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Review

Compartmental Analysis of the Insulin-induced GLUT4 Recruitment in Adipocytes

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Insulin stimulates glucose uptake in muscle and adipose tissue and thus maintains normal blood glucose level in our body. Derangement of this process causes many grave health problems. Insulin stimulates glucose transport primarily by recruiting GLUT4 from its intracellular storage sites to the plasma membrane. The process is complex and involves GLUT4 trafficking through multiple subcellular compartments (organelles) and many protein functions, details of which are poorly understood. This review summarizes a recent development to isolate and characterize the individual intracellular GLUT4 compartments and to illustrate how this compartmental analysis will help to identify the insulin-sensitive step or steps in the insulin-induced GLUT4 recruitment in rat adipocytes. The review does not cover the recent exciting development in identification of many proteins implicated in this process.

Keywords: Glucose transporter, Compartment, Kinetics, Insulin action, Adipocytes

GLUT4 recycling and insulin action

Regulation of glucose metabolism is a key element of glucose homeostasis in mammals and humans (Olefsky, 1999). Insulin plays a key role, primarily by stimulating glucose uptake in muscle and adipose cells (Birnbaum, 1992; James *et al.*, 1994). Dysfunction of this process produces grave health problems, including diabetes and obesity (DeFronzo and Ferrannini, 1991; DeFronzo *et al.*, 1992; Rea and James, 1997). Glucose uptake (transport) by mammalian cells are mediated by a family of intrinsic membrane proteins collectively known as the facilitative glucose transporters (GLUT) (Pessin and Bell, 1992; Olson and Pessin, 1996). Of the five isoforms known in this family, GLUT4 is the major player in stimulation of glucose uptake by insulin. GLUT4 is selectively expressed in insulin target cells as the major isoform, with a small amount of GLUT1 as a minor isoform. Like any biologically important protein, GLUT4 is regulated at several different levels, including its synthesis, degradation, and catalytic activity. The most important regulation of GLUT4 in these insulin target cells, however, occurs via subcellular compartmentalization and targeting to the plasma membrane where this protein functions. This mechanism of insulin action is similar if not identical in muscle and adipose cells, and the bulk of the studies on the mechanisms of the insulin action on GLUT4 regulation have been performed using adipocytes with their ready availability as a monocellular suspension.

GLUT4 in rat adipocytes is largely sequestered in intracellular sites. Only a small fraction (5% or less) is found at the plasma membrane in the absence of insulin, and insulin causes rapid redistribution of GLUT4 from these intracellular sites to the plasma membrane (Cushman and Wardzala, 1980; Suzuki and Kono, 1980). This GLUT4 recruitment accounts for most of the stimulation of glucose transport by insulin (Zorzano *et al.*, 1989; Holman *et al.*, 1990; Kern *et al.*, 1990; Hansen *et al.*, 1995). Steady state kinetic measurements further revealed that GLUT4 constantly and rapidly recycles between the plasma membrane and intracellular sites via membrane trafficking, and that insulin-induced GLUT4 recruitment is accompanied by a reduction in GLUT4 endocytic (internalization) rate constant and an increase in GLUT4 exocytic (externalization) rate constant (Jhun *et al.*, 1992; Czech and Buxton, 1993; Satoh *et al.*, 1993; Yang and Holman, 1993).

Evidence indicates that the itinerary of GLUT4 recycling involves not only membrane dynamics such as vesicle

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budding/fission and vesicle docking/fusion, but also a series of intracellular compartments (collectively known as endosomal compartments) such as early, sorting, sequestering and recycling endosomes. Thus, GLUT4 internalization appears to occur via clathrin-coated endocytosis (Slot *et al.*, 1991; Chakrabarti *et al.*, 1994; Al-Hasani *et al.*, 1998; Ceresa *et al.*, 1998; Volchuk *et al.*, 1998), the pathway shared by a number of membrane proteins including TfR and IGF-II receptors (Mellman, 1996; Mukherjee *et al.*, 1997). GLUT4 is nevertheless regulated by insulin selectively from other recycling proteins, indicating the presence of a mechanism and/or compartment where GLUT4 is sorted from other receptors (Livingstone *et al.*, 1996; Martin *et al.*, 1996). The presence of a GLUT4-specific storage compartment has been suggested by a large intracellular GLUT4 pool that does not appear to be directly involved in the recruitment (Slot *et al.*, 1991; Lee *et al.*, 1997).

