Interactions of sodium pentobarbital with D-glucose and L-sorbose transport in human red cells

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Abstract

Pentobarbital acts as a mixed inhibitor of net D-glucose exit, as monitored photometrically from human red cells. At 30°C the K_i of pentobarbital for inhibition of V_{max} of *zero-trans* net glucose exit is 2.16 ± 0.14 mM; the affinity of the external site of the transporter for D-glucose is also reduced to 50% of control by 1.66 ± 0.06 mM pentobarbital. Pentobarbital reduces the temperature coefficient of D-glucose binding to the external site. Pentobarbital (4 mM) reduces the enthalpy of D-glucose interaction from 49.3 ± 9.6 to 16.24 ± 5.50 kJ/mol ($P < 0.05$). Pentobarbital (8 mM) increases the activation energy of glucose exit from control 54.7 ± 2.5 kJ/mol to 114 ± 13 kJ/mol ($P < 0.01$). Pentobarbital reduces the rate of L-sorbose exit from human red cells, in the temperature range 45°C–30°C ($P < 0.001$). On cooling from 45°C to 30°C, in the presence of pentobarbital (4 mM), the $K_{i\ (sorbose,\ glucose)}$ decreases from 30.6 ± 7.8 mM to 14 ± 1.9 mM; whereas in control cells, $K_{i\ (sorbose,\ glucose)}$ increases from 6.8 ± 1.3 mM at 45°C to 23.4 ± 4.5 mM at 30°C ($P < 0.002$). Thus, the glucose inhibition of sorbose exit is changed from an endothermic process (enthalpy change = $+60.6 \pm 14.7$ kJ/mol) to an exothermic process (enthalpy change = $-43 \pm 6.2\ 7$ kJ/mol) by pentobarbital (4 mM) ($P < 0.005$). These findings indicate that pentobarbital acts by preventing glucose-induced conformational changes in glucose transporters by binding to 'non-catalytic' sites in the transporter. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Glucose transport; Erythrocyte; Pentobarbital

1. Introduction

Barbiturates and phenothiazines inhibit glucose uptake across cell membranes [1,2]. Both types of drug inhibit red cell net glucose transport more than exchange transport. This finding, together with the absence of any observed effect on the sugar affinity for the transporter, led El-Barbary et al. [2] to suggest that barbiturates are non-competitive in-hibitors of glucose transport, binding preferentially to the unoccupied form of the transporter.

Hypnotics and anaesthetics interfere with many other transport and receptor types, e.g., GABA [3,4], Na$^+$ channels, [5] glutamate, [6] dopamine, serotonin [7], nicotinic receptors [8] and Ca^{2+} ATPase [9]. They inhibit by interacting with non-polar sites in the protein interior. A wide range of affinities (K_d) for pentobarbital has been observed; from ca. 10 μM for GABA [3,4] to 10 mM for Ca^{2+} ATPase [9]. Such binding is expected to modify conformational substates of the enzyme or transporter and, thereby give a mixed inhibition, i.e., reduce the maximal rate of activity, V_m and substrate affinity, K_m.

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Recent studies on hexose transport in red cells showed that D-glucose interacts exothermically with the human erythrocyte glut 1 transporter. This leads to a conformation change that reduces the activation energy, E_a of net glucose transport, thereby facilitating transport. It was also shown that D-glucose inhibits L-sorbose transport across the glucose transporter by an endothermic process [10] (see Scheme 1. Sorbose is transported via the glut 1 transporter as a low affinity 'poor' substrate. Its transport differs from D-glucose, as it has very low affinity for the glut 1 transporter (K_t, ca 200 mM); it has a much lower maximal rate of transport than glucose, ca. 1/20, and it is not subject either to accelerated exchange or counterflow. An anomaly of D-glucose inhibition of L-sorbose transport is that it has a 4–10-fold higher K_i of inhibition of L-sorbose exit than expected, on the basis of the affinity of glucose for the transporter (2–4 mM). Additionally, it has been shown that the K_i of glucose-dependent inhibition of L-sorbose exit rises from 5–12 mM as temperature is reduced from 50°C to 30°C [10].

An explanation for this effect is that the glut 1 transporter is activated by interaction with D-glucose, but not by L-sorbose. Return to the transporter ground state after glucose dissociates is not instantaneous. If sorbose binds to the transporter after interaction with D-glucose in the interval prior to its return to ground state, then mobility of L-sorbose across the transporter is slightly raised. This raised mobility of sorbose raises the apparent K_i of glucose inhibition of sorbose exit, $K_{i\ (sorbose,\ glucose)}$. Since cooling slows conformational change, it follows that cooling will increase the probability of L-sorbose binding to the activated state of the transporter in the co-presence of D-glucose and thereby, raise the $K_{i\ (sorbose,\ glucose)}$ for inhibition of sorbose flux.

D-Mannose has a lower binding enthalpy than D-glucose for red cell sugar transporter and higher activation energy of net transport than glucose. Its effect on sorbose transport is less marked than that of glucose. This indicates that the extent of activation of the transporter relates to the energy of its interaction with substrate. It follows that a good substrate, e.g., D-glucose, is one which causes a larger conformational change than poorer ones, e.g., D-mannose, or very poor ones, like L-sorbose. Accelerated exchange, or a substantial reduction in activa-

tion energy of net flux, E_a, occur only when large conformational changes are induced by interaction with 'good substrates' [10].

Since accelerated exchange transport of sugars across the red cell sugar transporter has lower activation energy than net flux, this indicates that the transporter adopts a larger conformation shift from the ground state during the accelerated exchange mode than in net flux mode. These different transporter conformations during net and exchange flux modes may explain the differential effects of pentobarbital on net and exchange transport [1,2].

It seemed likely that pentobarbital might reduce the glucose-induced conformational changes in the transporter. It is for this reason that the effects of sodium pentobarbital on the thermodynamic interactions of D-glucose and L-sorbose with the human erythrocyte glucose transporter were investigated.

