Biochimica et Biophysica Acta 821 (1985) 37–44
Elsevier

BBA 72809

Monensin stimulates sugar transport in avian erythrocytes

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(Received May 29th, 1985)

Key words: Glucose transport; Sodium ionophore; Ionophore; Monensin; Ca^{2+}-dependence; (Pigeon erythrocyte)

The cell-medium distribution of the nonmetabolized glucose analog, 3-*O*-methyl-D-glucose was studied in pigeon erythrocytes. The sodium ionophore monensin increased in parallel and in a dose-dependent manner the influx of hexose and of Na^+. These effects were independent of external Ca^{2+} and there was no alteration in ^{45}Ca influx. If, as suggested previously, hexose transport in these cells is modulated by cytoplasmic Ca^{2+}, the stimulatory effect of monensin on hexose transport may be due to increased mitochondrial Ca^{2+} efflux via Na^+-Ca^{2+} exchange, owing to the elevation of cytoplasmic Na^+. Such a mechanism is consistent with the observed failure of monensin to affect 3-*O*-methyl-D-glucose transport in cells partially depleted of Ca^{2+}. Monensin also depressed cellular ATP levels but the data favour a Ca^{2+}-dependent mechanism of hexose transport regulation rather than a direct effect of metabolic depletion. The inhibitor of specific-mediated hexose transport, cytochalasin B was found to inhibit equally basal and stimulated 3-*O*-methyl-D-glucose uptake but there was a cytochalasin B-insensitive uptake component in excess of L-glucose uptake. This appears to reflect a greater diffusional permeability of 3-*O*-methyl-D-glucose than of L-glucose.

Introduction

Hexose transport in muscle and adipose tissue occurs by facilitated diffusion, is normally rate limiting for glucose utilization and is stimulated by insulin and other hormonal and metabolic factors. In contrast, hexose transport in mature human erythrocytes, the liver and some other tissues is more rapid than glucose metabolism and is not subject to regulatory control (see Refs. 1 and 2 for review). In avian erythrocytes [3,4] hexose transport is also subject to regulation, resembling that in muscle with respect to its link to the level of cytoplasmic Ca^{2+}, which has been proposed as a proximal regulator of the activity of the transport process [1,2]. However, Na^+-Ca^{2+} exchange does not occur at the plasma membrane of these cells [4], so that hexose transport is not altered by factors which affect Ca^{2+} influx via their effects on intracellular Na^+ levels.

These features make the avian erythrocytes highly suitable for the study of sugar transport regulation by some factors believed to act through releasing Ca^{2+} from intracellular storage sites. We have previously shown that the effects of A23187 [5], lithium [6] and monensin [7] to increase hexose transport in muscle and of A23187 [4], anoxia and adrenalin [8] in pigeon erythrocytes, all occur in the absence of external Ca^{2+} and are consistent with such a mechanism.

The data in the present report show that the sodium ionophore monensin stimulates hexose transport in pigeon erythrocytes in parallel to the release of intracellular Ca^{2+}, presumably through increasing mitochondrial Ca^{2+} loss via the Na^+-Ca^{2+} exchange pathway. Some of these results have been reported previously in abstract form [9].

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Abbreviation: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid.

Materials and Methods

Radiolabelled compounds were from New England Nuclear (Boston, MA) and from Amersham Corp. (Oakville, Ontario). The ATP bioluminescence reagent, type CLS, was from Boehringer Mannheim Ltd. All other chemicals and reagents were of the highest commercial quality.

