J. Membrane Biol. 87, 225–232 (1985)

The Journal of
Membrane Biology

Na^+-Sensitive Component of 3-O-Methylglucose Uptake in Frog Skeletal Muscle

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Summary. A Na^+-sensitive uptake of 3-O-methylglucose (3-O-MG), a nonmetabolized sugar, was characterized in frog skeletal muscle. A removal of Na^+ from the bathing solution reduced 3-O-MG uptake, depending on the amount of Na^+ removed. At a 3-O-MG concentration of 2 mM, the Na^+-sensitive component of uptake in Ringer's solution was estimated to be about 26% of the total uptake. The magnitude of Na^+-sensitive component sigmoidally increased with an increase of 3-O-MG in bathing solution, whereas in Na^+-free Ringer's solution the uptake was proportional to the concentration. The half saturation of the Na^+-sensitive component was at a 3-O-MG concentration of about 13 mM, and the Hill coefficient was 1.4 to 1.6. Phlorizin (5 mM), a potent inhibitor specific for Na^+-coupled glucose transport, reduced the uptake in a solution containing Na^+ to the level in Na^+-free Ringer's solution. Glucose of concentrations higher than 20 mM suppressed 3-O-MG uptake to a level slightly lower than that in Na^+-free Ringer's solution. These observations indicate that there are Na^+-coupled sugar transport systems in frog skeletal muscle which are shared by both glucose and 3-O-MG.

Key Words 3-O-methylglucose uptake · frog · skeletal muscle · insulin · sugar transport

Introduction

Sugar uptake in skeletal muscle is increased during exposure to insulin, and supposed to play an important role to moderate hyperglycemia during periods of sugar absorption from the intestine. The study of sugar transport in muscle has been carried out by several methods; such as measurement of disappearance of sugar from a known volume of medium (Park et al., 1961; Clausen, 1965), analytical measurement of glucose taken up by muscle (Narahara et al., 1960), isotopic measurement of muscle extract (Narahara & Özand, 1963), and isotopic measurement with internal dialysis (Baker & Carruthers, 1983; Carruthers, 1983). Kinetic analyses generally indicate that insulin increases the capacity (V_{max}) of the transport mechanism. Such an effect might be brought about either by an increase in number of active sites available for transport in plasma membrane or by an increase in turnover rate of a fixed number of transport systems. Besides the effect on sugar transport, insulin has a stimulating effect on Na^+/K^+ transport (Moore, 1973; Erlij & Grinstein, 1976; Flattman & Clausen, 1979; Kitasato et al., 1980). It is well known that the sugar transport in apical membrane of epithelial cells in intestine and urinary tubulus is facilitated by a carrier mechanism associated with passive inward movement of Na^+ (Schultz, 1977). Thus, it may be rational to suppose some causal relation between the activation of Na^+/K^+-pump and the stimulation of sugar transport. Present studies were aimed at finding out the Na^+-dependent process of sugar transport in skeletal muscle. However, against the first expectation, results of experiments indicated that the relative magnitude of the Na^+-sensitive component was not large enough to account for the increase in sugar transport observed when insulin was added. We report here characteristics of Na^+-sensitive glucose transport in skeletal muscle.

Materials and Methods

Materials

Paired sartorius muscles were carefully dissected from bullfrog (*Rana catesbeiana*) under a microscope, and used for providing control and test muscles. Tendons and connective tissues were removed as much as possible. Isolated muscles were stored in Ringer's solution at 4°C for 15 to 18 hr prior to starting an experiment. Muscles having any damages were discarded.

Solutions

Normal external solution used in the present studies was Ringer's solution whose composition was (mM): NaCl 110, KCl 2, $CaCl_2$ 2.5, Tris-HCl 2.5 (pH 7.4). Solutions of various Na^+ concentrations were prepared by replacing NaCl with Tris-HCl. 50 mM Na^+ Ringer's solution means the solution whose ionic composition was the same as that of normal Ringer's solution except

Table 1. Magnitudes of 3-O-MG uptake at various Na^+ concentrations[a]

Exp. No	Magnitude of uptake (μmol/kg of muscle water/min)			Uptake ratio	
	a, 0 mM Na^+	*b*, 50 mM Na^+	*c*, 110 mM Na^+	*b*/*a*	*c*/*a*
(1–6)	8.4 ± 0.3	10.1 ± 0.4	—	1.19 ± 0.01	—
(7–12)	9.6 ± 0.6	—	12.9 ± 0.5	—	1.36 ± 0.06
(1–12)	9.0 ± 0.5	10.8[b]	12.3[c]	—	—