2. Materials and methods

2.1. Solutions

The composition of the buffered saline was as follows (in mM): NaCl 140; KCl 2.5; MgCl$_2$ 2.0; Hepes (N-2-hydroxyethylpiperazine-N'-(2-ethanesulfonic acid)) 5. All chemicals including L-sorbose, D-glucose and sodium pentobarbital were obtained from Sigma Chemical Co., Dorset. All solutions were buffered to pH 7.4 with HCl, using appropriate temperature corrections and corrections for the buffering effect of the drug.

2.2. Cells

Fresh human erythrocytes were obtained by venepuncture, washed three times in isotonic saline by repeated centrifugation and resuspension. The cells were then suspended in solutions containing sugars at the preloading concentration-usually 100 mM, final haematocrit 10%. The cells were incubated for at least 2 h, in the case of D-glucose and pentobarbital, or in the case of L-sorbose 5 h, to allow the sugars to equilibrate with the cell water. The cells were then recentrifuged to obtain a thick cell suspension ca. 95% haematocrit. This cell suspension was kept at 4°C until required. Aliquots of pre-warmed cell suspen-

sion (7.5 μl) were added to a 1-cm^2 fluorescence cuvette containing 3 ml of saline solution which had been pre-warmed to the required temperature. The cell suspensions were mixed vigorously and photometric monitoring was started within 5 s of mixing. The final glucose concentration in nominally glucose-free solution with this regime is maximally 0.25 mM, which is at least 10-fold less than the lowest K_m of D-glucose measured here: so contamination of the external solution with glucose has a negligible effect on D-glucose exit. Pentobarbital was added to the external solutions at the same concentrations as that in the pre-loaded cells.

2.3. Photometric monitoring

2.3.1. Sorbose exit

The effects of varying equimolar concentrations of D-glucose, added to both cell water and external solutions, on the exit rates of L-sorbose from cells were monitored photometrically, using a Hitachi 2000-F fluorescence spectrometer with a temperature-controlled and monitored cuvette; $E_{ex} = E_{em} = 650$ nm. The output was recorded and stored directly using a MacLab 2 e (AD Instruments). Data were collected at a rate of 0.33–5 points s^{-1}, depending of the time course of exit; each run consisted of 200–2000 data points. The photometric response was found to be approximately linear for osmotic perturbations ± 50 mM NaCl.

The time courses of L-sorbose exit were fitted to mono-exponential curves of the form $y_t = A\{1 - B \cdot \exp(C \cdot t)\}$ using the curve fitting program in Kaleidagraph 3.08 (Synergy Software): y_t is the voltage recorded at time, t; the coefficient, A is a scaling factor which fits the curves to the voltage signal; B and C are the exponential coefficients; and t is the time in seconds at which the observed data y_t were obtained. These fits gave correlation coefficients, $r > 0.98$ and standard errors of the means of the rate coefficients. Since the loading concentration of L-sorbose was the same for all experiments (100 mM), the rate coefficient C can be used to monitor the effects of temperature and of either D-glucose, or sodium pentobarbital concentration on L-sorbose exit permeability. Complete sets of data covering the entire temperature range were collected over 48-h periods from single venepuncture samples, i.e.,
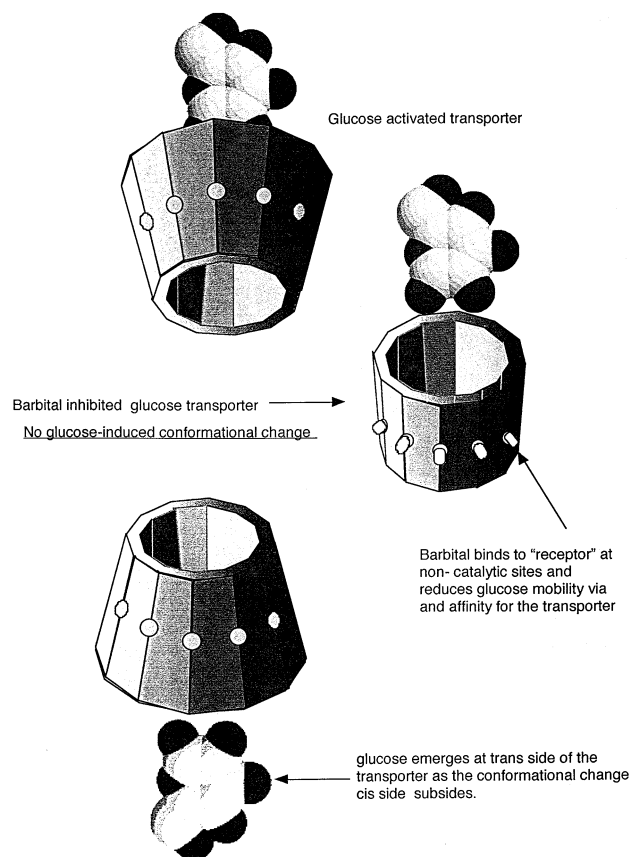
Scheme 1. The diagram shows space-filling models of glucose entering and leaving a hypothetical glucose transporter. The conformational change in the glut results from interaction with glucose [10]. This conformational change facilitates glucose transport through the transporter. Pentobarbital is viewed as a mixed inhibitor, which binds to the transporter at some site external to the sugar-binding domains. Pentobarbital binding prevents the glucose-induced conformational changes that facilitate glucose transport and alters the kinetics of sorbose transport.

ca. 120 flux determinations per session. No obvious changes in rates were noted during the sessions.

D-Glucose exit rates were similarly estimated; however, the initial rates of D-glucose exit were calculated from the following equation: sugar exit rate (mmol l^{-1} cells s^{-1}) = $D \cdot C$, where D = {loading concentration − external [sugar])/100}. It should be noted that representation of D-glucose exit as a monoexponential gives a very good approximation both to the initial zeroth order saturation kinetics and to the later hyperbolic relationship of flux with cell concentration $r \sim 0.98$.

