Biochimica et Biophysica Acta, 508 (1978) 174—183

BBA 77965

CYCLIC AMP TRANSPORT IN HUMAN ERYTHROCYTE GHOSTS

G.D. HOLMAN

Department of Biochemistry, University of Bath, Bath BA2 7AY (U.K.)

(Received September 27th, 1977)

Summary

10^{-5} M cyclic AMP has high permeability in human erythrocyte ghosts (p = $0.061 \cdot 10^{-6}$ cm $\cdot$ s^{-1}). Saturation of influx and efflux occurs. K_{zt}^{oi} = 4.43 mM. V_{zt}^{oi} = 259.6 μM $\cdot$ min^{-1}. K_{zt}^{io} = 0.475 μM. V_{zt}^{io} = 28.3 μM $\cdot$ min^{-1} at 30°C. Equilibrium exchange entry of cyclic AMP has similar kinetics to zero *trans* influx, though the system does show counterflow. Cytochalasin B is an apparent competitive inhibitor of cyclic AMP exit. (K_i = $3.9 \cdot 10^{-7}$ M).

Control experiments indicated that cyclic AMP remains intact during incubation with red blood cell ghosts and is contained within the intravesicular space during the transport experiments.

Introduction

Despite the wealth of information on cyclic AMP and its role in the control of intracellular events such as glucose metabolism, in hormone mediated effects and in cell contact and recognition phenomena [1], its transport across membranes has not been studied in any detail.

The red blood cell has been used as a model for the study of many facilitative transport systems. These include the glucose transport system [2] and the anion transport system [3] which have long been extensively studied. More recently a nucleoside transport system has been identified [4] and kinetically characterised [6]. The present study reports the presence of a facilitative transport system for cyclic AMP and some of the kinetic parameters of this system have been measured.

The effect of cytochalasin B on cyclic AMP transport has also been studied. This compound is known to inhibit cytokinesis and to induce changes in cell shape [28]. Some of the effects of this drug were originally attributed to an effect on cell microfilaments [29] but it is also known to be an inhibitor of certain transport systems. Bloch [26] showed that it was an inhibitor of D-glucose transport in erythrocytes while Plageman and Estersen [7] have shown

that it is an inhibitor or uridine and thymidine transport in Novikoff rat hepatoma cells.

Materials and Methods

Chemicals

Cyclic AMP (sodium salt) and theophylline were obtained from Sigma. Cyclic[8-^{3}H]AMP was obtained from the Radiochemical Centre, Amersham. Cytochalasin B was obtained from the Aldrich Chemical Company. Other chemicals were obtained from B.D.H. Ltd. (reagent grade).

Red blood cell ghost preparation

Outdated human erythrocytes, stored in citrated buffer, were washed three times in 30 times the volume of phosphate/saline buffer (154 mM NaCl/25 mM sodium phosphate, pH 7.2). The cells obtained from 1 ml of blood were then haemolysed in 30 ml of 12.5 mM sodium phosphate buffer (pH 7.2) and membranes were isolated as described by Dodge et al. [8]. The membranes (approximately 15 mg protein/ml) were resealed in phosphate/saline buffer containing sufficient NaCl to restore the NaCl concentration to 154 mM. Initially, samples were agitated briefly on a rotamixer and were then incubated at 30°C for 1 h in a shaking water bath.

Zero trans entry experiments

The kinetic parameters for zero *trans* entry (influx with the initial absence of internal substrate) were estimated as follows. 0.1 ml of membranes were released in a total of 0.2 ml buffer for 1 h. The suspension of ghosts was then transferred to a further 0.2 ml of buffer containing appropriate concentrations of cyclic[^{3}H]AMP at 30°C. Initial mixing was vigorous followed by a gentle shaking in a water bath. 0.05 ml samples were removed at the time intervals indicated in Results (usually 1, 3, 5 and 90 min) and added to 15 ml of ice-cold phosphate/saline buffer containing 0.1 ml of ethanol. A zero time sample was prepared by adding this stopping solution before the addition of label. These solutions were filtered immediately through glass fibre filters (Whatman GF/F; 0.7 μm pore size, fast flow rates). Radioactivity trapped in the ghosts was then estimated by extraction into 1 ml of 1% trichloroacetic acid in scintillation vials. After the addition of scintillant (PPO 0.25%, Triton 50%, toluene 50%) samples were counted in a Packard Liquid Scintillation counter.

