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CHOLINERGIC STIMULATION OF GLUCOSE TRANSPORT IN HUMAN ERYTHROCYTES

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The effects of cholinergic stimulation on glucose equilibrium exchange rate have been studied in human erythrocytes. Carbamylcholine increases the V of equilibrium exchange by 20% but has no significant effect on K_m. The cholinergic effect is abolished by the muscarinic antagonist atropine or by alterations in intracellular calcium concentrations induced by the calcium ionophore A23187.

Introduction

Cholinergic stimulation of human erythrocytes leads to an alteration in membrane structure that increases the order parameter of incorporated fatty-acid spin labels [1,2]. Increases in this spectroscopic parameter signify decreased rotational mobility of the labeled molecule, and in the case of a spin-labeled membrane have been interpreted as decreases in the bulk fluidity of the membrane lipid. Glucose transport is one of numerous erythrocyte membrane functions affected by changes in membrane fluidity. For example, membrane fluidity can be manipulated by treatment of cells with phospholipase A_2, sphingomyelinase, or phospholipase C to modify phospholipids [3–6], incorporation of lipid-soluble molecules into the bilayer [7], alteration of the cholesterol/phospholipid ratio in the membrane [8–11], exchange of cholesterol for other steroids [8,11,12], and degradation of the membrane cytoskeleton [13]. All of these procedures affect hexose transport across the membrane in a way that suggests that increases in membrane fluidity are correlated with decreases in transport rate. A similar effect has been seen for uridine [11] and lactate [10] transport in erythrocytes, and for the activity of succinate oxidase in mitochondrial membranes from sheep and rats [10].

These correlations between membrane fluidity and transporter activity led us to examine the rate of glucose transport in erythrocytes exposed to cholinergic stimulation. We find that cholinergically-stimulated erythrocytes display an increase in V of equilibrium exchange of glucose, and no change in K_m. This is consistent with previously reported inverse correlations between membrane fluidity and glucose transport rate.

Materials and Methods

Carbamylcholine chloride and atropine sulfate were purchased from Sigma Chemical Co. The Ca^{2+}-ionophore, A23187, was the product of Calbiochem. New England Nuclear supplied D-[U-^{14}C]glucose.

Erythrocytes were separated from plasma by centrifugation at $3600 \times g$ for 10 min, and washed three times by supernatant aspiration, resuspension in 0.15 M NaCl, and centrifugation. Packed cells (20 ml) were loaded to a given concentration of D-glucose by suspension in 20 ml of a buffer containing 1.7-times that concentration of D-glucose as well as 0.14 M NaCl, 5 mM KH_2PO_4,

2.5 mM Na_2HPO_4, 1 mM $MgSO_4$, and 50 μCi of D-[U-^{14}C]glucose, adjusted to pH 7.4 with 1 M NaOH. After 20 min at room temperature the loaded cells were packed by centrifugation at 3600 $\times g$ for 15 min, and isolated by supernatant aspiration. These cells were allowed to warm to the temperature of the experiment.

One ml of the packed, loaded cells was added rapidly to 9 ml of stirred, thermostatically controlled buffer containing the effector being studied and D-glucose at the cell-loading concentration. At 6 to 8 s intervals, a total of four 1 ml aliquots was removed and added to 3 ml of the same buffer containing 4 mM $HgCl_2$, 1.2 mM KI, and 5 mM D-glucose. A fifth aliquot (the 'infinite time' point) was removed after 5 min and treated similarly. The cells were pelleted by brief centrifugation at 3200 $\times g$, and 1 ml aliquots of the supernatants were removed for scintillation counting.

Data were analysed essentially as in Ref. 9. The slope (m) of plots of $\ln(S(\infty) - S(t))$ versus t (where $S(t)$ is the amount of radioactivity in cpm in the supernatant of the sample taken at time t) is described by:

$$m = \frac{V}{[\mathrm{Glc}] - K_m}(1 + (v_i/v_e))$$

where V and K_m are the kinetic parameters for equilibrium exchange, and v_i and v_e are the total intracellular and extracellular volume, respectively. The ratio of v_i/v_e was approximated to be 0.08 in these experiments.

Results

Representative results of a glucose equilibrium exchange experiment are shown in Fig 1. Plots of $\ln(S(\infty) - S(t))$ are linear with time through 30 s. The slope of this function is increased by approx. 20% in the presence of 10 μM carbamylcholine. This rate enhancement is not observed in the presence of 10 μM of the muscarinic antagonist atropine. Thus, the effect appears to be mediated by interaction of the carbamylcholine with a receptor, rather than resulting from some non-specific interaction.