Blood was obtained from the neck vein of locally bred domestic pigeons, following decapitation. The erythrocytes were centrifuged, washed and incubated by the procedure of Wood and Morgan [3] as described previously [4,8]. An imidazole-glycylglycine buffer (pH 7.4) was used which contained (mM): 137.0 NaCl, 5.9 KCl, 2.4 $MgSO_4$, 1.2 KH_2PO_4, 4.2 imidazole and 7.6 glycylglycine and, where indicated, 1.25 mM $CaCl_2$. The medium without added Ca^{2+} is referred to as 'Ca^{2+}-free' although it does contain low levels of Ca^{2+} originating as contamination of other reagents or leaking from cells. Its calcium content as determined previously [4] is between $0.5 \cdot 10^{-7}$ and $1.1 \cdot 10^{-7}$ M. For the calculation of Ca^{2+} influx in Table I, an average concentration of $4 \cdot 10^{-7}$ M was conservatively assumed for the nominally Ca^{2+}-free medium. In some instances a mixture of metabolic substrates was added [10] which consisted of 2.7 mM adenine, 5.0 mM inosine, 5.0 mM fumarate and 10.0 mM KH_2PO_4. Where shown, monensin or A23187 dissolved in dimethyl sulfoxide (DMSO) were added; the same volume of solvent was added to controls. All media were equilibrated with 100% O_2 and maintained at 40.5°C.

Unless otherwise indicated, the cells were suspended at 10% hematocrit and preincubated for the period shown with all additions. A mixture of ^{14}C-labelled and unlabelled 3-*O*-methyl-D-glucose (total concentration, 0.1 mM) and tracer amounts of [^{3}H]inulin, serving as extracellular marker, was added and incubation continued for the indicated time. Incubation was stopped by diluting 0.5 ml of cell suspension with 1.0 ml of ice-cold buffer, followed by immediate centrifugation for 30 s in a high-speed Eppendorf microfuge. The supernatant was removed, the tubes drained by a standardized procedure and the cell pellet weighed. Preparation of tissue extracts, double label scintillation counting and calculation of results were done as described previously [11]. Samples of incubation media were treated similarly. For measurements of Ca^{2+} uptake the same procedure was followed using ^{45}Ca.

Results are expressed as rates (nmol/l intracellular water per min) or as percent equilibration, i.e. the concentration of ^{14}C-labelled 3-*O*-methyl-D-glucose in the intracellular water is given as a percentage of the concentration in the medium at the end of incubation. These values include a correction for the inulin space determined in the same sample; the average water content of the cell pellet was determined separately. The use of 3-*O*-methyl-D-glucose as a nonmetabolized glucose analog in transport studies has been repeatedly validated [12].

$^{45}Ca^{2+}$ efflux experiments were done as described previously [4]: The cells were first loaded with ^{45}Ca in the presence of 6 μM A23187 and then the ionophore was removed and the original low Ca^{2+} permeability restored by repeated washing of the cells with an ice-cold solution containing 160 mM KCl, imidazole-glycylglycine buffer and 0.5% bovine serum albumin [13]. The loss of ^{45}Ca from the cells into Ca^{2+}-free medium was then followed. Results are given as percent of the initial ^{45}Ca remaining in the cells.

To deplete the cells of internal calcium, they were treated in the same manner using a Ca^{2+}-free medium containing 6 μM A23187 and 1.0 mM EGTA.

ATP was determined by a luciferin-luciferase bioluminescence assay as described previously [4]. Statistical evaluation was by Student's *t*-test.

Results

Dose-response curves for the effect of monensin on 3-*O*-methyl-D-glucose uptake and on intracellular Na^+ content are shown in Fig. 1. There is a good correlation between the increases in these two parameters at almost all monensin concentrations. Sugar transport began to decline with the highest level of monensin, 10^{-5} M, and this was associated with visible hemolysis of the erythrocytes.

Fig. 2 shows 3-*O*-methyl-D-glucose transport into pigeon red cells for two concentrations of monensin as a function of duration of preincuba-

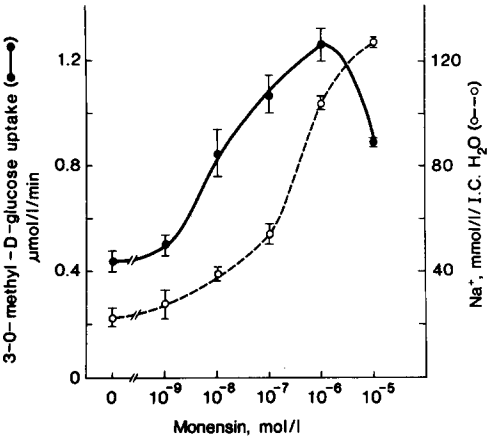

Fig. 1. Effect of monesin on 3-*O*-methyl-D-glucose transport (μmol/l per min) and Na^+ content (mmol/l intracellular water) in pigeon erythrocytes. Cells were preincubated for 30 min and incubated for an additional 30 min with the indicated concentration of monensin in medium containing 1.25 mM Ca^{2+}. The solvent, DMSO, was present in controls. The data are means ±S.E. of a single representative experiment done in quadruplicate.