Initial rates were estimated from samples taken over the first 5 min and from the equilibrium sample obtained by incubating with the lowest concentration of cyclic AMP for 90 min at 30°C. At 100% filling of the ghosts $[\text{cyclic AMP}]_i = [\text{cyclic AMP}]_o$. The concentration of cyclic AMP inside was determined from the measurement of the internal D-glucose space.

Exchange entry experiments

These were performed as described for the net entry except that ghosts were resealed in the presence of cyclic AMP (total volume 0.35 ml) and the reaction was started by the addition of 0.05 ml of cyclic[^{3}H]AMP in phosphate/saline buffer.

Zero trans exit experiments

0.2 ml of membranes were resealed in the presence of appropriate concentrations of cyclic[^{3}H]AMP (total volume 0.3 ml). In preliminary experiments the concentration range studied was similar to that described for entry but was subsequently lowered when a low saturation constant was evident. After resealing these solutions were transferred to 10 ml of phosphate/saline buffer at 30°C. Initially mixing was vigorous followed by gentle shaking in a water bath. 1 ml samples were added to the stopping solution at the times indicated in the results section (usually 15 s, 1, 3, 5, 10, 20, 30 min) and filtered immediately and treated as described above. Zero time samples were obtained by adding stopping solution before the addition of the washout buffer. Estimates of contamination of filters by extracellular isotope were obtained from 90-min samples which were added to 1% trichloroacetic acid followed by ice-cold stopping solution. Preliminary experiments showed that exit was linear for 3 min at concentrations from 10^{-5}—10^{-4} M cyclic AMP. Subsequently, initial rates were estimated from samples obtained over the first 3 min at these concentrations and from samples obtained over the first 5 min at high concentrations.

Cytochalasin B was added in the exit medium in 0.05 ml of ethanol before the addition of resealed ghosts. (This concentration of ethanol did not effect exit of control experiments).

Permeability estimates

These were calculated from the average initial rate constant k (in min^{-1}) for entry and exit. $P = k \times \text{volume}/(\text{Area} \times 60) = k \times 10^{-6}$ cm $\cdot$ s^{-1} [31].

Chromatography

0.1 ml of red blood cell membranes were incubated at 30°C for 90 min in phosphate/saline buffer containing 10^{-5} M cyclic[^{3}H]AMP, (total volume 0.4 ml). The [^{3}H]cyclic AMP was then extracted into 90% ethanol and non-radioactive cyclic AMP was added to bring the concentration to 4 mg/ml. This extract was separated by paper chromatography (Whatman No. 1, run in ethanol : 0.5 M ammonium acetate (5 : 2)). Spots were identified under ultraviolet light and the chromatogram was then cut into 1 cm sections which were counted in scintillation vials. 97—98% of the recovered radioactivity co-chromatographed with authentic cyclic AMP.

Fitting the Michaelis-Menten equation

As discussed by Cleland [9] and by Cornish-Bowden [10] reciprocal plots require weighting procedures. The K_m and V values and their variances reported here were determined by linear regression using a weighting factor v^2/s^2 [10]. This was considered appropriate as the variance in v at each concentration is assumed to be a constant proportion of v. Other weighting procedures assume that the variance at high v is small compared with that at low v. This assumption may be erroneous in isotope flux experiments as isotope flux is low when s and v are high (unless constant specific activity label is used). The weighting procedure used gives similar results to unweighted regression of mean values of v. The variance in K_m and V are lower in the latter case in spite of the smaller sample number. This may indicate that a large source of error is due to dif-

ferences in day to day technique which can be partially compensated for by taking means from values of v at each concentration.

Results

Net entry of cyclic AMP

Fig. 1a shows the time cours for net entry of cyclic AMP. Entry is linear for the first 5 min and reaches equilibrium within 60—90 min. To eliminate the possibility that adenosine 3′5′ cyclic monophosphate diesterase may be present and be hydrolysing the substrate, 2 mM theophylline, a known inhibitor of this enzyme, was added to the incubation medium. As shown in Fig. 1a, theophylline had no effect on the entry rate or on the equilibrium radioactivity which was identical to that in a control experiment.

