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Insulin stimulation of glucose and amino acid transport in mouse fibroblasts with elevated membrane microviscosity[1]

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Summary. Basal and insulin-stimulated transport of 2-deoxy glucose and of α-aminoisobutyric acid in mouse 3T3 fibroblasts were modulated by increasing the lipid microviscosity of the cell plasma membrane. The kinetics indicate that the insulin effect is induced either by recruitment of new transport carriers or by reduction of the translocation activation energy.

Carrier-mediated glucose transport in human erythrocytes and mouse fibroblasts of modified membrane microviscosity, $\bar{\eta}$, does not obey simple diffusion considerations[4]. Upon slight increase in $\bar{\eta}$ the maximal transport rate, V_{max}, reaches a peak activity beyond which further increase in $\bar{\eta}$ reduces it. The effect of increased $\bar{\eta}$ could be accounted for by 2 counteracting trends; a reduced turnover rate of each transport carrier and an apparent increased number of operating carrier sites[4]. In the following study we examined the insulin stimulation of transport of 2-deoxy glucose (2dG) and of α-aminoisobutyric acid (AIB) in 3T3 mouse fibroblasts[5,6] with increased membrane microviscosity[4]. Transport experiments were carried out at 37 °C with quiescent cell-monolayers in which the plasma membrane microviscosity was selectively increased by incorporation of cholesteryl hemisuccinate (CHS) under mild conditions[4]. Steady-state fluorescence polarization with DPH as a probe, in addition to a special quenching technique, were employed to determine and resolve the $\bar{\eta}$ value at 37 °C of the cell plasma membranes in the monolayers[4]. Although $\bar{\eta}$ is a complex combination of dynamics and order of the membrane hydrocarbon region[7] it is of a direct relevance to membranal activities[8]. A full description of the experimental details and rationale is given in a previous publication[4]. At each level of membrane viscosity transport experiments were repeated 6–12 times. Figure 1 summarizes the effect of increased membrane microviscosity on the maximal rate of 2dG transport (V_{max} at 37 °C) in the absence and in the presence of 100 ng/ml of insulin[9]. As shown, within the experimental microviscosity range, the effect of insulin on the maximal transport rate of 2dG is increased by about 50%. The patterns described in figure 1 reveal a distinct maximal V_{max} value at about 30–50% increase in the apparent $\bar{\eta}$-value. Analogous experimental results for AIB transport are shown in figure 2.

Following our previous approach[4] for analysing the effect of $\bar{\eta}$ on a membranal activity, V_{max} can be presented as the product of the transport rate constant, k_{tr}, and the actual concentration of the operating carriers, C_+:

$$V_{max} = k_{tr} \cdot C_+ = k_{tr} \cdot \alpha \cdot C_0 \qquad [1]$$

where α is the accessible fraction of the total operateable carriers C_0, which is operationally dependent on $\bar{\eta}$.

Analysis of the dependence of α and k_{tr} on $\bar{\eta}$ leads to the following general expression[4]:

$$\frac{1}{V_{max}} = A \cdot \eta \cdot \frac{1+\tilde{\eta}^{-m}}{1+f \cdot \tilde{\eta}^{-m}} \qquad [2]$$

where $\tilde{\eta}$ is the normalized lipid microviscosity, $\bar{\eta}$, in units of $\bar{\eta}_{1/2}$, the latter corresponds to half accessibility ($\tilde{\eta} = \bar{\eta}/\bar{\eta}_{1/2}$), and m is a plasticity parameter which characterizes the degree of cooperativity between the lipid viscosity and the statistical accessibility of the carrier sites.

Fitted parameters (± SE) for the dependence of the maximal rate of transport (V_{max} at 37 °C) on membrane microviscosity ($\bar{\eta}$), according to Eq. 2

Ligand	Insulin stimulation (100 ng/ml)	A $\left(\frac{\text{nmole} \cdot \text{poise}}{10^6 \text{ cell} \cdot \text{min}}\right)^{-1}$	$\bar{\eta}_{1/2}$, 37 °C (poise)	m	f
2dG	–	3.2 (±0.2)	1.41 (±0.03)	4.8 (±1.1)	~0
2dG	+	2.6 (±0.2)	1.42 (±0.03)	4.7 (±1.1)	~0
AIB	–	0.68 (±0.02)	1.32 (±0.03)	>8	~0
AIB	+	0.24 (±0.01)	1.32 (±0.02)	>8	~0

The factor A is a constant given by:

$$A = \frac{A_0}{C_0} \cdot e^{\Delta E^*/RT} \quad [3]$$

where A_0 is a proportionality factor and ΔE^* is the activation energy of the translocation process. The lines presented in figures 1 and 2 are computer fits according to Eq. 2. The fitted parameters obtained for figures 1 and 2 are summarized in the table.