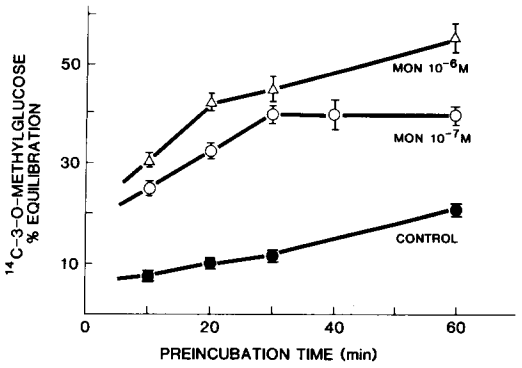

Fig. 2. Influence of preincubation time with monensin on the transport of 3-*O*-methyl-D-glucose. Pigeon erythrocytes were preincubated for the period shown, followed by 30 min incubation in medium with 1.25 mM Ca^{2+}. Data are means ±S.E. of two experiments in quadruplicate. ●, no monensin; ○, 10^{-7} M monensin; △, 10^{-6} M monensin.

tion. Transport was significantly ($P < 0.001$) stimulated by 10^{-7} M monensin and to a further significant ($P < 0.02$ or better) extent by 10^{-6} monensin. There was a gradual increase in transport with duration of preincubation. This increase was about the same in the presence and absence of monensin and probably reflects the progressive depletion of cellular energy reserves (see Fig. 3). Such depletion has been shown to increase hexose transport in these cells [3] and to release Ca^{2+} from intracellular storage sites [8].

Table I compares the effects of 10^{-6} M monensin on the cellular content of Na^+ and K^+ and on the influx of 3-*O*-methyl-D-glucose and ^{45}Ca in the presence and absence of external Ca^{2+}. As shown previously [4], basal hexose uptake was not affected by external Ca^{2+}, and the present results show that the stimulatory effect of monensin is also independent of external Ca^{2+}. The increase in internal Na^+ level and the reciprocal drop in the level of K^+ elicited by the ionophore were also not altered by the presence or absence of Ca^{2+} in the incubation medium; the values shown in Table I are pooled from experiments with and without Ca^{2+}. In contrast to the clearcut and parallel effects of monensin to increase Na^+ levels and hexose transport, there was no significant change in ^{45}Ca influx, either from a medium con-

TABLE I

EFFECTS OF MONENSIN ON 3-*O*-METHYL-D-GLUCOSE AND ^{45}Ca TRANSPORT AND ON Na^+ AND K^+ LEVELS

Cells were preincubated 20 min and incubated 30 min with or without 10^{-6} M monensin as shown in Materials and Methods. The data for Na^+ and K^+ content were pooled from both ^{45}Ca and 3-*O*-methyl-D-glucose experiments.

	Control	Monensin	*P*
Na^+ content (mmol/l cell water)	26.0 ± 2.4 (16)	69.6 ± 2.0 (18)	< 0.001
K^+ content (mmol/l cell water)	104.1 ± 1.3 (16)	62.6 ± 0.9 (18)	< 0.001
^{45}Ca uptake (mmol/l per min)			
in 1.25 mM Ca^{2+} medium	542.0 ±33 (4)	383.0 ±83 (5)	
in Ca^{2+}-free medium	4.15± 0.19 (4)	3.85± 0.15 (5)	
3-*O*-methyl-D-glucose uptake (mmol/l per min)			
1.25 mM Ca^{2+} medium	96.7 ±10.0 (4)	270.0 ±23.3 (4)	< 0.001
Ca^{2+}-free medium	60.0 ± 9.3 (4)	260.0 ±20.0 (4)	< 0.001