Effects of varying Pentobarbital on Glucose exit at 30°C

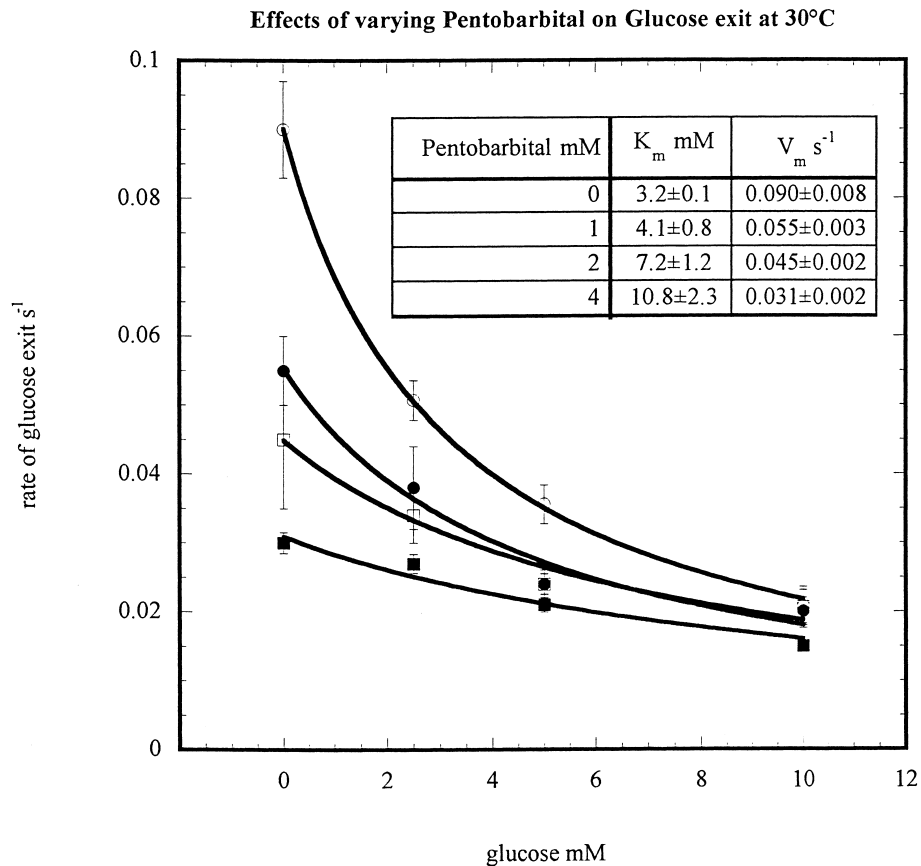| Pentobarbital mM	K_m mM	V_m s^{-1}
0	3.2±0.1	0.090±0.008
1	4.1±0.8	0.055±0.003
2	7.2±1.2	0.045±0.002
4	10.8±2.3	0.031±0.002

Fig. 1. Effects of varying concentrations of pentobarbital on net glucose exit from human erythrocytes at 30°C. The lines through the points are the best-fit least-square linear regression lines of the equation $K_m \cdot V_m / (K_m + G_o)$. The derived Michaelis–Menten parameters ± S.E.M. are shown in the table inset. The S.E.M are derived from means of 4–5 data points obtained from traces from 3–4 samples of blood.

2.4. Statistics

All the probabilities were estimated from two-tailed Student's *t*-values for unpaired means, even although the data were always matched. The *n* values were estimated from the number of degrees of freedom all data points were obtained from the means of 3–5 sets of data. Each data set was repeated on separately drawn blood samples.

3. Results

3.1. Effects of varying concentrations of sodium pentobarbital on the kinetics of D-glucose exit from human red cells at 30°C

The results shown in Figs. 1 and 2 indicate that

pentobarbital inhibited D-glucose exit from human red cells at 30°C. The maximal velocity, V_m of *infinite-cis* net glucose exit decreased from a control value of 9.0 ± 0.8 mmol l^{-1} cell water s^{-1} to 3.1 ± 0.15 mmol l^{-1} cell water s^{-1} as pentobarbital concentration is raised from 0 to 4 mM. The concentration of pentobarbital giving a 50% reduction in the rate of glucose exit was 2.16 ± 0.14 mM, which is the same value obtained by El-Barbary et al. [2] for inhibition of glucose influx into human red cells at 23°C (Fig. 2). The results also demonstrated an increase in the apparent K_m (*infinite-cis* exit) of glucose as [pentobarbital] increased. The concentration of pentobarbital required to increase the control K_m from 3.2 ± 0.1 mM to 6.4 mM was 1.66 ± 0.06 mM (Fig. 2). Hence, pentobarbital behaves as a mixed (un-competitive) inhibitor of glucose transport at 30°C.

An interpretation of these data is that pentobarbi-

**Effect of Pentobarbital on normalized Km and Vm
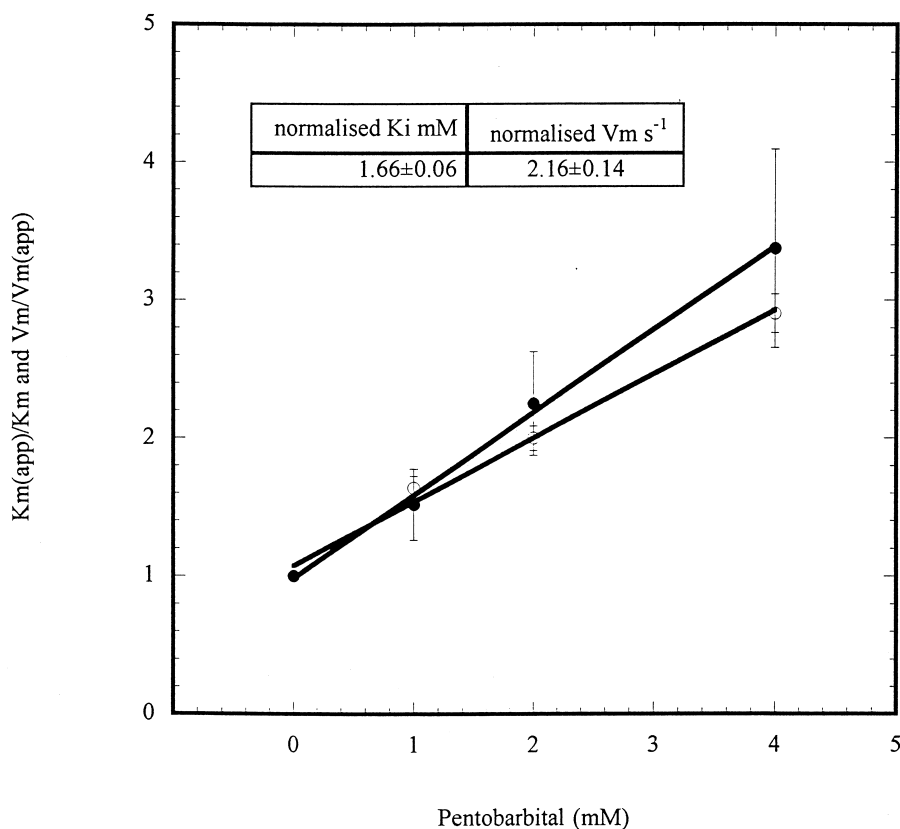of glucose exit from human red cells at 30°**

