004)). The small magnitude of these rates makes their numerical analysis somewhat difficult, however, when the model was simulated with a constant insulin input of 100 nM for 240 minutes an increase of approximately 4.4% in total receptor numbers, mainly in the variables $x_2$ and $x_6$, was observed (results not shown). Indeed, an increase of 0.4% was found even when the model was simulated for 240 minutes with zero insulin input. The Jacobian of the system in the basal condition was also found, and numerical calculation of the eigenvalues confirmed that the differential equations of the IR subsystem are stiff (Liu & Yuan (2010); Ulfhielm (2006)).

3.2 Sensitivity Profiles

Results of the PSA are shown in Figures 2 and 3. Figure 2 shows the sensitivity profiles of parameters from the IR subsystem of the Sedaghat model, with $\Delta GT$ versus insulin pulse concentration. The majority of parameters from the IR subsystem were insensitive across all four insulin levels; the parameters PTP and IRp were sensitive at all insulin levels; and the rate constants $k_1$ and $k_-3$ were sensitive at low insulin levels only. For all sensitive parameters, a decline in sensitivity at higher concentrations of insulin was evident.

Sensitivity profiles of rate constants from the downstream portion of the Sedaghat model are shown in Figure 3. A greater number of sensitive parameters is evident in this figure, demonstrating that the signal is amplified as it propagates through the signal transduction cascade. A notable feature of many of the profiles, such as $k_8$, $k_9$ and $k_{12}$, is an obvious vertical asymmetry in the graph: sensitivity on the upper side of the profile is depressed at high insulin concentrations. This is due to a saturation phenomenon caused by the GLUT4 expression approaching its steady state maximum early in the simulation, as has been noted in Liu and Yuan (2010). Consequently, in order to minimize bias resulting from saturation, entries in the four-tuples were calculated using the central difference of the $+5\%$ and $-5\%$ perturbations. That is, if $C(x_i)$ represents the central difference of the sensitivity profile of the $i$th parameter, then

$$C(x_i) = \Delta GT(1.05x_i) - \Delta GT(0.95x_i).$$
PSA was also carried out using a constant insulin input (Gray (2013)). Parameter sensitivity rankings for both the fifteen minute step input and the constant input data are listed in ascending order in Table 2. It should be noted that, although the rank order changes somewhat with this alternative insulin input.
Fig. 3: Sensitivity profiles of rate constants from the downstream subsystem of the Sedaghat model. Increased parameters are shown as closed blue squares (10%) and open cyan squares (5%). Decreased parameters are shown as closed red triangles (10%) and open magenta triangles (5%). In all simulations a fifteen minute insulin pulse at a concentration of either 0.1 nM, 1 nM, 10 nM or 100 nM was used.
profile, no parameter switches from the sensitive to the insensitive group or vice-versa.

3.3 Model Reduction

PSA of the model revealed that only two processes in the Sedaghat IR subsystem are sensitive for glucose transport. These processes consist of the forward reaction pathway from $x_2$ through to $x_4$ and $x_5$ via $x_3$, which features the sensitive rate constant $k_1$; and the backward pathway from $x_4$ and $x_5$ to $x_2$, containing the multiplicative combination of PTP and $k_{-3}$. Thus a simplified receptor state space model that features only these parameters and state variables was proposed. The equations for this model, the Receptor State Space model, are as follows:

$$\frac{dy_2}{dt} = r_1y_1y_2 - r_{-1}y_5$$  \hspace{1cm} (3.1)

$$\frac{dy_5}{dt} = r_1y_1y_2 - r_{-1}y_5,$$  \hspace{1cm} (3.2)

where $y_2$ is the concentration of unbound IR and $y_5$ is the concentration of both singly- and doubly-bound IR. In other words, $y_2$ corresponds to $x_2$ and $y_5$ corresponds to the sum of the variables $x_4$ and $x_5$ from the Sedaghat model. The rate constant of the forward pathway is $r_1$, which most closely corresponds to $k_1$ in the original model and $r_{-1}$ is the backward rate constant, corresponding to (PTP)$k_{-3}$.

Rate constants for the model were determined by simulated annealing (SA), using the output of the Sedaghat model as a benchmark. The corresponding initial conditions and rate constants from the Sedaghat model ($k_1$ and $k_{-3}$) were used as initial values for the SA algorithm. It should be noted that the set of values to which the algorithm eventually converged differ from the Sedaghat ones in the fourth or fifth significant figure only. Full details of the model (differential equations, initial conditions and parameter values) are given in Table 3.