[a] All the measurements were carried out on paired muscles. Temperature was 22°C. Figures represent mean and standard error of the mean.
[b] A normalized magnitude of uptake at 50 mM Na^+ which was calculated from the averaged magnitude at 0 mM Na^+ in experiments (1–12) and average uptake ratio at 50 mM Na^+ to that at 0 mM Na^+.
[c] A normalized magnitude of uptake at 110 mM Na^+ calculated using the average magnitude at 0 mM Na^+ in experiments (1–12).

for reducing NaCl to 50 mM and adding Tris-HCl. 3-O-methyl-D-glucose (3-O-MG) was used for uptake experiments. The external solutions used for determining concentration dependence of 3-O-MG uptake were prepared by adding 3-O-MG to 50 mM Na^+ Ringer's solution to provide desired concentrations. Osmolarity was adjusted by adding Tris-HCl, and checked by the freezing-point depression method. 3-O-methyl-D-[U-^{14}C]glucose was added to make the solution radioactive. Additions of radioactive compound raised the 3-O-MG concentration by less than 100 μM.

Measurement of 3-O-MG Uptake

One of a pair of muscles was incubated in test uptake solution, while the other was in control uptake solution. Incubation period was usually 30 min. Following exposure to 3-O-MG each muscle was blotted on filter paper, and then weighed. Mounting on a platinum frame, the muscle was transferred to a series of test tubes filled with Ringer's solution which was continuously stirred by a small magnetic stirrer. After washing, the muscle was dissolved in tissue solubilizer, Sintilamine® (Dotite, Kumamoto). Radioactivities of solutions in washing test tubes and in muscle were counted by using scintillator cocktail, ACS II (Amersham, USA). Radioactivities in muscle during washing period were estimated by back add method, and plotted semilogarithmically with respect to washing time. Extrapolation of a straight line through the points except the first three points to the zero time gave the radioactivity transferred into the intracellular compartment during incubation. The amount of 3-O-MG transferred into the intracellular compartment was calculated from the count thus estimated and the specific radioactivity of 3-O-MG in the incubation solution. In the first 30 min of incubation with 2 mM 3-O-MG the amount of 3-O-MG transferred into the intracellular compartment was linear to the incubation period. The volume of the extracellular compartment was estimated from muscle wet weight and the radioactivity, which could be rapidly washed out. On average, the extracellular space was about 35% of muscle wet weight. The uptake was expressed by the amount transferred into the intracellular compartment per intracellular volume per min.

Chemicals

Bovine insulin, phlorizin, phloretin and cytochalasin B were purchased from Sigma Chemical Co. Ouabain was from Merk. [U-^{14}C]3-O-MG was the product of Amersham. All chemicals used were of analytical grade.

Results

Removal of Na^+ from External Solution Reduces 3-O-MG Uptake

3-O-MG is a nonmetabolized derivative of glucose. Since the absolute magnitude of 3-O-MG uptake differed greatly from animal to animal, one of a pair of muscles was always used as a control in each experiment, and the effect of various maneuvers was determined from the ratio of the uptake in test muscle to that in control muscle. In experiments to determine a relationship between 3-O-MG uptake and concentration of Na^+ in uptake solution, test uptake was measured at a variety of Na^+ concentrations, while control uptake was always measured in Na^+-free Ringer's solution. The concentration of 3-O-MG in uptake solutions was 2 mM throughout these experiments. All the experiments were performed at 22°C. Magnitudes of the uptake at Na^+ concentrations of 0, 50 and 110 mM are summarized in Table 1. The uptake in 50 mM Na^+ Ringer's solution was 1.19 ± 0.01 (mean ± SEM) fold that in Na^+-free solution, whereas the uptake from normal Ringer's solution where Na^+ concentration was 110 mM was 1.36 ± 0.06-fold that at 0 mM Na^+. The normalized magnitude of uptake at 110 mM Na^+ was 12.3 moles $\cdot$ min^{-1} $\cdot$ kg^{-1} of muscle water. This value is about twofold the value reported by Narahara and Özand (1963). Curve (*a*) in Fig. 1 shows the relationship between 3-O-MG uptake and external Na^+ concentration.