normalised Ki mM	normalised Vm s^{-1}
1.66±0.06	2.16±0.14

Fig. 2. The lines displayed are obtained from the Michaelis–Menten parameters in Fig. 1. The normalised K_m is the ratio K_m(apparent) obtained with varying concentrations of pentobarbital, to the K_m obtained with zero pentobarbital present. The normalised V_m is the ratio of V_m obtained with zero pentobarbital to the V_ms with pentobarbital. The lines joining the points are the least-square linear regression lines. Hence, the normalised K_i is obtained from the points on the lines where the normalised ratio = 2.

tal binds both to the glucose binding site (EG) and to some additional 'non-catalytic' site (E) of the transporter, thereby affecting both K_m and V_m (see Section 4).

3.2. The effects of temperature and pentobarbital 4 mM on the flux parameters K_m and V_m of D-glucose exit

The effects of temperature on the flux parameters K_m and V_m of net D-glucose exit from red cells in the presence or absence of pentobarbital 4 mM are shown in Fig. 3. Arrhenius and van't Hoff plots derived from the above data are shown in Fig. 4. In addition to raising the K_m of the external site of the transporter for D-glucose, pentobarbital (4 mM) re-

duced the temperature coefficient of the K_m of glucose interaction with the transporter (Fig. 3). The enthalpy of glucose interaction, decreased from 49.3±9.6 to 16.24±5.5 kJ/mol – a reduction of 66% ($P < 0.05$); the corresponding entropy change on glucose interaction with the transporter was decreased by 85% from 14.12.±3.82 J/mol K° to 2.2±2.2 J/mol K° by pentobarbital (4 mM) ($P < 0.05$).

The Arrhenius plots of the maximal rates of glucose exit show that pentobarbital (8 mM) increased the activation energy of glucose exit from control 54.7±2.5 kJ/mol to 114±12.8 kJ/mol ($P < 0.01$).

These data indicate that pentobarbital reduced the energy of glucose interaction with the transporter and concurrently increased the activation energy re-

Effects of temperature on Pentobarbital (4mM)
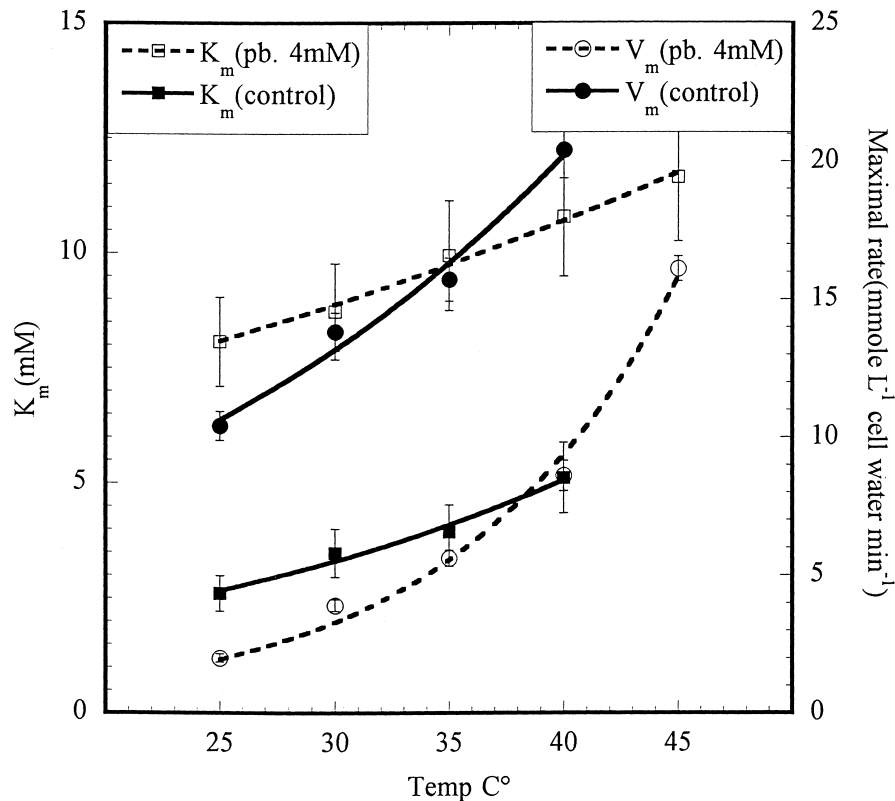## inhibition of glucose exit from human red cells

Fig. 3. Effect of temperature variation in the range 25–45°C on the apparent K_m and V_m obtained as in Fig. 1 at 30°C ± pentobarbital (4 mM). The lines through the points are the least square exponential regression equations according to the formula $A \cdot \exp(B \cdot T^{\circ C})$, where A and B are exponential coefficients and $T^{\circ C}$ is the temperature centigrade.

quired for glucose to traverse the transporter. These findings strongly suggest the pentobarbital impairs the capacity of the transporter to undergo glucose-induced conformational changes.