3.4 Analysis of the Receptor State Space Model

3.4.1 Fixed Points

Since it is assumed that the number of IR is conserved, $y_2 + y_5 = R$, where $R$ represents the total IR. Substituting this into Equation (3.2), we have

$$\frac{dy_5}{dt} = r_1y_1y_2 - (r_1y_1 + r_{-1})y_5 = f(y_5).$$  \hspace{1cm} (3.3)

It can be seen that there is a fixed point of the system at $y_1 = 0$, $y_5 = 0$, representing the basal (unstimulated) state. If we assume positive values of $y_1$, then $y_5^*$, the insulin stimulated steady state value of $y_5$, is given by

$$y_5^* = \frac{r_1y_1R}{r_1y_1 + r_{-1}} = R - \frac{Rr_1}{r_1y_1 + r_{-1}}.$$

The stability of this fixed point can be determined by linearizing about $y_5^*$. We can see that since $y_1 \geq 0$,

$$f'(y_5^*) = -(r_1y_1 + r_{-1}) < 0,$$

implying that $y_5^*$ is stable, with characteristic time scale $\tau$ given by

$$\tau = \frac{1}{|f'(y_5^*)|} = \frac{1}{r_1y_1 + r_{-1}}.$$
Table 2: Sensitivity rankings of parameters from the Sedaghat model. A fifteen-minute insulin pulse and a constant level of insulin were used as inputs to the model. Rate constants are listed in order from most to least sensitive.

<table>
<thead>
<tr>
<th>Sensitive Parameters</th>
<th>15 Min Insulin Pulse</th>
<th>Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td>$k_{-13}$</td>
<td>$k_{13'}$</td>
</tr>
<tr>
<td>PTEN</td>
<td>$k_{-9}$</td>
<td>$k_{9}$</td>
</tr>
<tr>
<td>PTP</td>
<td>$k_{9}$</td>
<td>$k_{12}$</td>
</tr>
<tr>
<td>PTP</td>
<td>$k_{8}$</td>
<td>$k_{8}$</td>
</tr>
<tr>
<td>$k_{13'}$</td>
<td>$k_{-9}$</td>
<td>$k_{-13}$</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>$k_{8}$</td>
<td>$k_{-12}$</td>
</tr>
<tr>
<td>$k_{8}$</td>
<td>$k_{-8}$</td>
<td>$k_{13}$</td>
</tr>
<tr>
<td>IR_p</td>
<td>$k_{-7}$</td>
<td>$k_{-8}$</td>
</tr>
<tr>
<td>$k_{-7}$</td>
<td>$k_{-8}$</td>
<td>PTP</td>
</tr>
<tr>
<td>$k_{-8}$</td>
<td>$k_{7}$</td>
<td></td>
</tr>
<tr>
<td>$k_{-12}$</td>
<td>$k_{1}$</td>
<td></td>
</tr>
<tr>
<td>$k_{-3}$</td>
<td>IR_p</td>
<td></td>
</tr>
<tr>
<td>$k_{7}$</td>
<td>$k_{-7}$</td>
<td></td>
</tr>
<tr>
<td>$k_{13}$</td>
<td>$k_{-11}$</td>
<td></td>
</tr>
<tr>
<td>$k_{1}$</td>
<td>$k_{3}$</td>
<td></td>
</tr>
<tr>
<td>$k_{11}$</td>
<td>$k_{13}$</td>
<td></td>
</tr>
<tr>
<td>$k_{-11}$</td>
<td>$k_{11}$</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insensitive Parameters</th>
<th>15 Min Insulin Pulse</th>
<th>Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{14}$</td>
<td>$k_{14}$</td>
<td></td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>$k_{10}$</td>
<td></td>
</tr>
<tr>
<td>$k_{2}$</td>
<td>$k_{4'}$</td>
<td></td>
</tr>
<tr>
<td>$k_{-4}$</td>
<td>$k_{-2}$</td>
<td></td>
</tr>
<tr>
<td>$k_{4}$</td>
<td>$k_{2}$</td>
<td></td>
</tr>
<tr>
<td>$k_{3}$</td>
<td>$k_{-4}$</td>
<td></td>
</tr>
<tr>
<td>$k_{6}$</td>
<td>$k_{4}$</td>
<td></td>
</tr>
<tr>
<td>$k_{-5}$</td>
<td>$k_{6}$</td>
<td></td>
</tr>
<tr>
<td>$k_{5}$</td>
<td>$k_{3}$</td>
<td></td>
</tr>
<tr>
<td>$k_{4'}$</td>
<td>$k_{5}$</td>
<td></td>
</tr>
<tr>
<td>$k_{-1}$</td>
<td>$k_{-5}$</td>
<td></td>
</tr>
<tr>
<td>$k_{4'}$</td>
<td>$k_{-4'}$</td>
<td></td>
</tr>
<tr>
<td>$k_{-14}$</td>
<td>$k_{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k_{-2}$</td>
<td>$k_{-14}$</td>
<td></td>
</tr>
<tr>
<td>SHIP</td>
<td>SHIP</td>
<td></td>
</tr>
<tr>
<td>$k_{-10}$</td>
<td>$k_{-10}$</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: State variables, differential equations, initial conditions and parameter values of the Receptor State Space model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chemical Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y_1$</td>
<td>Insulin input</td>
</tr>
<tr>
<td>$y_2$</td>
<td>Unbound surface IR</td>
</tr>
<tr>
<td>$y_3$</td>
<td>Bound surface IR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Differential Equation</th>
<th>Initial Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{dy_2}{dt} = r_1y_1y_2 - r_{-1}y_5$</td>
<td>$y_2(0) = 9 \times 10^{-13}$ M</td>
</tr>
<tr>
<td>$\frac{dy_3}{dt} = r_1y_1y_2 - r_{-1}y_3$</td>
<td>$y_3(0) = 0$ M</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y_1$</td>
<td>$1 \times 10^{-7}$ if $t &lt; 15$, 0 otherwise</td>
</tr>
<tr>
<td>$r_1$</td>
<td>$6 \times 10^7$ M$^{-1}$ min$^{-1}$</td>
</tr>
<tr>
<td>$r_{-1}$</td>
<td>0.202 min$^{-1}$</td>
</tr>
</tbody>
</table>