The effect of insulin on uptake at various Na^+ concentrations was also examined. Insulin (200 mU/ml) was added to test uptake solutions of vari-

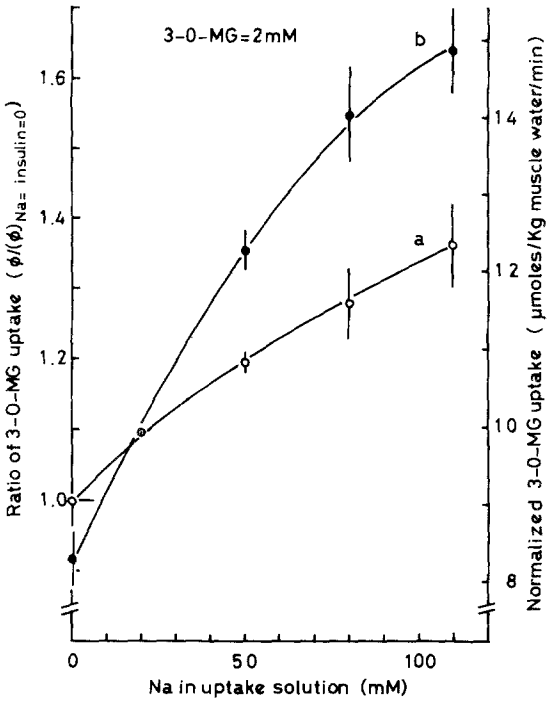

Fig. 1. Relationship between 3-O-MG uptake and external Na^+ concentration. (*a*) The relationship in solution without insulin. (*b*) The relationship in solution with insulin (200 mU/ml). Curves were drawn by eye. Normalized magnitude of uptake was calculated from the ratio of uptake at a variety of Na^+ concentrations to that at 0 mM Na^+ and the average magnitude of uptakes at 0 mM Na^+. Each uptake ratio was obtained from experiments conducted on paired muscles. Relationship in solution with insulin was estimated from the ratio of uptake in solution with insulin to that in solution without insulin of the identical Na^+ concentration and the normalized magnitude of uptake in the absence of insulin at the corresponding Na^+ concentration, which had been obtained from experiments giving curve (*a*). Temperature was 22°C. Each point is the mean of uptake ratios obtained from six paired measurements. Vertical bar is SEM

ous Na^+ concentrations. Solution for control experiments was of the same Na^+ concentrations as in the corresponding test uptake solution. Uptake-ratios were calculated from paired measurements. The normalized magnitude of uptake in insulin-containing solution at a variety of Na^+ concentrations was estimated from the uptake ratio and the magnitude of uptake in insulin-free solution at the corresponding Na^+ concentration, which had already been estimated from experiments for determining the dependence of uptake in insulin-free solution. Curve (*b*) in Fig. 1 shows the relationship thus obtained between 3-O-MG uptake and concentration of Na^+ in insulin-containing solution. In the absence of Na^+ insulin did not increase 3-O-MG uptake, but rather decreased by about 7%. However, at Na^+ concentrations higher than 20 mM, insulin increased the uptake. At the concentration of 110 mM the ratio of uptake in insulin-Ringer's solution to that in insulin-free Ringer's was 1.20 ± 0.04. Findings from these experiments, that 3-O-MG uptake increases with an increase of external Na^+, and that the stimulation of the uptake by insulin requires external Na^+, suggest that insulin increases only the Na^+-sensitive component of the uptake.

Table 2. Ratios of uptake in 50 mM Na^+ Ringer's to that in Na^+-free Ringer's at various 3-O-MG concentrations[a]

3-O-MG (mM)	Uptake ratio (mean ± SE)
2.0	1.21 ± 0.02
5.0	1.25 ± 0.02
10.0	1.26 ± 0.02
15.0	1.30 ± 0.02
20.0	1.26 ± 0.02
30.0	1.19 ± 0.01
40.0	1.18 ± 0.01
50.0	1.12 ± 0.01

[a] Each uptake ratio was calculated from six paired measurements. Temperature, 22°C.

Na^+-Sensitive Component of 3-O-MG Uptake is Saturable

To characterize the Na^+-sensitive component of 3-O-MG uptake, a relationship between uptake and concentration of 3-O-MG was studied. The procedure to estimate the concentration dependence of the uptake in Na^+-free Ringer's solution was similar to that used to estimate the relationship between 3-O-MG uptake and external Na^+ concentration, except that a variable was 3-O-MG concentration instead of Na^+ concentration in this case. The concentration of 3-O-MG in control uptake solution was 2 mM. The normalized magnitude of uptake at a given concentration of 3-O-MG was calculated from the average uptake ratio and the average magnitude of uptake at 2 mM 3-O-MG, and plotted with respect to the concentration. As curve (*a*) in Fig. 2*A* shows, in the absence of Na^+ the uptake linearly increases with an increase of external 3-O-MG.