Biochemical studies, on the other hand, have revealed a number of proteins that colocalize with GLUT4 in vesicles isolated from microsomes, implicating them in GLUT4 regulation (James *et al.*, 1987; Tanner and Lienhard, 1989; Slot *et al.*, 1991; Smith *et al.*, 1991; Cain *et al.*, 1992; Czech and Buxton, 1993; Laurie *et al.*, 1993; Thoidis *et al.*, 1993; Herman *et al.*, 1994; Volchuk *et al.*, 1995; Kandror and Pilch, 1996; Martin *et al.*, 1996; Kandror and Pilch, 1998). Since multiple subcellular compartments of endosomal system are involved in GLUT4 regulation (Pessin *et al.*, 1999), it is expected that each of these proteins function in a distinct compartment. However, the compartment-specific association of these proteins and the roles they play in GLUT4 sequestration or recruitment are largely not known, mainly due to inability to separate these GLUT4 compartments individually and study their roles in GLUT4 recycling.

Separation of GLUT4 compartments in rat adipocytes

Conventionally, isolation of subcellular compartments is achieved by cell homogenization followed by differential density gradient centrifugation. This separates the plasma membrane GLUT4 and intracellular GLUT4 in PM and LDM fractions, respectively (Simpson *et al.*, 1983; Jhun *et al.*, 1991). Attempts at further fractionation of intracellular GLUT4 in LDM into individual compartments have been not successful, partly because the cell homogenization causes an extensive vesiculation of organelles, which impedes separation of individual organelles with distinct origin. Recently, we (Lee *et al.*, 1999) have largely overcome this problem by using hypotonic lysis in lieu of mechanical homogenization.

Hypotonic lysis creates tears in the plasma membrane, through which a majority of the intracellular GLUT4 is released into the medium, free from the plasma membrane. The remainder is trapped inside ruptured plasma membrane sheets, which is readily recovered as a pellet of low-speed centrifugation pellet (900 g pellet). The GLUT4 released to the medium is readily and quantitatively recoverable from the 900 g-supernatant by high-speed centrifugation (185,000 g pellet). The procedure is illustrated in Fig. 1.

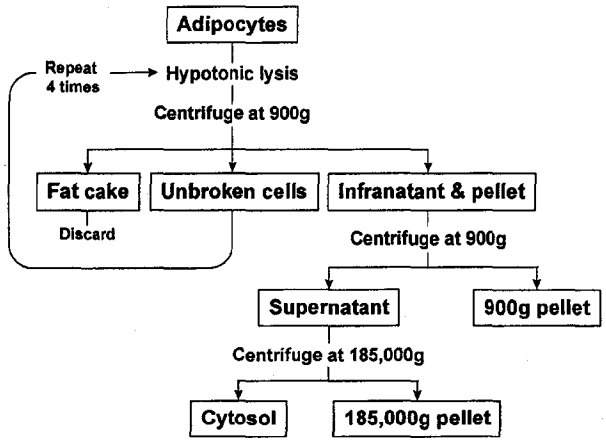

Fig. 1. The flow sheet illustrating the hypotonic lysis of rat adipocytes and the preparation of 900 g-pellet and 185,000 g-pellet.

Typically, the 185,000 g pellet recovers 80 and 70% of the intracellular GLUT4 pool (or approximately 75 and 45% of the cellular GLUT4) for the basal and insulin-stimulated sates, respectively. The remaining portion of the intracellular GLUT4 pool (trapped intracellular pool, or T) occurs in the 900 g pellet together with ruptured plasma membrane sheets (PM). The 185,000 g pellet can be further separated on glycerol density gradients into two fractions, H (heavy) and L (light).

Electron microscopy revealed that the organelles in each of these fractions are largely intact with little vesiculation artifacts and are morphologically distinct. Thus, 900 g pellet contained intact nuclei and mitochondria sequestered in cell boundaries with large membranous structures similar to rough and smooth endoplasmic reticulum. Fraction H showed only large membranous structures with relatively few and variously sized vesicular structures. Fraction L, on the other hand, contained only small vesicular particles of uniform size (estimated to be 50-100 nm in diameter, together with ribosome-like structure and abundant amorphous protein fragments.