The effect of carbamylcholine concentration on glucose equilibrium exchange is shown in Fig. 2.

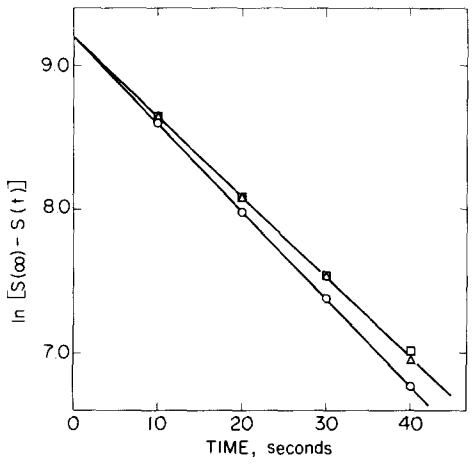

Fig. 1. Equilibrium exchange of D-[U-^{14}C]glucose across the erythrocyte membrane. Erythrocytes were loaded with 100 mM D-glucose containing D-[U-^{14}C]glucose. Efflux of the label into solutions containing 100 mM D-glucose was observed. $S(t)$ is the cpm in an aliquot of supernatant at any time. Control (□), cells exposed to 10 μM carbamylcholine (○), cells exposed to 10 μM carbamylcholine and 10 μM atropine (△). Temp. 25°C.

The maximum rate enhancement is observed in the concentration range 1 to 10 μM carbamylcholine.

The experiments described above were conducted in buffered saline containing 10 μM $CaCl_2$. When the cells were suspended instead in buffer containing 10 μM EGTA to chelate Ca^{2+}, the cholinergic enhancement of glucose exchange was diminished (Table I). The effects of intracellular

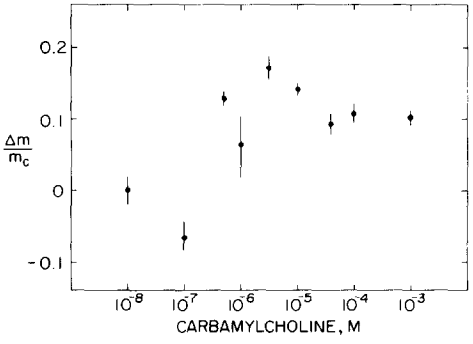

Fig. 2. Response of D-glucose equilibrium exchange to carbamylcholine. $\Delta m = m - m_c$ where m and m_c are slopes of a plot of $\ln(S(\infty) - S(t))$ versus time in the presence and absence, respectively, of carbamylcholine. Hence, $\Delta m/m_c$ is the fractional change in equilibrium rate in the presence of carbamylcholine. Results are averages ± S.E. $n = 7$ for 10^{-5} M; $n = 5$ for 10^{-8} M; $n = 4$ for 10^{-7} M; $n = 2$ for $3 \cdot 10^{-6}$ M and 10^{-4} M. Temp. 25°C.

TABLE I

STIMULATION OF D-GLUCOSE EQUILIBRIUM EXCHANGE BY CARBAMYLCHOLINE

The relative stimulation of equilibrium exchange of glucose was measured in the presence of carbamylcholine using cells with normal and modified intracellular Ca^{2+} concentrations. Averages ± S.E. $\Delta m/m_c$ is as in Fig. 2.

Addition	$\Delta m/m_c$	N
None	0.00	
10 μM carbamylcholine + 10 μM Ca^{2+}	0.17 ± 0.02	10
5 μM A23187 + 10 μM Ca^{2+}	−0.09 ± 0.01	11
10 μM carbamylcholine + 5 μM A23187 + 10 μM Ca^{2+}	−0.11 ± 0.01	9
10 μM carbamylcholine + 10 μM EGTA	0.07 ± 0.01	8
5 μM A23187 + 10 μM EGTA	0.02 ± 0.01	7
10 μM carbamylcholine + 5 μM A23187 + 10 μM EGTA	0.00 ± 0.01	7

Ca^{2+} on the cholinergic response were examined using the Ca^{2+}-specific ionophore A23187 [17]. In buffer containing 10 μM Ca^{2+}, the ionophore alone inhibited glucose exchange and abolished the stimulatory effect of carbamylcholine. In buffer containing no Ca^{2+} and 10 μM EGTA, the ionophore alone did not affect glucose exchange, but inhibited the effect of carbamylcholine. The former conditions (ionophore + 10 μM extracellular Ca^{2+})