By measuring the initial rates of entry over the first 5 min at various concentrations of cyclic AMP, saturation of influx was observed (Fig. 2). As shown in Table I, $K_{zt}^{oi} = 4.43 \pm 0.41$ mM; $V_{zt}^{oi} = 259.6 \pm 16.1$ $\mu M \cdot min^{-1}$. Thus, although saturation of influx is observed, it is not in the physiological concentration range but is of interest as it shows that a specialised transport system is available to cyclic AMP and these kinetic constants will be of use in the elucidation of how this specialised transport system operates mechanistically.

Exchange entry of cyclic AMP

Exchange of tracer substrate for *trans* cold substrate results in kinetics different from those of net flux for substances such as glucose [23], chloride [3] and uridine [6] when these compounds interact with their respective transport systems. However, as shown in Fig. 1 and 2, the kinetics of exchange influx are not significantly different from those of net influx for cyclic AMP. This may mean that substrate interacting with the transport system at the inner surface does not lower the resistance of the transport system to substrate flowing from outside to inside.

Zero trans exit of cyclic AMP

Exit of 10^{-5} M cyclic AMP is rapid and initial rates can only be estimated

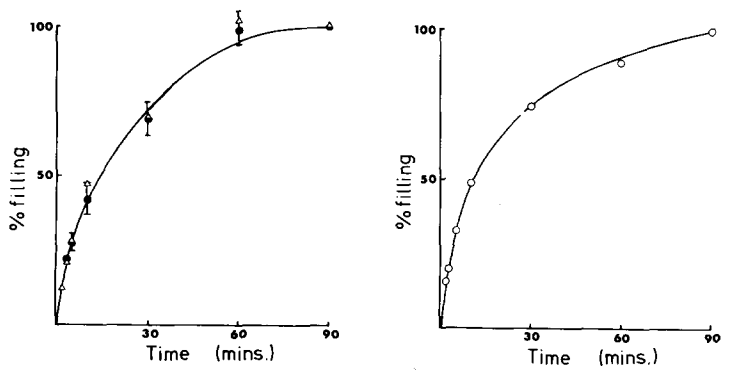

Fig. 1. (a) A time course for net influx of 10^{-5} M cyclic AMP in the presence (△) and in the absence (●) of 2 mM theophylline. Error bars represent the S.E.M. of 4 experiments. (b) A single experiment showing the time course for exchange influx of 10^{-5} M cyclic AMP.

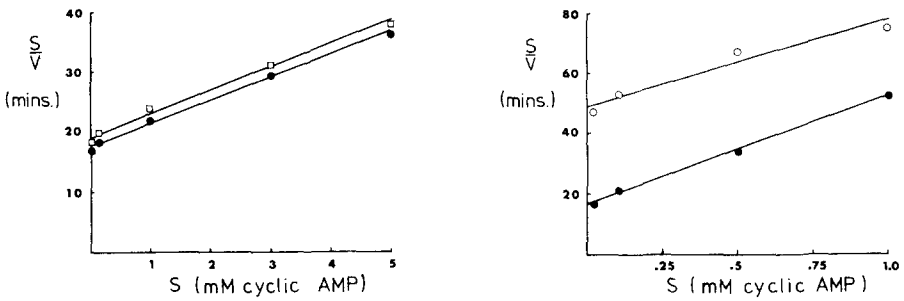

Fig. 2. A reciprocal plot of cyclic AMP influx vs. concentration (●, zero *trans* influx; □, exchange influx). Each point represents the mean of at least 3 determinations of v. The lines are derived from unweighted linear regression.

Fig. 3. A reciprocal plot of cyclic AMP efflux vs. concentration in the presence (○) and in the absence (●) of 10^{-6} M cytochalasin B. Each point represents the mean of at least 2 determinations of v in the presence and at least 3 determinations of v in the absence of cytochalasin B. The lines are derived from unweighted linear regression.

from samples taken over the first 3 min. At 1 mM, samples over the first 5 min can be used for estimating the exit rate. Similarly, in the presence of cytochalasin B (10^{-6} M) exit is sufficiently slowed to estimate exit over 5 min. Theophylline (2 mM) was added to an exit experiment where ghosts were incubated with 10^{-5} M cyclic AMP prior to the exit, but no effect of this preincubation on the exit rate was observed (not shown).