Organelle-specific marker distribution data (Lee *et al.*, 1999) have further revealed that the entire plasma membrane (PM) and endosomal structures (T) including the nucleus, mitochondria and the endoplasm reticulum were recovered in the 900 g-pellet, whereas the fractions H and L were made of exclusively intracellular compartments. PM and T in 900 g pellet are not physically separable at this time, and are separately characterized indirectly by studying 900 g-pellet and a highly enriched PM fraction that can be readily prepared by the conventional subcellular fractionation method after homogenization (Simpson *et al.*, 1983; Jhun *et al.*, 1991).

Table 1. Percent distribution of protein and GLUT4 in the absence and presence of insulin.

		Basal		Insulin	
		Protein	GLUT4	Protein	GLUT4
900 g	T	55.3 ± 5.8	25.0 ± 2.5	59.4 ± 6.3	55.0 ± 4.1
	(T_H)	22.6 ± 3.9	21.5 ± 3.4	25.8 ± 4.1	43.2 ± 6.3
	(T_L)	32.7 ± 1.4	3.5 ± 0.5	33.6 ± 1.9	11.9 ± 1.7
185,000 g		45.2 ± 4.2	74.3 ± 2.9	41.8 ± 3.9	45.1 ± 2.2
	H	24.7 ± 2.2	56.2 ± 4.1	22.2 ± 2.1	41.7 ± 3.7
	L	20.3 ± 0.3	18.1 ± 1.3	19.6 ± 0.5	3.7 ± 0.7

Values represent mean and SD from three independent experiments.

The GLUT4 compartments in PM, T, H, and L will be referred to as G4PM, G4T, G4H and G4L, respectively. Insulin treatment changed these GLUT4 compartment sizes in a characteristic manner without affecting protein contents of the compartments. This is summarized in Table 1. Typically, insulin treatment reduced G4H and G4L and increased G4T as will be discussed later quantitatively. G4L has been purified in its native state by immunoadsorption, while G4H and part of G4T have been purified by immunoadsorption after sonication. They were shown to be distinct in their protein composition.

GLUT4 compartment in L may represent GLUT4-exocytic vesicles

The GLUT4 compartment in L (G4L) is readily and quantitatively purifiable by 1F8-immunoadsorption in its native state. L is a homogeneous population of uniformly small vesicles (50-100 nm in diameter). L is free of vesiculated plasma membranes, endoplasmic reticulum, TGN, mitochondria and recycling endosomes. The fraction L, on the other hand, contains PI3K, Akt-2, and γ-adaptin, in addition to GLUT4 and IRAP. G4L is enriched not only in IRAP, but also in VAMP2, a v-SNARE protein found in exocytic vesicles. Furthermore, G4L was exceptionally sensitive to insulin stimulation, being reduced from 18% of total cellular GLUT4 to less than 4% by insulin-stimulation, a 75-80% reduction (Table 1). Insulin typically increases PM GLUT4 from its basal value of 5% to 35% after insulin treatment, a recruitment amounting to 30% of cellular GLUT4 from intracellular pool to the plasma membrane. Thus, G4L is the most insulin-sensitive GLUT4 compartment, and accounts for as much as a half of the insulin-induced GLUT4 recruitment in adipocytes.

The role of G4L in insulin-induced GLUT4 recruitment and the molecular mechanisms by which insulin reduces this compartment are yet to be identified. An interesting possibility is that these presumably exocytic GLUT4 vesicles are structurally associated with a certain retention molecule or apparatus, and insulin interrupts this association, allowing the vesicles to undergo exocytosis. The presence of such a retention mechanism has been suggested by the observation that GLUT4 C-terminal (Lee *et al.*, 1997) or IRAP N-terminal (Waters *et al.*, 1997) recombinant peptides introduced in excess caused GLUT4 translocation to the plasma membrane in adipocytes, partly mimicking insulin action. This putative retention molecule is yet to be identified. Alternatively, insulin may stimulate the fusion of GLUT4 exocytic vesicles to the plasma membrane, thus reducing G4L compartment size. Fusion machinery proteins such as v- & t-SNAREs have been shown to participate in insulin stimulated GLUT4 translocation (Pessin *et al.*, 1999) and may be targets of insulin regulation as might be the recently identified Syntaxin 4-interacting protein, Synip (Min *et al.*, 1999). These two possibilities are not necessarily mutually exclusive.