The effect of Na^+ on the relationship between uptake and concentration of 3-O-MG was determined by comparing the uptake in 50 mM Na^+ Ringer's solution with that in Na^+-free solution of the same 3-O-MG concentration as in 50 mM Na^+ solution. Uptake ratios at various 3-O-MG concentrations are summarized in Table 2. A normalized magnitude of uptake in 50 mM Na^+ Ringer's solution as a function of external 3-O-MG concentration was calculated from the average uptake ratio and the magnitude of uptake in Na^+-free Ringer's solution

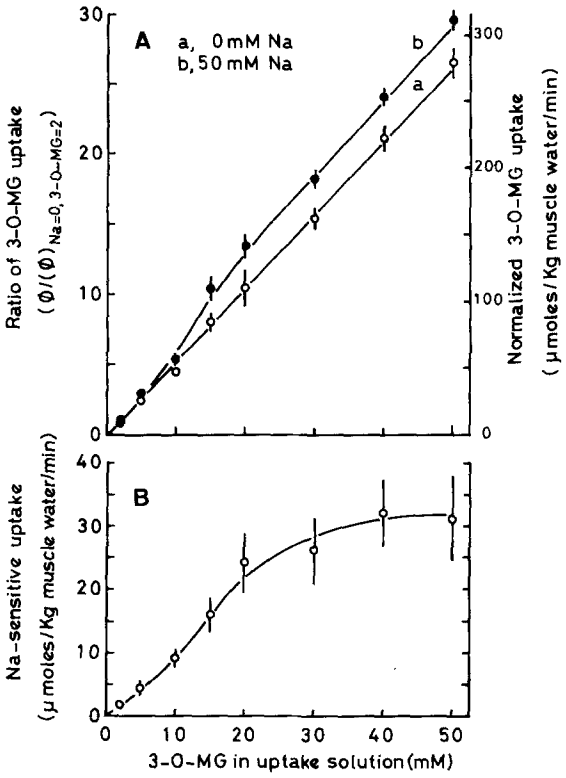

Fig. 2. Concentration dependences of 3-O-MG uptake in solutions with and without external Na^+. (*A*) Relationship between uptake and external concentration of 3-O-MG. (*a*) The relationship in solution without Na^+. (*b*) The relationship in solution with 50 mM Na^+. (*B*) The concentration dependence of Na^+-sensitive component of the uptake. Curves were drawn by eye. Temperature was 22°C. Each point is a mean from six paired estimations. Vertical bar represents SEM

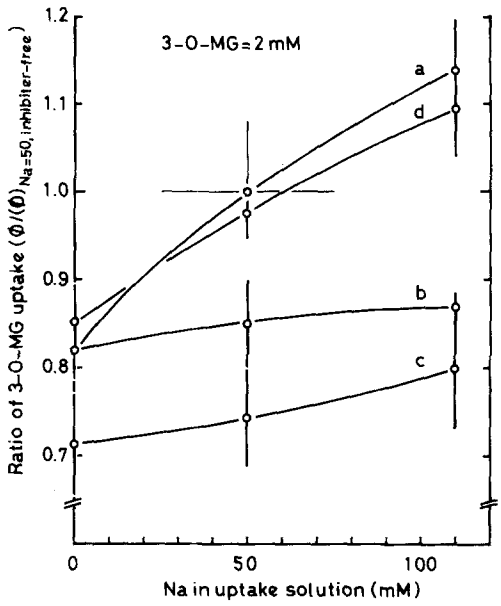

Fig. 3. Effects of inhibitors on 3-O-MG uptake at various Na^+ concentrations: (*a*) without inhibitor; (*b*) with phlorizin (5×10^{-3} M); (*c*) with cytochalasin B (1×10^{-5} M); (*d*) with phloretin (2×10^{-4} M). Curves were drawn by eye. Experiments were carried out using muscle pairs in solutions with and without inhibitor. A magnitude of uptake in inhibitor-free 50 mM Na^+ solution was taken as unity. 3-O-MG concentration in uptake solutions was 2 mM. Temperature was 22°C. Vertical bar represents SEM from six paired estimations

of corresponding 3-O-MG concentration, which had been estimated from experiments for determining the concentration dependence in Na^+-free Ringer's solution. Curve (*b*) in Fig. 2*A* shows thus obtained concentration dependence of uptake in 50 mM Na^+ Ringer's solution.