TABLE I

KINETIC CONSTANTS FOR CYCLIC AMP TRANSPORT IN HUMAN ERYTHROCYTE GHOSTS AT 30°C

Results are derived from weighted linear regression or from nonweighted regression of mean values of v. Subscripts zt and ee refer to zero *trans* and equilibrium exchange parameters in the directions outside to inside (oi) or inside to outside (io).

	Weighted regression (±S.D.)	Nonweighted regression of mean values of v (±S.D.)
K_{zt}^{oi}	4.33 ± 1.06 mM	4.43 ± 0.411 mM
V_{zt}^{oi}	271.5 ± 54.0 $\mu M \cdot min^{-1}$ ($n = 23$)	259.6 ± 16.1 $\mu M \cdot min^{-1}$ ($n = 5$)
K_{ee}^{oi}	3.88 ± 1.36 mM	4.67 ± 0.57 mM
V_{ee}^{oi}	230.6 ± 63.0 $\mu M \cdot min^{-1}$ ($n = 16$)	246.0 ± 20.4 $\mu M \cdot min^{-1}$ ($n = 5$)
K_{zt}^{io}	0.368 ± 0.17 mM	0.475 ± 0.048 mM
V_{zt}^{io}	29.3 ± 8.9 $\mu M \cdot min^{-1}$ ($n = 15$)	28.3 ± 1.4 $\mu M \cdot min^{-1}$ ($n = 4$)
K_{zt}^{io} + Cytochalasin B	1.80 ± 0.54	1.69 ± 0.38 mM
V_{zt}^{io} + Cytochalasin B	37.5 ± 9.3 $\mu M \cdot min^{-1}$ ($n = 9$)	35.1 ± 6.4 $\mu M \cdot min^{-1}$ ($n = 4$)

Fig. 3 shows the saturation of exit in the presence and in the absence of 10^{-6} M cytochalasin B. Table I shows that zero *trans* exist of cyclic AMP has a lower K (0.475 ± 0.048 mM) than that of zero *trans* entry and a lower V (28.3 ± 1.4 μM · min^{-1}) than entry. $K_{zt}^{oi}/V_{zt}^{oi} \simeq K_{zt}^{io}/V_{zt}^{io}$, which indicates that the data is consistent with the Haldane relationship and that cyclic AMP transport is not active. The permeability calculated from exit and entry data at 10^{-5} M cyclic AMP is $0.061 \cdot 10^{-6}$ cm · s^{-1}.

Davoren and Sutherland have studied the synthesis and release of cyclic AMP in pigeon erythrocytes [32]. They showed that the loss in intracellular cyclic AMP and its recovery in the medium was rapid and suggested that it may be transported against a concentration gradient in this system.

Cytochalasin B produces a large increase in the apparent K_{zt} exit with an insignificant effect on V (Table I). The K_i, assuming competitive inhibition, is $3.9 \cdot 10^{-7}$ M, indicating that cytochalasin B is a potent inhibitor of cyclic AMP transport. This K_i value is comparable with the K_i value found for cytochalasin B inhibition of sugar transport in the red blood cell [27].

The effect of increasing osmolarity on the equilibrium level of cyclic AMP

Increasing the osmolarity of the buffer has often been used to vary the size of the intravesicular space in transport experiments [11]. This experiment was carried out in the present case to check that the ghosts reseal and also to estimate the amount of isotope not contained in the intravesicular space. This value is obtained from the 'y' intercept of a plot of equilibrium isotope level vs. 1/osmolatiry. Isotope in the ghosts was measured after incubation of resealed ghosts for 90 min at 30°C in the presence of 10^{-5} M cyclic AMP plus 0, 100, 200, and 500 mM sucrose added to phosphate/saline buffer. As shown in Fig. 4, the isotope in the ghosts at infinite osmolarity is not significantly different from zero, which indicates that cyclic AMP is not significantly metabolised or bound to membrane material but is contained within the free solution in the vesicles. The binding of cyclic AMP to particulate fractions from a