GLUT4 comartment in fraction H is the major insulin-sensitive storage pool

The GLUT4 compartment in the H fraction (G4H) is the largest intracellular GLUT4 compartment that is also insulin-sensitive. Typically, it contains 55% of total GLUT4 in basal adipocytes and 40% of total GLUT4 in insulin-stimulated adipocytes. G4H and G4L contribute equally to the recruitment to the plasma membrane. H is particularly enriched in TGN38 and γ-adaptin. G4H (the immunopurified GLUT4-compartment of the fraction H) is free of GLUT1 (Lee *et al.*, 2000). These findings suggest that G4H may be the putative GLUT4-specific storage or sequestration compartment.

GLUT4 intracellular compartment (T) in 900G pellet is largely insulin-insensitive

900 g-pellet contains plasma membrane sheets (PM) and trapped intracellular organelles (T) as already discussed. 900 g-pellet contains more than 95% of integrin and α-adaptin, indicating that this fraction contains practically all of the plasma membrane. 900 g-pellet typically contains 26 and 55% of total GLUT4 of basal and insulin treated adipocytes, respectively (Table 1). The amount of plasma membrane GLUT4 (G4PM) determined by cell surface labeling, on the

other hand, amounted to 6 and 36% of total cellular GLUT4 in basal and insulin-stimulated sates, respectively (Jhun *et al.*, 1992). Thus by a simple subtraction, one can realize that the pool size of this intracellular GLUT4 compartment in T (G4T) was not much affected by insulin, amounting to approximately 20% of the total cellular GLUT4 for both basal and insulin-stimulated adipocytes. However, this estimation is based on assumption that G4T itself is a single compartment, for which we have no data to support. Indeed, G4T may include GLUT4 endocytotic vesicles. G4T can be separated into heavy and light fractions (denoted as $G4T_H$ and $G4T_L$, respectively), after sonication and glycerol density sedimentation (Lee *et al.*, 1999), and $G4T_L$ may be GLUT4 endocytic vesicles as suggested by its enrichment in alpha-Adaptin and Rab5. Thus insulin sensitivity of this putative sorting compartment can not be studied directly at this time.

Possible roles of G4T, G4H and G4L in the GLUT4 itinerary; a model

Possible roles of the GLUT4 endosomal compartments, G4T, G4H, and G4L to the GLUT4 recycling pathway is proposed as a working model in Fig. 2. G4T, with its modest size that is less or not affected by insulin, is most likely the early or sorting endosomes. Such a sorting compartment must exist as GLUT4 is believed to be endocytosed via the clathrin coat-mediated route together with many other proteins and receptors. G4H, the largest GLUT4 compartment supplying approximately a half of insulin-induced GLUT4 recruitment, on the other hand, is likely the long-sought, putative GLUT4 storage compartment. This compartment may correspond to the tubulovesicular structure as seen in electron microscopy (Slot *et al.*, 1991), from which exocytic vesicles may bud. G4L may be an authentic vesicular compartment of GLUT4 exocytic vesicles in "ready-to-go" state for fusion to the plasma membrane. Insulin reduces this compartment by some 80%, indicating that this compartment, specifically at its SNARE-mediated fusion to PM, is the major control point for the insulin-induced GLUT4 recruitment. G4H and G4L as purified by 1F8-immunoadsorption are free of GLUT1 (Lee *et al.*, 2000), indicating that they are the endosomal compartments specific to GLUT4 recycling. It has been suggested that separate endosomal GLUT4 compartments exist, one undergoing constitutive recycling, and the other is responsible to insulin-induced GLUT4 recycling (Volchuk *et al.*, 1996; Kao *et al.*, 1998), the relationship of which to G4T, G4H and G4L are yet to be determined.