3.3. Effects of pentobarbital (4 mM) on glucose-dependent inhibition of L-sorbose exit from human red cells

The effects of pentobarbital (4 mM) on glucose-dependent inhibition of sorbose exit in the temperature range 45°C to 30°C are shown in Fig. 5A,B. Pentobarbital reduced the rate of sorbose exit from human red cells into glucose-free solutions at all temperatures tested ($P < 0.001$). A plot of the derived flux parameters shows that on cooling, the $K_{i \, (glucose)}$ in control cells increased from 6.8 ± 1.3 mM at 45°C to 23.4 ± 4.5 mM at 30°C ($P < 0.002$)

(Fig. 6). The temperature-dependent changes in sorbose exit were similar, but slightly higher than those reported previously [10]. Pentobarbital increased the $K_{i \, (sorbose, \, glucose)}$ of sorbose exit at high temperatures and also reversed the sign of the temperature coefficient of glucose dependent inhibition. On cooling from 45°–30°C in the presence of pentobarbital (4 mM) the $K_{i \, (sorbose, \, glucose)}$ decreased from 30.6 ± 7.8 mM to 14 ± 1.9 mM. Thus glucose inhibition of sorbose exit changes from an endothermic process (enthalpy change = $+60.6 \pm 14.7$ kJ/mol) to an exothermic process (enthalpy change = -43.3 ± 6.2 kJ/mol) in the presence of pentobarbital (4 mM) ($P < 0.005$).

4. Discussion

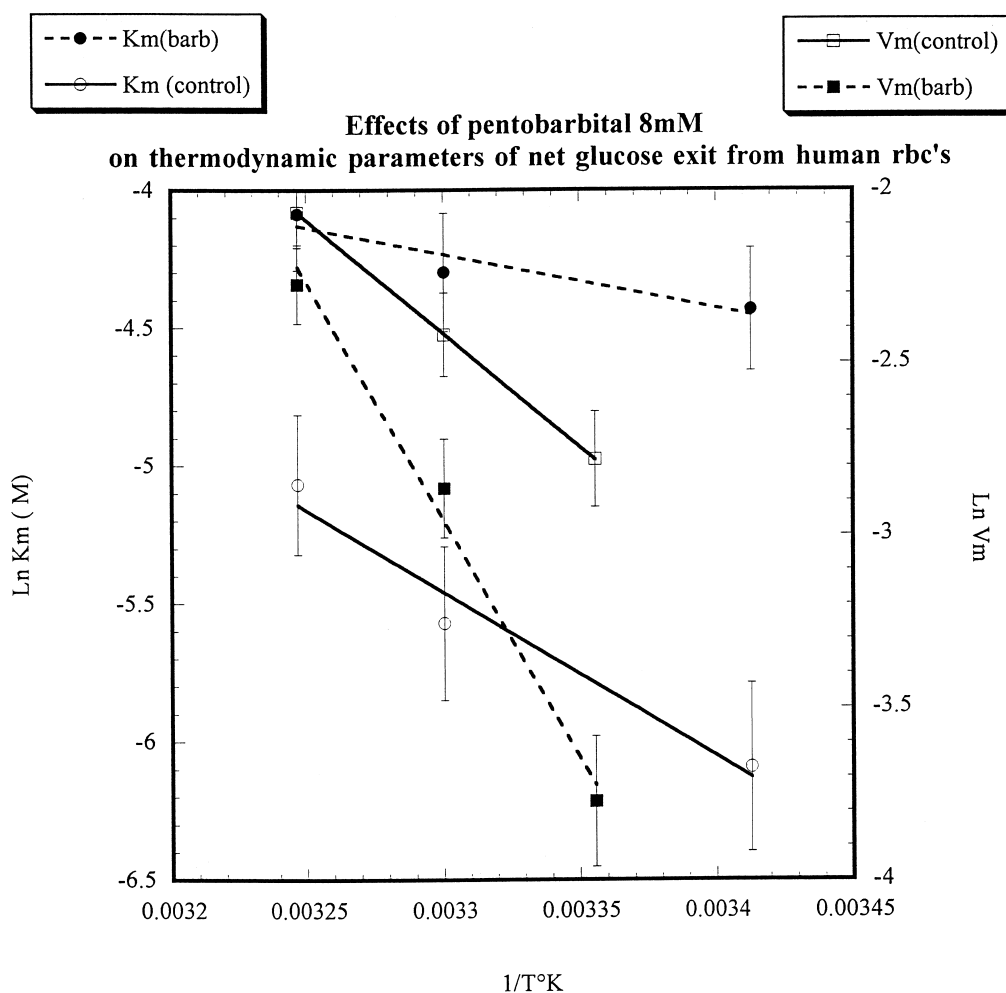The results reported here show that pentobarbital

Fig. 4. Van't Hoff plots (circles) and Arrhenius plots (squares) of the data shown in Fig. 3 where K_m is transformed to $\ln(K_m M)$ and V_m is transformed to $\ln(V_m)$ and $T^{\circ C}$ to $1/K^{\circ}$. The enthalpies and entropies are obtained from the slopes and intercepts of van't Hoff plots of $\ln(K_m M)$ versus $1/K^{\circ}$ and activation energies of glucose exit from the slopes of $\ln(V_m)$ versus $1/K^{\circ}$. The lines joining the points are the least-square linear regression lines.

is a mixed inhibitor (un-competitive) of glucose transport, reducing both V_m and apparent affinity of D-glucose for the transporter. This contrasts with an earlier report [2] that pentobarbital acts as an non-competitive inhibitor of glucose transport, merely reducing V_m without affecting K_m. The difference between these findings may be due to differing methodologies. The optical measurement of rates of D-glucose exit is a very discriminating means of estimating the affinity of sugars for the external site of the transporter.

This new finding is consistent with known effects of pentobarbital on other biological processes, e.g., Ca^{2+} ATPase [9] and activation of the nicotinic receptor [8]. Barbiturates are amphipathic drugs, which

have a higher efficacy in hydrophobic environments, possibly owing to their tendency to be concentrated at the water–lipid interface. It has been suggested that the drug binds to hydrophobic regions in the interstices of the protein folds and thereby reduces, or prevents conformational changes induced by substrate binding [8].

The following results fit with this view: pentobarbital (a) reduces the enthalpy and entropy of glucose binding; (b) increases the activation energy of glucose exit from the transporter; and (c) converts the endothermic response of the $K_{i \text{(sorbose, glucose)}}$ on L-sorbose transport to an exothermic response.