The difference between normalized magnitudes of uptake in 50 mM Na^+ Ringer's solution and in Na^+-free solution was plotted in Fig. 2*B*. The Na^+-sensitive component increased sigmoidally with an increase of external 3-O-MG. The half-saturation was at a 3-O-MG concentration of 13 mM. The Hill coefficient was 1.4 to 1.6. These experiments show that the Na^+-sensitive component is saturable, while the Na^+-insensitive component is unsaturable. In squid giant axon also Na^+-sensitivity of sugar transport has been reported (Baker & Carruthers, 1981).

Phlorizin Reduced the Uptake of 3-O-MG to the Level in Na^+-Free Ringer's Solution

Phlorizin is reported to inhibit specifically the Na^+-coupled glucose transport in epithelial cells of small intestine and urinary tubulus (Aronson & Sacktor, 1975; Kimmich & Randles, 1975; Kinne et al., 1975). In order to get more information about the properties of the Na^+-sensitive component in skeletal muscle, effects of inhibitors were examined. All the uptake solutions used for these experiments contained 2 mM 3-O-MG. Phlorizin (5 mM) was added to uptake solutions, whose Na^+ concentrations were 0, 50 and 110 mM. As curve (*b*) in Fig. 3 shows, in Na^+-containing solution phlorizin suppressed the uptake close to the level observed in Na^+-free Ringer's solution. In Na^+-free Ringer's solution phlorizin had no effect on the uptake. These findings seem to indicate that the Na^+-sensitive component of 3-O-MG uptake in skeletal muscle is mediated by Na^+-coupled transport systems.

Cytochalasin B, a fungal metabolite, inhibits glucose transport in a variety of cells (Block, 1973;

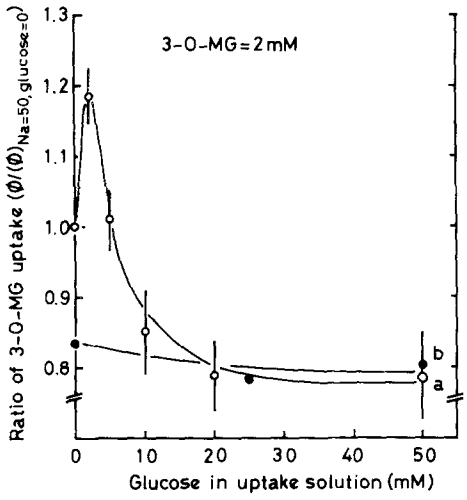

Fig. 4. Inhibition of 3-O-MG uptake by glucose: (*a*) in solution with 50 mM Na^+, (*b*) in solution without Na^+. Magnitude of uptake in glucose-free 50 mM Na^+ Ringer's solution is taken as unity. Curves were drawn by eye. 3-O-MG in uptake solutions was 2 mM. Ratios of uptake were taken from measurements carried out on paired muscles. Vertical bar represents SEM obtained from six paired experiments. Temperature was 22°C

Taverna & Langdon, 1973; Kimmich & Randles, 1979; Simons 1983). Cytochalasin B binding to plasma membrane of rat diaphragm is blocked by D-glucose (Wardzala & Jeanrenaud, 1981). Thus, effect of cytochalasin B on 3-O-MG uptake in frog muscle was investigated at a variety of Na^+ concentrations. As shown by curve (*c*) in Fig. 3, cytochalasin B (1×10^{-5} M) reduced the uptake to the level lower than that observed in Na^+-free Ringer's solution. In the presence of cytochalasin B, the relationship between uptake and external Na^+ concentration was less steep than that in inhibitor-free Ringer's solution (curve (*a*) in Fig. 3). These observations suggest that transport systems in muscle responsible for Na^+-sensitive component are slightly susceptible to cytochalasin B.

Phloretin is known to inhibit the passive facilitated glucose transport in basolateral membrane of urinary tubulus (Kimmich & Randles, 1975), that are insensitive to electrochemical potential gradient for Na^+. Present experiments showed that phloretin was a much less effective inhibitor than phlorizin for both Na^+-sensitive and Na^+-insensitive components of 3-O-MG uptake in skeletal muscle (curve (*d*) in Fig. 3). Ratios of uptake in 50 mM Na^+ Ringer's solution with phloretin to that in solution without phloretin at 0 and 110 mM Na^+ were 0.96 ± 0.07 and 0.94 ± 0.11, respectively. These pharmacological experiments strongly suggest that the Na^+-sensitive component is not mediated by passive facilitated transport systems, but by Na^+-coupled transport systems.