Kinetic analysis reveals that insulin modulates GLUT4 recycling at multiple steps

The development of techniques to separate and measure the four distinct GLUT4 compartments G4T, G4H, G4L and G4PM discussed above would also provide us with valuable data to analyze the kinetic properties of the GLUT4 trafficking

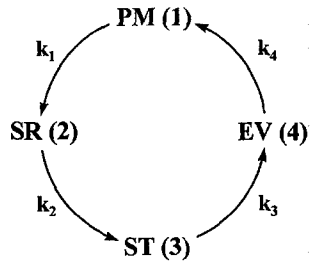

Fig. 2. Four major GLUT4-compartments identified in rat adipocytes, and a simple kinetic model used to analyze the GLUT4 recycling. See text for details.

in this classical insulin target cell. Here, we have simulated the system using a simple, four-compartment model. By adjusting model parameters to reproduce as faithfully as possible the observed behavior of the system, both in the basal state and maximally stimulated by insulin, we hoped to further clarify the changes in kinetic coefficients induced by insulin stimulation. Having available estimates of steady state pool sizes, both in the presence and absence of insulin, greatly reduced the number of free parameters to be adjusted, and thereby enhanced the relevance of the model results to the actual system.

The structure of the model was assumed to be a simple circular arrangement, as included in Fig. 2. The four compartments, denoted Plasma Membrane (PM), Sorting (SR), Storage (ST), and Exocytotic Vesicle (EV), each receive inflow from the preceding compartment, and in turn, provide an inflow to the following compartment. We further assume that G4PM, G4T, G4H and G4L represent PM, SR, ST and EV, respectively. This assumption is largely supported by the findings described above. The flow between compartments is assumed to be unidirectional, with a rate linearly dependent upon only the content of the source compartment.

Thus the flux, J_1, of process (1), endocytosis, is given by the simple linear kinetic law

$$J_1 = k_1 * (PM)$$

and the rate of change of the plasma membrane compartment becomes

$$d(PM)/dt = k_4 * (EV) - k_1 * (PM)$$

Similar equations apply to each of the individual compartments. Although the model system comprises four compartments, only three are independent since we assume that total GLUT4 is conserved; hence:

$$PM + SR + ST + EV = constant$$

Four was selected as the number of compartments in the model in accordance with the number of fractions distinguishable experimentally at this time (G4PM, G4T, G4H, and G4L), and as a number sufficiently large to adequately represent the presumed kinetic complexity of the transporter system. Although the real system is undoubtedly

more complex, modeling with a greater number of compartments would not be justified in terms of the precision with which the experimental data can be analyzed. For example, a discrete compartment representing the endocytotic vesicles, while likely to be a physical reality, does not appear in the model, since the content of this pool may be a negligible fraction of the total GLUT4 within the system as suggested by the absence of any observable, related fraction in our fractionation procedure.

The assumption that each process is unidirectional, while plausible in light of the physical nature of the processes involved, necessarily means that the kinetic model, as presented, is incomplete from a thermodynamic point of view. That is, at least one step of the cycle must be coupled with an energy-providing reaction to avoid the construction of a perpetual motion machine. This energy source, presumably metabolic, is imbedded in one or more of the kinetic coefficients. This is consistent with the well-documented observation that membrane dynamics involved in vesicle trafficking are complex and metabolically regulated.

Steady state in the distribution of GLUT4 among the four compartments is achieved when the flux of each process is the same, and the time derivative of each pool size vanishes. Under these conditions, a simple relationship exists between the kinetic coefficients and the steady state level of the pool sizes; namely,

$$J_{ss} = k_1 * (PM)_{ss} = k_2 * (SR)_{ss} = k_3 * (ST)_{ss} = k_4 * (EV)_{ss}$$

in which the subscript "ss" denotes steady state values. Thus the *relative* magnitudes of the four rate coefficients can be determined from the steady state compartment contents. Since the flux is an undetermined quantity, however, the absolute values of the coefficients cannot be deduced solely from steady state measurements. Measurements of the time dependent behavior of the model must be employed for this purpose. By scaling the sets of rate coefficients, such that the experimentally observed and simulated time constants are as similar as possible, absolute values can be assigned to the rate coefficients. Separate scaling factors are employed in the basal and insulin stimulated states, since these two states are kinetically independent.

Available in literature are two types of time dependent data for this system obtained from rat adipocytes and 3T3-L1 adipocytes (Jhun *et al.*, 1992; Yang *et al.*, 1992a,b; Holman *et al.*, 1994). The first, denoted TRAN, is the time course followed by the system upon the sudden change in kinetic parameters from those characteristic of the basal state to those attained under insulin stimulation, and the converse. The initial values of the pool sizes in this type of transition are then the steady state values of the state, either basal or insulin-stimulated, occupied by the system just prior to the transition. The second class of time dependent data, denoted SS, is that generated by labeling the plasma membrane pool with a tracer, and measuring the subsequent redistribution of the marker throughout all four compartments. In this instance, the corresponding initial conditions are 100% in PM, zero elsewhere. That is, since the system is mathematically linear, the tracer distribution behaves independently from the majority component, which is at its steady state distribution.