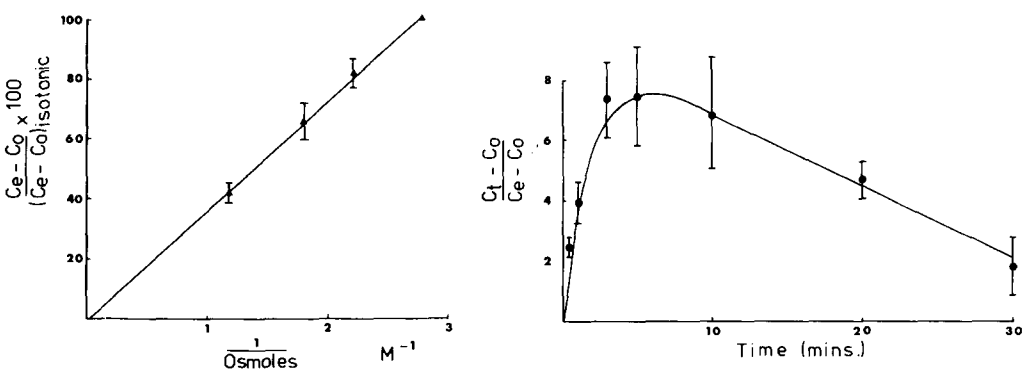

Fig. 4. A plot of equilibrium isotope level against 1/osmolarity. Error bars represent the S.E.M. of 3 experiments. The line is derived from linear regression (r = 0.94, y = —1.6).

Fig. 5. A time course for cyclic AMP counterflow. The isotope accumulation is calculated as a fraction of the equilibrated isotope in the 90-min sample. Results are the mean and the standard error of 3 experiments.

number of sources including erythrocytes [12] has been reported. Rubin et al. [12] studied binding of cyclic[^{3}H]AMP to human erythrocyte membranes at 0°C and found an apparent saturation of binding with a half saturation constant of 3.3 nM. However, some of this apparent binding may have been material trapped within or transported into an intra-vesicular space. Because of the results from the osmolarity experiment at 30°C described above it seems reasonable to assume that the amount of cyclic AMP binding to membrane material in the ghosts used in the transport experiments is much less than that contained in the vesicle space and consequently does not interfere with the estimates of kinetic parameters for transport. Rubin et al. [12] also reported that the membrane-associated cyclic AMP could be completely recovered and identified chromatographically. Similarly, Sheppard and Burghardt [13] noted no breakdown of cyclic AMP during its incubation with erythrocyte ghosts. These findings support the experiments described above using theophylline which indicate that phosphodiesterase activity in erythrocyte ghosts may be low (possibly due to the extensive dilutions used in the haemolysing procedure). Nevertheless, it was decided to test directly whether breakdown of cyclic AMP occurs under the conditions used in the transport experiments reported here. As described in Materials and Methods, 97—98% of the recovered radioactivity co-chromatographed with authentic cyclic AMP.

Counterflow of cyclic AMP

A membrane transport system that shows saturation kinetics should also show uphill movement of isotope driven by the movement of cold substrate down its concentration gradient. As shown in Fig. 5, cyclic AMP transport shows this phenomenon. Influx of 10^{-5} M cyclic AMP into ghosts containing 1 mM cyclic AMP was measured at the time intervals shown. The experimental protocol was the same as for zero *trans* exit except for the inclusion of cyclic-[^{3}H]AMP in the 10 ml of external solution only. This provides a greater dilution of the resealed ghosts than the zero *trans* influx experiment.

As expected, a transient accumulation of label occurs; however, the time course for this effect is more rapid than would be anticipated from the influx data shown in earlier experiments. As pointed out by Miller [14], errors in transport experiments due to experimental design should always be suspect before data can be considered as being incompatible with a particular transport model. The technical problem encountered in the counterflow experiment is that the ratio of ghosts to incubation medium is, of necessity, low. This ensures that unlabelled material from the ghosts does not appreciably lower the specific activity of the external label. However, it also means that the relative dilution of medium to cells is lower than in the zero *trans* influx experiment and consequently the equilibrium level of isotope, used in the calculation of label accumulation, is more difficult to estimate. An experiment that tests more directly the possibility of the presence of a transport component that exhibits a suitably low K^{oi}/V^{oi} ratio that would be compatible with the counterflow data is the infinite *trans* entry experiment [15].

At present the main conclusion from the counterflow experiment, which is independent of the above argument, is that it is consistent with the presence of a saturable transport component on the inner surface of the membrane.