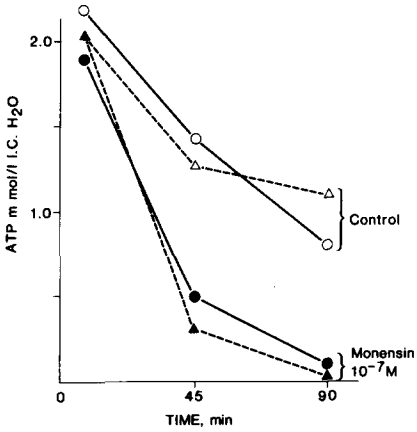

Fig. 3. Effect of monensin on ATP content of pigeon erythrocytes. Cells were incubated for the periods shown in the presence and absence of 1.25 mM Ca^{2+} and 10^{-7} M monensin. Data are means of two experiments in duplicate.

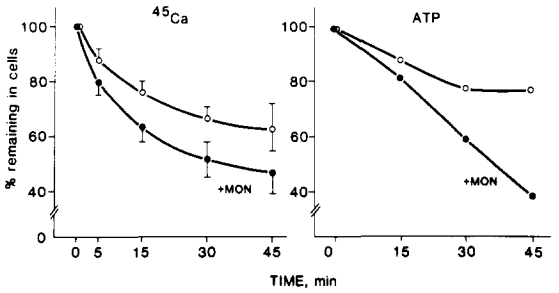

Fig. 4. Time-course of ^{45}Ca efflux and of simultaneous changes in ATP content. Cells were loaded with ^{45}Ca as described in Materials and Methods and its efflux into Ca^{2+}-free medium containing a mixture of metabolic substrates was followed in the presence and absence of 10^{-7} M monensin (MON). Data are means ± S.E. of six experiments. The drop in ATP content was determined in the same cells in three experiments in duplicate.

taining 1.25 mM Ca^{2+} or from a nominally Ca^{2+}-free medium. This disparity is not unexpected as earlier observations [4] indicated that a Na^{+}-Ca^{2+} exchange system is not present (or not functional) in the plasma membrane of pigeon erythrocytes.

As shown in Fig. 3, in cells incubated in the absence of metabolic substrates ATP declined during 90 min of incubation to about 40% of the initial value. In the presence of 10^{-7} M monensin ATP content dropped much more steeply to about 20 and 3% after 45 and 90 min, respectively. The presence or absence of external Ca^{2+} had no effect on ATP levels.

To determine if the ionophore could alter cytoplasmic Ca^{2+} levels by releasing Ca^{2+} from intracellular storage sites, cellular Ca^{2+} efflux was measured as shown in Fig. 4. This parameter is normally increased when cytoplasmic Ca^{2+} is elevated [14]. The efflux of ^{45}Ca from preloaded cells appeared to be more rapid in the presence of

TABLE II

EFFECTS OF MONENSIN IN Ca^{2+}-DEPLETED PIGEON RED CELLS

For Ca^{2+}-depletion cells were first incubated for 20 min in Ca^{2+}-free medium containing adenine/inosine/fumarate/KH_2PO_4, 6 μM A23187 and 1 mM EGTA and then washed to remove the A23187 as described in Materials and Methods. Control cells underwent the same treatment but in media containing 1.25 mM Ca^{2+} and devoid of A23187 and EGTA. The cells were incubated for 15 min in Ca^{2+}-free medium containing adenine/inosine/fumarate/KH_2PO_4 with or without 10^{-7} M monensin. Samples were taken for ATP and Ca determination, and 3-*O*-methyl-D-glucose uptake was determined for an additional 15 min incubation period. Data are means ± S.E. of two or three experiments done in triplicate.