Time courses of IR activation for the Receptor State Space model with different initial conditions are shown in Figure 4(a), clearly demonstrating the existence of a single, stable fixed point. In all cases, a constant insulin input of 10 nM was used. In contrast, Figure 4(b) shows time courses of IR activation for varying insulin concentrations but with an initial condition of zero, demonstrating that both $y_3^*$ and $\tau$ are functions of the insulin concentration.

3.4.2 Analytic Solution The simplified equations of the Receptor State Space model are amenable to analytic solution. Provided that the derivative $f(y_5)$ is non-zero, reciprocals of both sides of Equation (3) can be taken to obtain

$$\frac{dy_5}{dt} = \frac{1}{y_1y_1R - (r_1y_1 + r_{-1})y_5} = \left(\frac{1}{r_1y_1 + r_{-1}}\right)\left(\frac{1}{y_5^* - y_5}\right).$$

Thus

$$y_5(t) = y_5^* - \alpha e^{-(r_1y_1 + r_{-1})t},$$

where $\alpha = y_5^* - y_5(0)$.

Time courses of IR activation in response to a fifteen-minute insulin pulse for the Sedaghat and Receptor State Space models are shown in Figure 5(a). IR activation ($x_4 + x_5$ in Sedaghat; $y_5$ in the Receptor State Space model) represents the output of the IR subsystem that is propagated downstream. As can be seen in the figure, there is a slight divergence between the two models during the ‘plateau’
Fig. 4: Time course of IR activation for the Receptor State Space model for (a) constant insulin (10 nM) and varying initial conditions; and (b) varying insulin concentrations. Parameter values used are listed in Table 3.

phase of IR activation at high insulin concentrations. At low insulin concentrations, neither of the models reaches the plateau and so the two outputs match closely. In contrast, Figure 5(b) shows a comparison of the final output—the GLUT4 expression at the PM—of the two models. In this figure, the output of the system utilising the Receptor State Space model is practically identical to that of the Sedaghat model at all insulin levels tested.
FIG. 5: A comparison of (a) IR activation; and (b) GLUT4 translocation for the two models for varying insulin concentrations. Receptor State Space model outputs are indicated by dashed lines; Sedaghat model outputs by unbroken lines. The input was a fifteen-minute insulin pulse of 0.1 nM (red), 1 nM (magenta), 10 nM (cyan) or 100 nM (blue).
4. Discussion

Mathematical analysis of the Sedaghat model revealed a lack of conservation of IR and stiffness in the equations of the IR subsystem. In at least two recent papers utilising the Sedaghat model (Luni & Doyle (2011); Magrofuoco et al. (2012)), the IR subsystem was modified by removing the IR degradation/synthesis terms to make a closed subsystem. Furthermore, the drift in the system to higher numbers of IR under insulin stimulation is counter to experimental results: upregulation of IR numbers occurs in response to long-term insulin withdrawal, rather than insulin stimulation (Puig & Tjian (2005)). Thus, it is desirable to reduce the model in such a fashion that the IR is conserved and, if possible, create a non-stiff system of equations.