Discussion

For the red blood cell membrane the permeability to cyclic AMP is high ($0.061 \cdot 10^{-6}$ cm $\cdot$ s^{-1}). This is higher than would be predicted from the size of the molecule and is comparable with the permeability of the membrane to a low affinity sugar such as L-sorbose [16,17]. This observation indicates that a specialised transport system may be available for cyclic AMP. The following reservations need to be considered, however. Firstly, the red blood cell ghosts are probably more leaky than the intact cell would be and consequently the measured permeability may be an overestimate of the real permeability. Also, if cyclic AMP induces significant morphological changes in ghosts the estimates of kinetic parameters may be unreal. The observed permeability cannot be due entirely to a passive leak as saturation, counterflow and inhibition by cytochalasin B can be demonstrated. In their review [1] Robison et al. make it clear that many tissues do not respond to exogenous cyclic AMP but do respond to butyryl derivatives of cyclic AMP. Also, cyclic AMP added to homogenates generally has greater effects than cyclic AMP added to intact tissue preparations. By implication then, cyclic AMP permeability in most tissues is considered to be low. Several unknown factors such as the amount of exposed surface area and the extent of substrate loss due to metabolism may be important in these estimates of relative permeability and the possibility of cyclic AMP transport was not completely excluded by Robison et al.

Intracellular levels of cyclic AMP may, to some extent, be dependent on transport. Combinations of noninstantaneous changes in adenyl cyclase activity, in phosphodiesterase activity and in membrane permeability may be expected to produce large and rapid transients in cyclic AMP accumulation. As discussed in Results, the proportion of cyclic AMP associated with binding proteins in the ghost preparations used for the transport experiments may be small. However, in intact cells binding sites closely associated with transport sites may be of physiological importance in the control of compartmentalisation of cyclic AMP.

Nucleosides are now well known to show facilitative diffusion [4,5,18]. Cabatchik and Ginsburg [6] have clearly defined the kinetic characteristics of the uridine transport system of the red blood cell. It would be of interest to determine whether cyclic nucleotides share the nucleoside transport system. Paul et al. [19] have shown that adenosine derivatives do inhibit uridine transport in human erythrocytes but the effect of nucleotides was not investigated. The cyclic AMP transport system is asymmetric (high affinity inside). Apparent asymmetry in red blood cell transport systems is not unusual. The sugar transport system shows a 10-fold asymmetry for glucose [20] and galactose transport [21] (high affinity outside) in whole cells but not in ghosts [22], which is similar to the 4-fold asymmetry found for uridine transport [6] (high affinity outside). It has been difficult to reconcile the transport data of the sugar transport system with the conventional carrier model [23] and it has been postulated that asymmetrics may arise due to peripheral membrane proteins and haemoglobin [24]. This may be appropriately termed extrinsic asymmetry [25] to distinguish it from asymmetries that may be present in the transport channels themselves (intrinsic asymmetry). In red blood cell ghosts, haemo-

globin the major erythrocyte protein, is lost, but approximately 0.1% of mean corpuscular haemoglobin remains bound to the membrane [8]. The major extrinsic protein in red cell ghost preparations must be spectrin which is bound to the inner surface. Whether the cyclic AMP asymmetry is extrinsic cannot be evaluated with the present data and the determination of the kinetic constants from the infinite *cis* and infinite *trans* procedures [23] may be useful. The infinite *cis* procedure has produced data inconsistent with the carried model for glucose transport [23] but not for uridine transport [6].

The effect of cyctochalasin B on cyclic AMP exit is greatest at low substrate concentrations. This gives apparent competitive inhibition. Cytochalasin B is well known to be a powerful inhibitor of glucose transport in red blood cells. Bloch [26] reported that inhibition of entry was non-competitive. Recently, Baketter and Widdas [27] have confirmed this finding and have also shown that it is a competitive inhibitor of exchange exit of sugars. These findings were considered consistent with cytochalasin B acting directly or indirectly with an internal glucose transport site ($K_i = 1.1 \cdot 10^{-7}$ M). Plagemann and Estersen [7] found that cytochalasin B was a competitive inhibitor of uridine and thymidine transport in Novikoff rat hepatoma cells (K_i values = 2 and 6 μM, respectively). More recently [33] mixed kinetic effects for cytochalasin B have been reported. The effect of cytochalasin B on cyclic AMP transport is quantitatively similar to these effects on glucose and uridine transport ($K_i = 3.9 \cdot 10^{-7}$ M) though more extensive kinetic characterisation of this effect is required.