Corresponding to the three independent pool sizes characterizing the model system, the time course followed by the system in relaxing into its steady state will be composed of a weighed linear sum of three characteristic exponential functions. It is important to note that the relative contribution of each of these characteristic exponentials will vary, depending upon the initial conditions imposed upon the system. Consequently, the apparent time constant empirically observed for a particular compartment will depend upon the type of transient experiment, TRAN or SS, which is being conducted. Experimentally, only the plasma membrane compartment has been assayed with sufficient time resolution to provide useful transient data; further, we assume that the time course of PM in relaxing into its steady state is expressed by means of a single lumped parameter, an apparent half-time. In like fashion, the curves generated by the model simulation are analyzed in terms of a single, apparent half-time, estimated simply as the period required for the PM pool to relax half of the distance to its ultimate value.

It has been assumed, in modeling the transient behavior of the system, that the perturbation resulting in the subsequent relation into the steady state is essentially instantaneous. That is, for example, the pulse of GLUT labeling in the PM pool occurs over a time period much shorter than the characteristic time constants of the system. In particular, any time delay in the signal transmission from insulin receptor to the resultant change in kinetic coefficients is ignored.

The steady state pool sizes as measured, and the corresponding scaled kinetic constants are shown in Table 2 below. Since there has been some discussion in the literature of the PM pool size under basal conditions (Jhun *et al.*, 1992; Yang *et al.*, 1992a; Satoh *et al.*, 1993), two values representative of those reported have been considered, 6% (Jhun *et al.*, 1992; Satoh *et al.*, 1993) and 2% (Satoh *et al.*, 1993).

Also shown in the Table are observed half-times ($Expt_{1/2}$) taken from the literature (Jhun *et al.*, 1992) under various conditions, and the corresponding $t_{1/2}$ values measured from the simulated system ($Simt_{1/2}$). Unfortunately, the experimental data reported with regard to the transition from insulin stimulation to the basal state is scattered (6.8-12. 3min) and seems less reliable; this perhaps reflects the experimental difficulty in removing insulin from the system in a period short relative to the intrinsic kinetics of the trafficking system. The time course for each of the four compartments during relaxation under the four different experimental conditions were computed for both the 6% and 2% PM pool size models. The curves for 6% PM pool size model are shown in Fig. 3. The simulation showed that the apparent half-time of the PM compartment is nearly the same in both the basal steady state experiment, and in the transition into the basal state from the

Table 2. Steady state contents of each of the four compartments, expressed as per cent of total GLUT4, under both basal and insulin stimulated conditions. Rate coefficients are scaled to achieve the best correspondence between the experimental and simulation half-times. The columns labeled Ins/Bas indicate the ratio between the values of each rate coefficient in the insulin stimulated and basal states as derived from the model. Values shown within parentheses were not used in the analysis for the reasons discussed in text.

	6% BASAL		INSULIN		2% BASAL	
PM	6		36.0		2	
SR	20		15.9		24	
ST	56		44.5		56	
EV	18		3.6		18	
	SS	TRAN	SS	TRAN	SS	TRAN
Exp $t_{1/2}$	1.7	(11)	3.2	2.4	1.7	(11)
Sim $t_{1/2}$	1.7	1.5	4.2	1.9	1.7	1.6
		Ins/Bas		Ins/Bas		
k_1	0.380	0.24	0.092	0.22	0.471	
k_2	0.114	1.82	0.208	5.94	0.035	
k_3	0.040	1.82	0.074	4.93	0.015	
k_4	0.127	7.24	0.920	20.00	0.046	