To guide the model reduction, a PSA of the Sedaghat model was carried out by individually perturbing model parameters and measuring changes in the output. The time integral of GLUT4 expression at the plasma membrane was chosen as the model output, as it reflects a biologically important measure—the ability of the cell to uptake glucose from the blood, which is widely thought to be the rate-limiting step of glucose metabolism (Holman & Cushman (1996); Rowland et al. (2011)).

The PSA of the Sedaghat model revealed few sensitive parameters in the IR subsystem. Only $k_1$, $k_{-3}$ and PTP were noticeably sensitive, and these parameters exhibited a decline in sensitivity at high insulin concentrations. Furthermore, when the PSA was repeated with a constant insulin input, none of the parameters changed from sensitive to insensitive or vice versa. (See Table 2.)

Of the sensitive parameters in the IR subsystem, the factor PTP is a somewhat anomalous case. In the Sedaghat model, PTP impinges on the signalling pathway at four locations: the dephosphorylation of twice- and singly-bound internalised IR; the dephosphorylation of once-bound surface IR; and the dephosphorylation of IRS-1. It is the only factor to modulate several processes; in all cases, it appears as a multiplicative factor with another rate constant, either $k_6$, $k_{-3}$ or $k_{-7}$. As a result, its action can be assessed by considering the sensitivity of these rate constants as they are perturbed individually. It can be seen from Figures 2 and 3 that $k_6$ is insensitive across the whole insulin range, and that $k_1$ and $k_{-7}$ are sensitive, but only to a moderate degree. Thus, as far as the IR subsystem is concerned, the only significantly sensitive pathway modulated by PTP is the dephosphorylation of once-bound surface IR.

The Receptor State Space model results from eliminating all but the two sensitive pathways of the Sedaghat IR subsystem. That is, the forward reaction pathway from $x_2$ to $x_5$ and the backward pathway from $x_5$ to $x_2$. Hence the Receptor State Space model features only three state variables, namely: $y_1$, representing the insulin input; $y_2$, representing empty or unactivated surface IR; and $y_5$, representing activated surface IR. Thus, the processes of receptor internalization, recycling, synthesis and degradation have been omitted; and the secondary binding of insulin to the surface receptor has been elided into the receptor activation step. Consequently, only the state variable $y_5$, modulated by the factor IRp, propagates the signal downstream.

In the Receptor State Space model the conservation of IR is made explicit. As a result, for this subsystem at least, a fixed point at high insulin exists and an analytic solution can be found. (However, as the system reduction is limited to the top of the signalling cascade, this does not resolve the issue of non-conservation of GLUT4 in the downstream subsystem.)

It should also be noted that the total number of receptors differs by 10% between the two models, at least initially. In the Sedaghat model it is assumed that initially only 90% of the total receptor concentration is expressed at the PM, with the remainder present in the internalisation/recycling pathway. In contrast, the Receptor State Space model only includes surface receptors, leading to a lower number of receptors. Furthermore, since the number of IR is not conserved in the Sedaghat model, the disparity between the two models increases the longer the simulation is run.
Parameter values for the Receptor State Space model were determined by simulated annealing, using the output of the Sedaghat model as a benchmark. The parameter values obtained only differ from the Sedaghat ones in the fourth or fifth significant figure. As simulated annealing is a stochastic algorithm, a slightly different set of values will be obtained each time the algorithm is run, and hence this difference is not considered significant.

The output of the two models—Sedaghat and the system utilising the Receptor State Space model—are closely matched over the entire range of insulin concentrations tested, as seen in Figure 5.

5. Conclusion

In the current work, we have developed a potential alternative to the IR subsystem of the Sedaghat model. This model—the Receptor State Space model—retains the input-output relation of the IR subsystem of the Sedaghat model. However, the simplified equations of the Receptor State Space model are non-stiff, reducing computational time and resulting in a system that is analytically tractable. Furthermore, the non-conservation of IR in the Sedaghat model is eliminated. Consequently, we propose the Receptor State Space model as an ideal alternative to the IR subsystem of the Sedaghat model for situations where: the internal dynamics of the IR subsystem can be safely ignored; the model is to be embedded as a component of a larger multi-scale system; simulations over long time periods are necessary; or many simulations are required.

References