It has been shown [10] that glucose interaction with its transporter in the red cell is exothermic

A

**Effect of temperature on inhibition of L-sorbose exit
from human red cells by D-glucose**

B

**Effect of temperature on inhibition of L-sorbose exit
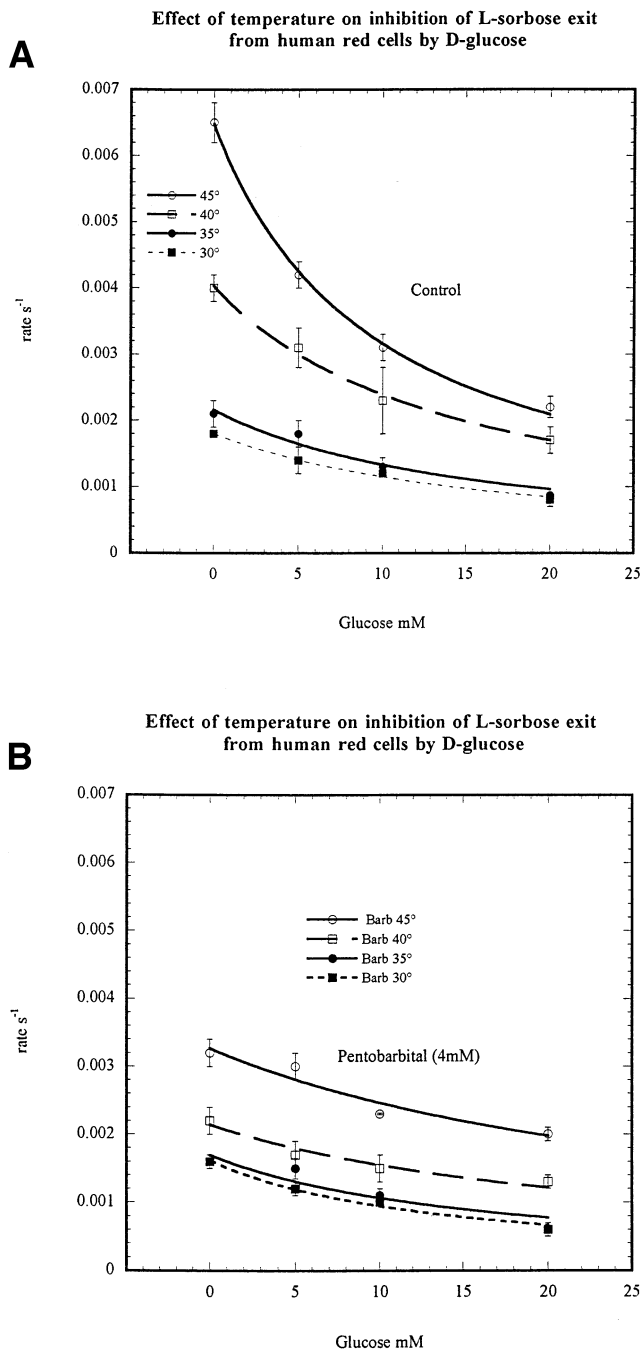from human red cells by D-glucose**

Fig. 5. Effects of temperature and varying internal and external [glucose] on rate of L-sorbose exit from an initial loading concentration of 100 mM. The lines through the data are the least square non-linear fits of the equation $K_i \cdot V_m/(K_i + G)$, where K_i is the inhibition constant giving half-maximal inhibition and V_m is the maximal velocity of sorbose exit. (A) Effects of temperature on sorbose exit; (B) same effects with 4 mM pentobarbital present in the bathing solution. Each point is obtained from at least three separate traces. The data were obtained from several repeat runs all showing similar responses.

and results in a conformational change which leads to facilitation of glucose exit. This is manifest as a reduction in the activation energy E_a of glucose exit. The activated state of the transporter has slow time decay after release of glucose (1–2 ms at 30°C). If L-sorbose, which does not itself activate the transporter, binds to the transporter within the relaxation time of the glucose-activated transporter it will have a higher mobility than if it were to bind only to the ground state of activation, with glucose absent.

At 30°C the $K_{i \, (sorbose, \, glucose)}$ of sorbose flux in control cells is 23.4 ± 4.5 mM (Fig. 6) and the K_m (*infinite cis*) is 3.2 ± 0.1 mM (Fig. 7). Thus the $K_{i \, (sorbose, \, glucose)}$ for inhibition of sorbose is 7.3 ± 1.4 higher than expected on the basis that glucose blocks sorbose flux by reversibly binding to the transporter ($P < 0.005$). With 4 mM pentobarbital present, the $K_{i \, (sorbose, \, glucose)}$ for inhibition of sorbose flux is 14.3 ± 1.9 mM and the K_m (*infinite cis*) $= 8.7 \pm 1.8$; hence the ratio of $K_{i \, (sorbose, \, glucose)}/K_{m \, (IC \, glucose)}$ falls from 7.3 ± 1.4 in control to 1.3 ± 0.32 with pentobarbital present (4 mM) ($P < 0.0025$). Additionally, in cells treated with pentobarbital, the ratio $K_{i \, (sorbose, \, glucose)}/K_{m \, (IC \, glucose)}$ increases as temperature is raised from 30°C to 45°C. This contrasts with the control condition where the ratio $K_{i \, (sorbose, \, glucose)}/K_{m \, (IC \, glucose)}$ decreases as temperature is raised from 30°C to 45°C (Fig. 8).

This pentobarbital-induced decrease in the ratio of $K_{i \, (sorbose, \, glucose)}/K_{m \, (IC \, glucose)}$ indicates that it prevents the glucose-induced conformational changes in the transporter which facilitate sorbose flux. This is another corroborating example for the view that pentobarbital prevents the glucose-induced activation of the transporter.