The value of $f \sim 0$ for both carriers (table) indicates that virtually all of the carrier sites are simultaneously affected by changes in $\bar{\eta}$.

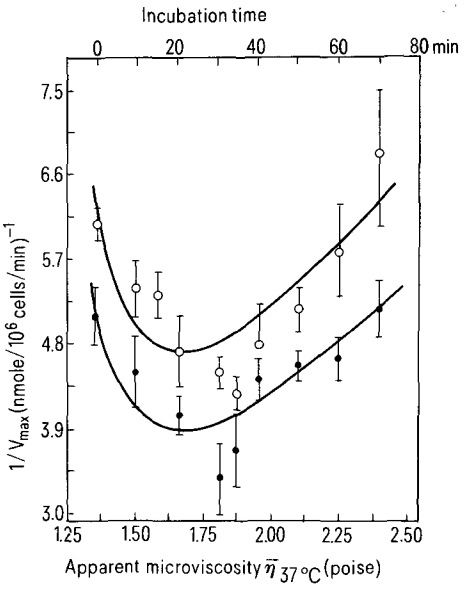

Figure 1. The effect of membrane microviscosity, or incubation time for CHS incorporation, on the basal (open symbols) and the insulin stimulated (solid symbols) maximal rate of ^{3}H-2dG uptake by 3T3 fibroblasts at 37 °C. The experimental points represent the mean and the SD of 6–12 independent experiments. The lines were computed for data fitting according to Eq. 2.

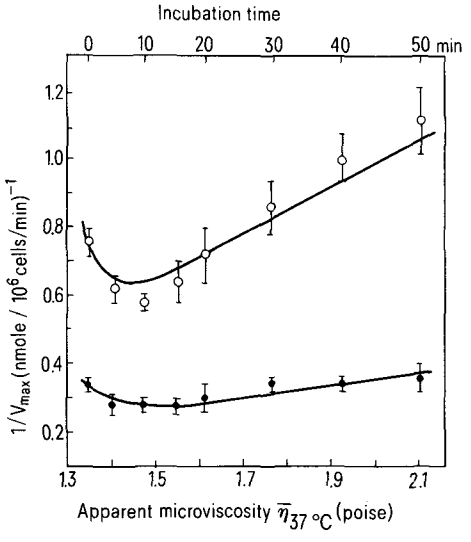

Figure 2. The effect of membrane microviscosity on the basal (open symbols) and the insulin stimulated (solid symbols) maximal rate of ^{3}H-AIB uptake of 3T3 fibroblasts at 37 °C. The experimental points represent the mean and the SD of 6 independent experiments. The lines were computed for data fitting according to Eq. 2.

The $\bar{\eta}_{1/2}$ values of both 2dG and AIB carriers are very close to that of the $\bar{\eta}$ value in untreated cells. This implies that under physiological conditions the cells retain about one-half of their glucose or amino acid carriers inaccessible, as has been suggested by others[10]. The m-values for both 2dG and AIB transports are characteristic of positive cooperativity between lipid viscosity and carrier accessibility[4]. The value of $m > 8$ for AIB transport (table) indicates that around the physiological state small changes in $\bar{\eta}$ can sharply modulate the number of operating amino acid carriers. The lack of any significant effect of insulin on either $\bar{\eta}_{1/2}$ or m indicates that the accessibility of neither the glucose nor the amino acid carriers change upon insulin stimulation.

According to Eq. 2, the most pronounced change induced by insulin stimulation of 2dG and AIB transports is of the factor A (see table). In principle, the observed decrease in A can be accounted for by either increase in C_0 or by decrease in ΔE (see Eq. 3). Increase in C_0, namely the introduction of new transport carriers into the plasma membrane upon insulin stimulation, should retain the relative fraction of accessible carriers since the accessibility factor, α, is unaltered. This mechanism of 'carrier recruitment' by insulin binding has been implied in several kinetic and binding studies[11–15]. Transformation of carrier precursors into active carriers by insulin could alternatively account for the increase in C_0. This mechanism could involve specific induction[16–18], or decrease[19] in protein phosphorylation upon insulin binding. The relative contribution of carrier recruitment, and the reduction of transport activation energy, to the overt transport stimulation by insulin, may be time-dependent. It is plausible, that immediately after hormone binding the spontaneous reduction in ΔE^* is the main mechanism of insulin stimulation, whereas after a few min carrier recruitment may reach a level where it becomes the dominant factor in the effect of insulin.

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