	Control	Monensin	Ca^{2+}-depleted	
			Control	Monensin
3-*O*-methyl-D-glucose uptake				
nmol/l per min	215.4 ±20.6 (8)	586.6 ±21.0 (7)	274.0 ±26.0 (7)	300.6 ±24.0 (1)
(% of respective control)		(272)		(111)
Ca content				
nmol/g wet wt.	136.0 ±15.5 (4)	116.2 ±11.3 (4)	83.1 ± 6.1 (4)	72 ± 5.8 (4)
(% of respective control)		(85)		(87)
ATP content				
mmol/l intracellular water	2.32±0.27 (3)	1.68± 0.12 (3)	2.98± 0.03 (3)	2.58± 0.16 (3)
(% of respective control)		(72)		(87)

TABLE III

INHIBITION OF BASAL AND STIMULATED 3-*O*-METHYL-D-GLUCOSE UPTAKE BY CYTOCHALASIN B

Cells were preincubated for 30 min and incubated with the hexose for an additional 30 min in the presence of the additions indicated, with and without 100 μM cytochalasin B (CB). Only in these experiments L-glucose was used to correct for extracellular space and 'nonspecific' entry. In experiment B, metabolic substrates (see Materials and Methods) were present throughout. The data are means ± S.E. of three experiments done in duplicate or triplicate.

Additions	3-*O*-Methyl-D-glucose uptake (nmol/l per min)		
	−CB	+CB	% Inhibition
Expt. A			
10 μl/ml DMSO	565.3 ± 32.0 (8)	113.3 ± 9.7 (8)	80.0
+1 μM A23187	1204.3 ± 64.3 (4)	289.3 ± 4.0 (4)	76.0
+6 μM A23187	1838.3 ± 57.7 (4)	525.0 ± 18.0 (4)	71.5
+2 mM KCN	1201.3 ± 22.0 (4)	180.0 ± 20.3 (4)	85.0
+0.1 μM monensin	1263.3 ± 32.3 (4)	167.7 ± 0.7 (4)	86.7
Expt. B with metabolic substrates			
Control	207.7 ± 11.7 (6)		
10 μl/ml DMSO	242.3 ± 6.0 (6)	119.0 ± 8.0 (6)	50.9
+0.1 μM monensin	447.7 ± 1.0 (6)	111.1 ± 15.7 (6)	75.2

10^{-7} M monensin but the difference at any given time was just below the 5% level of significance. The change in ATP content was also measured in these cells which were incubated in the presence of metabolic substrates (see Materials and Methods). In agreement with earlier results [4], the decline in ATP content was less steep in the combined presence of adenine, inosine and fumarate than in their absence (compare Fig. 3), but it was nevertheless enhanced by monensin.

Table II compares the effect of monensin on total calcium content and hexose transport in Ca^{2+}-depleted and control cells. Depletion of cellular calcium was achieved by exposing the cells to the Ca^{2+} ionophore A23187 in a Ca^{2+}-free medium containing EGTA, and subsequent removal of the ionophore and resealing of the cells, as described in Materials and Methods. The data show that the depletion procedure decreased ($P < 0.02$) cellular calcium content. Also, whereas monensin increased 3-*O*-methyl-D-glucose uptake in control cells 2.72-fold ($P < 0.001$), the rise in Ca^{2+}-depleted cells was 1.11-fold (not significant). Thus, Ca^{2+}-depletion did not affect basal hexose uptake but abolished its stimulation by monensin. The ATP content of Ca^{2+}-depleted cells was higher ($P < 0.05$) than in controls. Monensin significantly decreased ($P < 0.05$) the ATP content in both control and Ca^{2+}-depleted cells.

Because of contradictory reports in the literature [4,15] we have reinvestigated the effect of cytochalasin B, a specific inhibitor of hexose transport taking place by facilitated diffusion. Table III shows that a high concentration of the inhibitor depressed the transport of 3-*O*-methyl-D-glucose to a similar extent, about 80%, in untreated control cells and in those exposed to effective stimulatory concentrations of monensin, A23187 or cyanide. As basal uptake in fresh, untreated pigeon erythrocytes varies somewhat from experiment to experiment, reflecting presumably the metabolic status of the cells, adenine-inosine-fumarate was included in one experiment to support oxidative metabolism and preclude, as far as possible, any metabolic depletion of the cells. As expected from earlier results [8], the presence of metabolic substrates decreased basal hexose transport; its stimulation by monensin, although significant, was also diminished. Basal and monensin-stimulated hexose uptake were inhibited by cytochalasin B by about 50 and 75%, respectively, in the presence of metabolic substrates. The data also show that the small volume of DMSO, serving as solvent for cytochalasin B and for monensin, had a minor stimulatory effect of its own which was also inhibited by cytochalasin B. An alternative solvent, ethanol had virtually the same effect (not shown). Uptake of 3-*O*-methyl-D-glucose was