It also provides corroboration for the view that pentobarbital inhibition of D-glucose flux across the human red cell glucose transporter has no specific requirement for pentobarbital to bind to the 'unoccupied carrier site' [2]. Pentobarbital does not act by impeding the return of the empty carrier, as suggested by El-Barbary et al. [2]. This is evident from the finding that it reduces the affinity of glucose for the transporter. The mixed inhibition pattern merely requires that pentobarbital binds to non-catalytic sites on the protein surface and somehow prevents the conformational changes, which occur subsequent to substrate (glucose) binding. However, it remains a
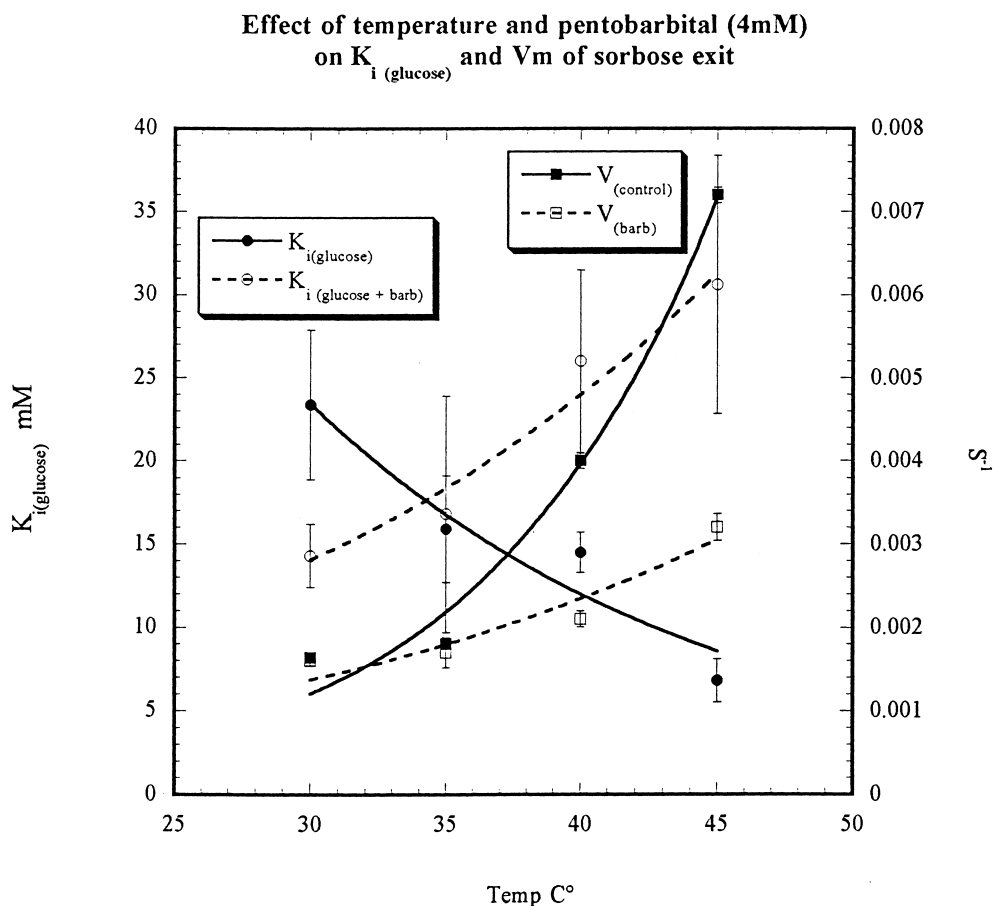
Fig. 6. Effects of temperature and pentobarbital (4 mM) on $K_{i\,(sorbose,\,glucose)}$ and V_m of sorbose exit. The primary data are from Fig. 5A,B. An important point of note is that $K_{i\,(sorbose,\,glucose)}$ decreases as temperature is raised in controls, whereas it rises as temperature is raised with pentobarbital (4 mM) present.

possibility that the transporter conformation in the accelerated exchange mode of transport has a lower affinity for pentobarbital, since access to the drug binding sites may be blocked by the large glucose-induced conformational changes. This pattern of inhibition has been previously noted also with phenothiazines as well as barbiturates [1,2] and suggests that access to the hydrophobic transporter sites may be blocked by double occupancy of the exchange mode of the fixed site transporter.

The results reported here and those published previously [10–13] are consistent with the view that the glucose transporter in human red cells (glut 1) is a fixed site transporter. Briefly, the conventional view that the glucose transporter is a single mobile site carrier in which the site alternates between inside and outside surfaces of membrane has several attractive features. It explains why accelerated exchange is

faster than net flux. It rationalises the apparent asymmetry of the transporter in which the transporter affinity for glucose inside is lower than outside and it is supported strongly by the finding that inhibitors binding to the inside site, like cytochalasin B, reduce the binding of inhibitors, like D-maltose to the outside sites.

However, the circulating carrier model implies that return of the empty carrier should be a slower process than movement of the sugar-carrier complex and hence should be the rate limiting step determining the maximal rate of zero-trans net sugar transport. The model implies that maximal rates of transport of all sugars sharing the glucose transporter and that the activation energies of net flux of these sugars should be similar, as all these should be determined by the rates of return of the empty carrier.

At 24°C, however, the maximal rate of uptake of

van't Hoff plots of Ki(glucose)
on sorbose exit ± Pentobarbital (4mM)