It seems unlikely that glucose and cyclic AMP share the same transport system, hence cytochalasin B may modify extrinsic membrane proteins which behave as a barrier in series with several red cell membrane transport systems. Such a protein lattice may control the opening of 'gates' to intrinsic membrane transport channels. This is consistent with the known action of cytochalasin B on actin-like filaments [30]. Alternatively, cytochalasin B may act on hydrophobic regions of several integral membrane proteins as suggested recently by Plagemann et al. [33].

Acknowledgements

I would like to thank Professor H.S. Bachelard for his help and encouragement and for a critical reading of the manuscript.

References

1 Robison, G.A., Butcher, R.W. and Sutherland, E.W. (1971) Cyclic AMP, pp. 5—142, Academic Press, New York
2 Widdas, W.F. (1954) J. Physiol. 125, 163—180
3 Rothstein, A.R., Cabantchik, Z.I. and Knauf, P.A. (1976) Fed. Proc. 35, 3—10
4 Lieu, T.S., Hudson, R.A., Brown, R.K. and White, B.C. (1971) Biochim. Biophys. Acta 884—893
5 Cass, C.E. and Paterson, A.R.P. (1971) Can. J. Biochem. 247, 3314—3320
6 Cabantchik, Z.I. and Ginsburg, H. (1977) J. Gen. Physiol. 69, 75—96
7 Plagemann, P.G.W. and Estensen, R.D. (1972) J. Cell. Biol. 55, 179—185
8 Dodge, J.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119—130
9 Cleland, W.W. (1967) Adv. Enzymol. 29, 1—32
10 Cornish-Bowden, A.J. (1976) Principles of Enzyme Kinetics, pp. 168—198 Butterworths, London
11 Hopfer, U., Nelson, K., Perrotto, J. and Isselbacher, K.J. (1973) J. Biol. Chen. 248, 25—32
12 Rubin, C.S., Erlichman, J. and Rosen, O.M. (1972) J. Biol. Chem. 247, 6135—6139

13 Sheppard, H. and Burghardt, C. (1969) Biochem. Pharmacol. 18, 2576—2578
14 Miller, D.M. (1968) Biophys. J. 8, 1329—1338
15 Ginsburg, H. and Stein, W.D. (1975) Biochim. Biophys. Acta 382, 353—368
16 Stein, W.D. (1967) The Movement of Molecules across Cell Membranes, Academic Press, New York
17 LeFevre, P.G. (1961) Pharmacol. Rev. 13, 39—70
18 Eilam, Y. and Bibi, O. (1977) Biochim. Biophys. Acta 467, 51—64
19 Paul, B.M., Chen, F. and Paterson, A.R.P. (1975) J. Med. Chem. 18, 968—973
20 Baker, G.F. and Widdas, W.F. (1973) J. Physiol. 231, 143—165
21 Ginsburg, H. and Ram, D. (1975) Biochim. Biophys. Acta 382, 369—376
22 Taverna, R.D. and Langdon, R.G. (1973) Biochim. Biophys. Acta 323, 207—219
23 Lieb, W.R. and Stein, W.D. (1972) Biochim. Biophys. Acta 265, 187—207
24 Naftalin, R.J. and Holman, G.D. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds.), pp. 257—299 Academic Press, New York
25 Whitesell, R.R., Tarpley, H.L. and Regen, D.M. (1977) Arch. Biochem. Biophys. 181, 596—602
26 Bloch, R. (1973) Biochem. 12, 4799—4801
27 Baketter, D.A. and Widdas, W.F. (1977) J. Physiol. 265, 39 p
28 Carter, S.B. (1967) Nature 213, 261—264
29 Spooner, B.S. and Wessells, N.K. (1970) Proc. Natl. Acad. Sci. U.S. 66, 360—364
30 Spudlich, J.A. and Lin, S. (1972) Proc. Natl. Acad. Sci. U.S. 69, 442—446
31 Whittam, R. (1964) Transport and Diffusion in Red Blood Cells. Monograph of the Physiological Society, pp. 2—3, Edward Arnold (Publishers) Ltd., London
32 Davoren, P.R. and Sutherland, E.W. (1963) J. Biol. Chem. 238, 3009—3015
33 Plagemann, P.G.W., Graff, J.C. and Wohlhueter, R.M. (1977) J. Biol. Chem. 252, 4185—4201