External Glucose Reduces 3-O-MG Uptake to the Level in Na^+-Free Ringer's Solution

Since the Na^+-sensitive component of 3-O-MG uptake was saturable, if glucose is transported by the same carrier systems that are available for 3-O-MG transport, an addition of glucose to the uptake solution will cause a suppression of 3-O-MG uptake to the level observed in Na^+-free Ringer's solution. To examine this view, 3-O-MG uptake was measured as a function of external glucose concentration in the presence and absence of Na^+. Test uptakes were measured at various glucose concentrations, while control uptakes were measured in glucose-free solution. The concentration of 3-O-MG in uptake solutions was 2 mM. The results of these experiments are shown in Fig. 4. Within a range of glucose concentration lower than 5 mM an addition of glucose to uptake solution of 50 mM Na^+ stimulated 3-O-MG uptake. Maximal stimulation was observed at 2 mM glucose. A further increase of glucose reduced 3-O-MG uptake. The final level of the uptake at a sufficiently high concentration of glucose (higher than 20 mM) was 0.78 of the control level observed in glucose-free 50 mM Na^+ Ringer's solution. The completely suppressed level was slightly lower than the level of uptake in Na^+-free Ringer's solution at the same concentration of 3-O-MG. This may indicate that in nominally Na^+-free solution a small amount of Na^+ still remains in extracellular space. Relating to this, in the absence of Na^+ an addition of glucose had no significant effect on 3-O-MG uptake (curve (*b*) in Fig. 4).

Effect of glucose on the concentration dependence of 3-O-MG uptake was also investigated. Test uptake was measured in 50 mM Na^+ Ringer's solutions with 5 mM glucose at a variety of 3-O-MG concentrations, while control uptake was measured in glucose-free solution whose 3-O-MG concentration was equal to that in solution for test uptake. Curve (*b*) in Fig. 5 shows the relationship between the Na^+-sensitive component of uptake and the concentration of 3-O-MG in 50 mM Na^+ Ringer's with 5 mM glucose, whereas curve (*a*) represents the relationship in the solution without glucose. At low concentrations of 3-O-MG, the uptake in 5 mM glucose solution was slightly larger than that in glucose-free solution. However, at concentrations of 3-O-MG higher than 5 mM the uptake in 5 mM glu-

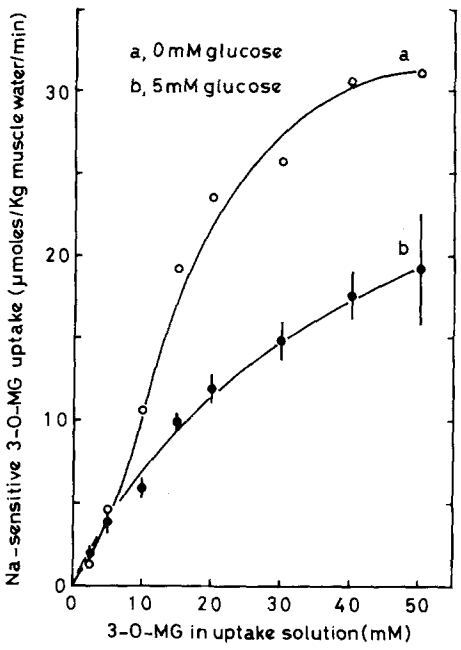

Fig. 5. Effect of glucose on the concentration dependence of Na^+-sensitive component of 3-O-MG uptake. (*a*) Concentration dependence in glucose-free solution. Data were taken from experiments shown in Fig. 2*B*. (*b*) Concentration dependence in 5 mM glucose solution. Na^+ concentration was 50 mM in all uptake solutions. Curves were drawn by eye. Uptake ratios were obtained from paired measurements. Magnitude of uptake in 5 mM glucose solution at a variety of 3-O-MG concentrations was calculated from the ratio of uptake in 5 mM glucose solution to that in glucose-free solution and the magnitude of uptake in glucose-free solution at the corresponding 3-O-MG concentration, which had been estimated from experiments for determining the concentration dependence in glucose-free solution shown in Fig. 2. Vertical bar represents SEM from six pairs of estimations. Temperature was 22°C

cose solution was always smaller than that in glucose-free solution. This finding implies that glucose competes with 3-O-MG at binding sites of transport systems. On the other hand, the finding that at low concentrations of 3-O-MG the uptake is stimulated by 5 mM glucose may be accounted for by an allosteric interaction between binding sites on a single carrier molecule.