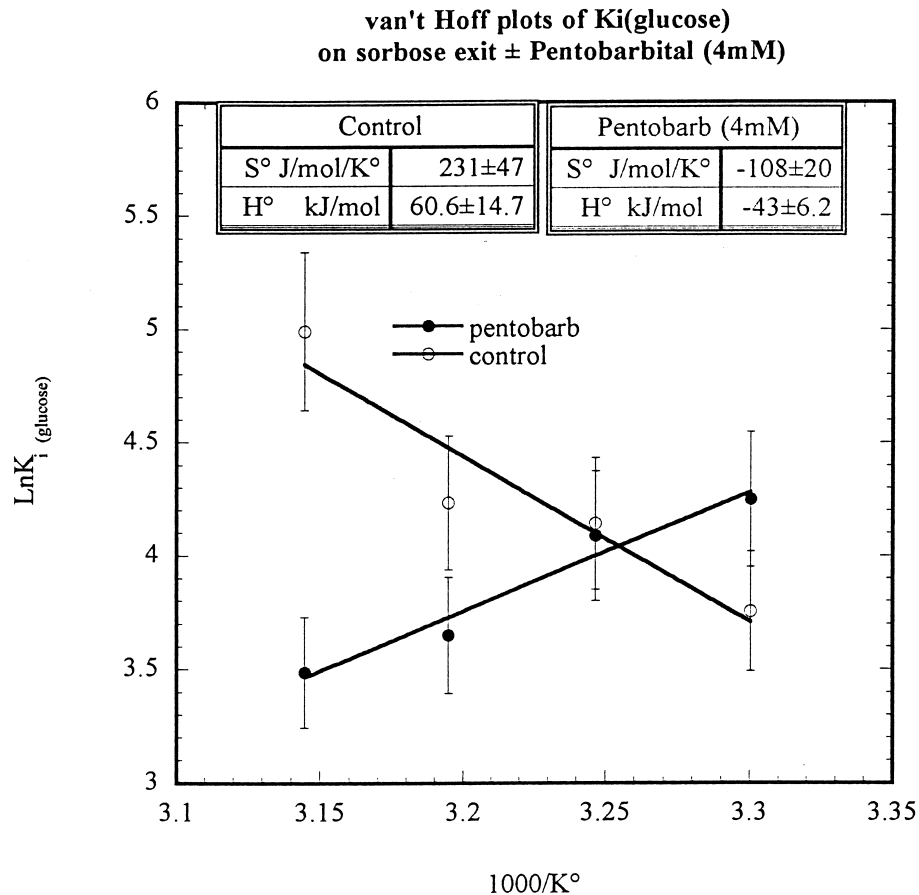| Control		Pentobarb (4mM)	
S° J/mol/K°	231±47	S° J/mol/K°	-108±20
H° kJ/mol	60.6±14.7	H° kJ/mol	-43±6.2

Fig. 7. Van't Hoff plots of the data shown in Fig. 6. The lines joining the points are linear regression lines. The enthalpy change resulting from glucose interaction with sorbose flux in controls is 60.6 ± 14.7 kJ/mol; with pentobarbital (4 mM) present the enthalpy change is −43 ± 6.2 kJ/mol ($P < 0.001$).

D-mannose into rat erythrocytes is only 26% of the maximal rate of uptake of 3-*O*-methyl-glucoside; whereas the self-exchange rates of these sugars are closely similar to each other [11]. Additionally, the activation energy of *zero-trans* D-mannose exit from human red cells is twice as large as that of D-glucose [10]. Hence, the maximal rates of net sugar transport cannot be described adequately by a single rate process.

The problems relating to flux asymmetry and the apparent occlusion of D-mannose binding to external sites by cytochalasin B have been resolved by Carruthers and co-workers [12,13]. They confirmed that there is a barrier to sugar diffusion at the cytosolic surface of the membrane, which reduces the apparent affinity of the transporter to glucose exit. They also showed that there is an allosteric interaction between the internal and external sites of the transporter, so that cytochalasin B binding to the inside reduces maltose affinity to the external surface and vice-versa.

Thus, the simplest hypothesis to explain glucose movement is a two-fixed-site model [10–13] which alters conformation according to the strength of ligand interactions. The transported sugar moves from one site to the other; consequently there is no necessity to postulate rate limitation by empty site movement.

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Effect of temperature and pentobarbital (4mM)
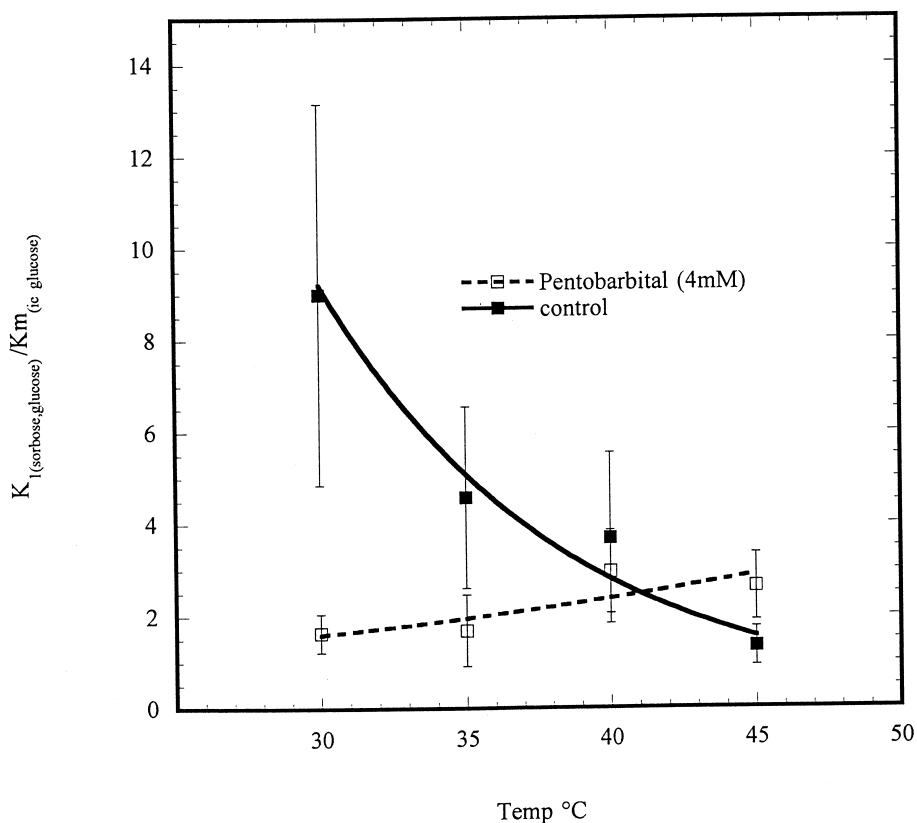**on the ratio $K_{i(sorbose,glucose)}$ to $K_{m(glucose,\ infinite\ cis)}$**

Fig. 8. Effects of temperature and pentobarbital (4 mM) on the ratio $K_{i\ (sorbose,glucose)}$ to $K_{m\ (glucose)}$. The lines joining the points are the least-square exponential regression lines to fit the equation $y = A\cdot \exp(B\cdot T^{\circ C})$, where A and B are the exponential coefficients and $T^{\circ C}$ is the temperature in °C.

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