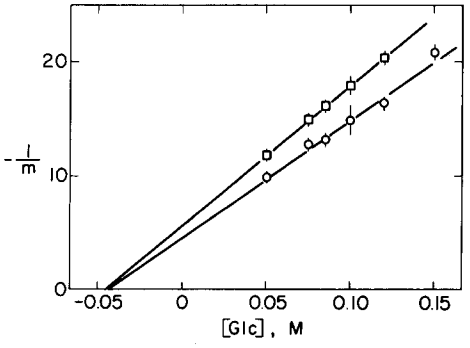

Fig. 3. Determination of K_m and V of D-glucose equilibrium exchange. Measurement of $-1/m$ at a variety of concentrations of D-glucose allows separation of K_m and V. Control (□), cells exposed to 10 μM carbamylcholine (○). Results are averages ± S.E. $n=3$ for 75 mM and 120 mM D-glucose; $n=6$ for 50 mM, 60 mM, and 85 mM D-glucose; $n=7$ for 100 mM D-glucose. Temp. 25°C.

should increase the intracellular Ca^{2+} concentration approximately 10-fold [17], while the latter (ionophore + extracellular EGTA) should deplete the cells of Ca^{2+}; thus it appears that the response of glucose exchange to cholinergic stimulation is dependent on normal intracellular Ca^{2+} levels.

The effects of carbamylcholine on V and K_m for glucose exchange were examined through studies at varying glucose concentrations (Fig. 3). Control values obtained were $V = 8.8$ mM/s and $K_m = 43$ mM, both of which are within normal ranges [18]. In the presence of 10 μM carbamylcholine, V was found to be 10.3 mM/s, while K_m was 46 mM. Within the accuracy of this graphic representation. K_m appears to be independent of cholinergic stimulation while V is enhanced by about 20%.

Discussion

Cholinergic receptors have been demonstrated in human erythrocytes both by radiolabel binding [16] and by spin-label studies of carbamylcholine-dependent changes in membrane fluidity [1,2]. The physiological function of these receptors is not understood, but the present study has shown that cholinergic stimulation has a measurable effect on glucose transport across the cell membrane. In the concentration range 1–10 μM, carbamylcholine increases the rate of this transport without detectable effect on the K_m of the sugar. In this same concentration range, carbamylcholine exerts its maximal effects on membrane fluidity [1,2] and on radiolabeled ligand binding [16]. Both the membrane fluidity change and acceleration of glucose transport appear to require normal intracellular calcium levels; depleting cells of their cytosolic calcium inhibits both responses. Increasing intracellular calcium to 10 μM also inhibits stimulation of glucose transport. This may be due to resultant morphological changes in the cell, but is not likely to arise from the calcium induced protein crosslinking which occurs only at 10-fold greater calcium concentrations [20].

An inverse relationship between red cell membrane fluidity and hexose exchange rate has been observed in other instances. For example, treatment with anesthetics or steroid substitution increase membrane fluidity and inhibit glucose ex-

change [11], while increasing membrane cholesterol content decreases fluidity [12,20], and accelerates exchange [10,12]. None of these treatments has measurable effects on the K_m of transport.

The effects of cholinergic stimulation are qualitatively similar to cholesterol enrichment; membrane fluidity is decreased and glucose transport is accelerated. However, the magnitude of the cholinergic effect is not strictly consistent with cholesterol enrichment effects. Micromolar carbamylcholine increases the glucose exchange rate by 20%, an enhancement comparable to that in red cells containing 1.3 [12] to 1.6 [10] times their normal cholesterol/phospholipid ratio. In a model phosphatidylcholine/cholesterol membrane, a 30% increase in cholesterol mole fraction would reduce the bulk lipid fluidity substantially; the order parameter of an incorporated fatty acid spin label would be expected to increase by at least 5% [21]. In intact red cells, however, cholinergic stimulation increases the spin-label order parameter by only 1.5–2% [1]. Thus, cholinergic stimulation appears to affect glucose transport to the same degree as cholesterol enrichment with less effect on membrane fluidity. It may be inappropriate to extrapolate from model membrane order parameters to natural membrane microviscosity; microheterogeneity of the cell membrane could give rise to the above disparity in a number of ways. For example, cholesterol could partition preferentially into regions of the membrane where its effect on glucose transport would be diminished. Alternatively, the cholinergic effect could be exerted on some particular function or region of the membrane rather than on bulk lipid properties. However, the qualitative correlation between glucose transport rate and fluidity changes induced by apparently diverse agents suggests that this function is sensitive to general membrane viscosity. Glucose transport in erythrocytes is 1000-times faster than is required for glycolysis, so the small rate changes observed in these studies are of dubious metabolic relevance. The significance of these observations lies rather in their implications for control of intrinsic protein function in membranes.

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