Discussion

Saturable Component

Present studies show that 3-O-MG uptake in frog skeletal muscle consists of saturable and unsaturable components. Saturability of sugar transport in muscle has been a conflicting subject. Morgan et al. (1961) have demonstrated that the intracellular sugar concentration in perfused rat heart increases with a rise in the concentration in medium. This means that unidirectional influx or an initial rate of uptake is proportional to the extracellular concentration. On the other hand, Narahara and Özand (1963) have reported that 3-O-MG uptake in frog muscle is saturable. This discrepancy may come from the difference in estimation of extracellular space in muscle. If the extracellular space is underestimated, the proportion of the actual uptake to the estimated one decreases. As a result, the saturable components in actual uptake become hardly recognizable. Conversely, an overestimation of extracellular space may result in an apparent reduction of unsaturable sugar space where sugar concentration is proportional to the external concentration. This may lead to an exaggeration of saturable component. In order to avoid such an error introduced from uncertainty of the estimation of extracellular space, in present studies the amounts of 3-O-MG transported into an intracellular space were determined from extrapolation of pseudo first-order decrease of radioactivity in muscle during washing period to the zero time. Results obtained from these experiments consist with observations from internal dialysis experiments in barnacle giant muscle fiber (Baker & Carruthers, 1983; Carruthers, 1983), in which the measurement of uptake is considered not to be influenced by the method employed to estimate the volume of extracellular space. This accordance indicates that estimations of the amount of sugar transported into intracellular space in these experiments are adequate. Perhaps the most striking observations from the present studies are that the removal of external Na^+ reduces 3-O-MG uptake at any concentrations of 3-O-MG, and that only this Na^+-sensitive component is saturable. Furthermore, the Na^+-sensitive and saturable component is completely suppressed by glucose. Phlorizin, a specific inhibitor of Na^+-coupled glucose transport, suppressed the Na^+-sensitive component while the Na^+-insensitive component remained unaffected. Taking all observations into consideration, it may be concluded that the Na^+-sensitive and saturable uptake is mediated by Na^+-coupled transport systems which are shared by both 3-O-MG and glucose.

Contribution of Na^+-Sensitive Component to the Stimulating Effect of Insulin on Glucose Transport

Up to now the view has been widely accepted that there is no active process in sugar transport in muscle. This view mostly comes from the observation that glucose space is almost equal to or less than

water volume in muscle. Present studies show that there is a Na^+-sensitive component in 3-O-MG uptake, although the Na^+-sensitive component is only about 26% of total uptake. This means that the contribution of Na^+-coupled process to the equilibrium distribution can hardly be detected, unless the driving force for Na^+ is very large. Insulin increases Na^+/K^+-pump activity in skeletal muscle (Moore, 1973; Erlij et al., 1976; Kitasato et al., 1980). The activation of Na^+/K^+ pump causes hyperpolarization (Flattman & Clausen, 1979; Zierler & Rogus, 1980) and a decrease in the intracellular Na^+ concentration. The resultant increase in the driving force for passive Na^+ movement is estimated to be about 30% at the highest. On the other hand, present studies have revealed that insulin increases the Na^+-sensitive component about twofold (Fig. 1). Such an increase in the Na^+-sensitive component cannot be interpreted as a sole result of an increase in the driving force for Na^+. Furthermore, the Na^+-sensitive component is only a small portion of total glucose uptake, as stated above. From these observations, against the first expectation, it should be concluded that the stimulation of glucose uptake by insulin is not a result only of an increase in driving force which may accelerate glucose transport, but is mostly due either to an increase of transport systems or to an activation of fixed number of transport systems. In concentration with this, however, it should be stated that newly incorporated or activated transport systems are those having Na^+-coupled transport mechanism.