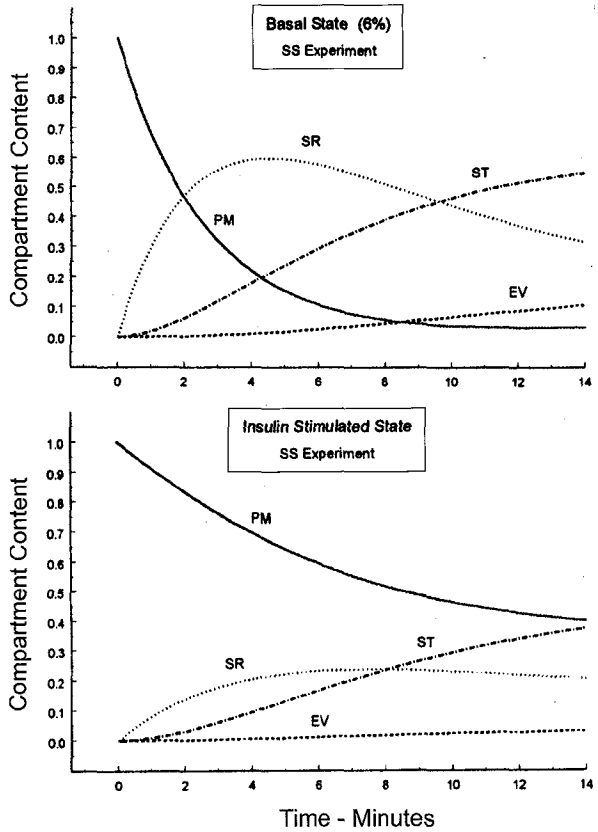

Fig. 3. Simulation of the relaxation time course for PM, SR, ST and EV.

insulin stimulated state, whether the 6% or 2% PM pool size model is considered. As shown in Table 2, these $t_{1/2}$ values are 1.7 and 1.5 (6% PM) or 1.6 (2% PM) minutes, respectively. In contrast to these results, under conditions of insulin stimulation, the apparent half-times differ by a factor greater than 2 in the compartment model. As it happens, however, only the sorting compartment, two types of experiment. In this instance, the apparent $t_{1/2}$ for label redistribution is 4.2 minutes, compared to 1.9 minutes in the basal to insulin transition. Undershoot or overshoot in the contents of any single compartment is not unexpected in the four SR, shows a significant overshoot under the particular sets of initial conditions placed upon the system. Although not immediately evident from the figures, the PM compartment falls slightly below its ultimate steady state value in both basal state experiments.

Significantly, the predictions of this model analysis concerning the effect of insulin upon the kinetic coefficients for the processes of endocytosis and exocytosis are reasonably consistent with those reported in the literature (as summarized in reference of Holman *et al.*, 1994). The endocytotic rate constant in our model decreases by a factor near four, while the exocytosis coefficient increases over seven-fold in the 6% model system, and 20 fold in the case of the 2% model. Interestingly, the other two rate coefficients involved in the system also increase in the presence of insulin, by factors between 2 and 6.

While a four-compartment model, such as the one considered here, is clearly inadequate to faithfully represent the full complexity of the GLUT4 recycling system in adipocytes, it is sufficient to capture the essential features of the system, and provide a reasonable approximation of its kinetic behavior. The model developed here, based upon steady state distribution of GLUT4 among four distinguishable fractions in adipocytes, G4PM, G4T, G4H and G4L, provides additional support to the idea that insulin stimulation modulates the rate coefficients of all of the processes involved in GLUT4 cycling. It acts to decrease the

rate coefficient of endocytosis while increasing all the other rate constants, including the two rate constants involved in intracellular compartments of the endosomal system.

Concluding remarks

Importance of the insulin-stimulated glucose transport function in human physiology and pathophysiology can not be over emphasized; dysfunction in this process is known to cause many fatal complications in Type 2 diabetes and obesity, a major health problem in many economically advanced and advancing countries. The insulin-induced GLUT4 recruitment, the major mechanism for the insulin action, is shown to be defective in Type 2 diabetic patient. The process is complex involving multiple compartments, membrane dynamics, and many proteins. The separation and kinetic characterization of G4T, G4H and G4L in response to insulin outlined here would allow us to directly assess the role of these three endosomal GLUT4 compartments in insulin-induced GLUT4 recruitment, and how they are controled in this process. Once we understand in detail how this insulin-induced GLUT4 recruitment works in normal physiological state, it will likely give us insights into the nature of the defect underlying insulin resistance, and eventually into how to improve prevention, diagnosis and treatment of this important health problem.

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