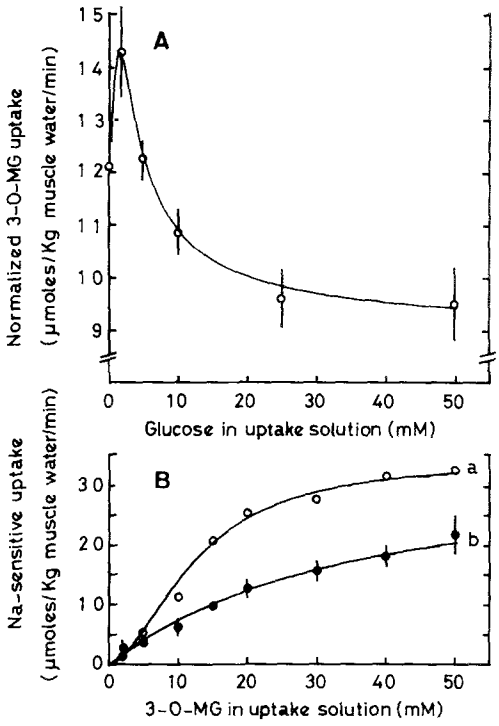

Fig. 6. Theoretical relationships derived from an allosteric carrier model. (*A*) Relationship between 3-O-MG uptake and external glucose concentration. (*B*) Concentration dependences of 3-O-MG uptake in solutions without (*a*) and with 5 mM glucose (*b*). Data were taken from experiments shown in Figs. 4 and 5. Continuous curves were drawn by using Eq. (1). $L = 14$, $K_1 = 0.5$ mM and $K_2 = 2.0$ mM

Properties of Na^+-Sensitive Glucose Transport Systems

Although the Na^+-sensitive component of glucose uptake is not large enough to account for the increase of glucose uptake in muscle observed when insulin is applied, the Na^+-sensitive component is persistently observed. The concentration dependence of Na^+-sensitive component is sigmoidal, and the Hill coefficient is 1.4 to 1.6. If Na^+-sensitive 3-O-MG uptake is proportional to the degree of saturation of transport systems with 3-O-MG at the outer surface of plasma membrane, then the concentration dependence may represent the relationship between saturation degree and external concentration. From the sigmoidal nature of the concentration dependence curve, it may be considered that the number of binding sites on a single carrier molecule is two or more. The finding, that glucose of low concentrations have stimulated 3-O-MG uptake from a solution containing 3-O-MG of relatively low concentration, implies that binding of glucose to one of the binding sites on a single carrier causes an elevation of the affinity of the other binding site or sites to 3-O-MG. Since the Hill coefficient of the concentration dependence is not so high, for simplicity, we tentatively took the number of binding sites on a single carrier at the outer surface as two. Assuming the presence of two states of high and low affinities to sugars, the relationship between the degree of saturation of the binding sites with 3-O-MG and sugar concentrations is expressed by the following Monod-Wyman-Changeux type equation:

$$Y = \frac{\frac{[M]}{K_2} + 2\frac{[G]}{K_1}\frac{[M]}{K_2} + 2\left(\frac{[M]}{K_2}\right)^2}{2\left[L + 1\frac{[G]}{K_1} + \frac{[M]}{K_2} + \left(\frac{[G]}{K_1}\right)^2 + 2\left(\frac{[G]}{K_1}\frac{[M]}{K_2}\right) + \left(\frac{[M]}{K_2}\right)^2\right]} \quad (1)$$

where $[G]$ and $[M]$ are concentrations of glucose and 3-O-MG, respectively. L represents the equilibrium ratio of carrier density in low-affinity state to that in high-affinity state. K_1 and K_2 are dissociation constants of glucose-site-complex and of 3-O-MG-

site-complex of carriers in high-affinity state, respectively. Binding of sugars to low-affinity carriers has been neglected. The theoretical curves obtained by calculation using Eq. (1) is shown in Fig. 6, where two relationships are drawn; one is inhibition of 3-O-MG uptake by glucose of various concentrations and another is the concentration dependence of 3-O-MG uptake in solution with 5 mM glucose. Theoretical curves are reasonably well fitted to points obtained from data of experiments shown in Figs. 3 and 4, when L, K_1 and K_2 are 14, 0.5 and 2.0 mM, respectively.

Kono, Robinson, Blevins and Ezaki (1982) have elegantly demonstrated that sugar transport activity of plasma membrane-rich fraction prepared from insulin-treated fat cells are 6.3- to 8.6-fold that of the fraction prepared from untreated cells, whereas the transport activity of Golgi-rich fraction decreased to one-half of the control value. On the other hand, cytochalasin B binding in plasma membrane-rich fraction prepared from insulin-treated adipocytes is fourfold the control binding (Wardzala, Cushman & Salans, 1978). This quantitative discrepancy between effects of insulin on transport activity and cytochalasin binding implies that more efficient transport systems are incorporated into plasma membrane during the activation of glucose transport by insulin. Allosteric and Na^+-sensitive properties of newly incorporated transport systems may be related to these observations.

This work was supported by Grants-in-Aid for Scientific Research (57570029, 58570036) from the Japanese Ministry of Education, Science and Culture. We are grateful to Mrs. Yuko Tanigaki-Matsuzuka for her secretarial assistance.

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Received 14 March 1985; revised 27 June 1985