not totally abolished by even a very high concentration of cytochalasin B. The residual uptake of 3-*O*-methyl-D-glucose was in all instances significantly in excess of the uptake of L-glucose which was used in these experiments to correct for 'nonspecific uptake', as well as for extracellular space. L-Glucose uptake was not significantly altered by any of the treatments in Table III; In the presence of KCN, DMSO alone or DMSO combined with 100 μM cytochalasin B, 6 μM A23187 or 0.1 μM monensin uptake ranged between 95 and 108% of control.

Discussion

The data in the preceding paper [7] indicate that the sodium ionophore monensin stimulates hexose transport in mouse diaphragm in parallel to the increased influx of Na^+ and Ca^{2+}, the latter occurring largely through Na^+-Ca^{2+} exchange. However, monensin also stimulated hexose transport in Ca^{2+}-freee medium suggesting that monensin may act to release Ca^{2+} from intracellular storage sites. Nucleated avian erythrocytes are highly suitable to test this proposition more directly. It has been shown [3,4,8,15] that hexose transport in these cells is subject to regulation and is Ca^{2+}-dependent but is not affected by agents whose action in muscle is linked to increased Na^+-Ca^{2+} exchange. Our data indicate [4] that Na^+-Ca^{2+} exchange does not occur in the plasma membrane of pigeon red cells.

The results of this study show that 3-*O*-methyl-D-glucose transport in pigeon erythrocytes was strongly stimulated by monensin in parallel to the influx of Na^+ but that the effect was independent of the presence of external calcium and was not accompanied by increased ^{45}Ca influx. If stimulation of hexose transport by monensin involves the release of Ca^{2+} from internal stores, one would expect [14] that an increase in cytoplasmic Ca^{2+} should result in accelerated Ca^{2+} efflux. This prediction was not unequivocally confirmed; the efflux of ^{45}Ca from preloaded pigeon erythrocytes appeared to be somewhat faster in the presence of monensin but the effect was not statistically significant perhaps for technical reasons, as Ca^{2+} fluxes across the pigeon red cell membrane are relatively slow [4].

Stimulation of hexose transport by monensin was, however, abolished in cells depleted of internal Ca^{2+} by treatment with A23187 and EGTA. Ca^{2+} permeability is greatly increased by treatment with A23187 and returns to normal after removal of the ionophore [4]. The present as well as earlier [4] data indicate that after such treatment hexose transport also returns to a low basal level. The same effect, abolition of hexose transport stimulation by adrenalin has been previously demonstrated in Ca^2-depleted cells [8]. It may be significant that the depletion procedure caused the loss of only about 40% of total cellular Ca^{2+} (in the absence of monensin). In earlier experiments [4,8] addition of EGTA to cells being loaded with ^{45}Ca in the presence of A23187 caused an almost total loss of label. Thus, the calcium ionophore might not affect equally all cellular calcium pools, and one particular cellular compartment could be more rapidly loaded and depleted than others. The observation that the stimulatory effects of monensin or adrenalin are abolished in the (partially) Ca^{2+}-depleted cells might indicate that, if Ca^{2+} is involved in hexose transport regulation, this more mobile intracellular Ca^{2+} pool might be involved.

It is conceivable that the failure of Ca^{2+}-depleted cells to respond to monensin may be due to some indirect effects of the depletion procedure rather than to Ca^{2+} loss. However, this is very unlikely because hexose transport returns to the normal low basal level after A23187 is removed at the end of the depletion procedure [4,8] and may then be stimulated again by A23187 in the presence of external Ca^{2+} (Bihler and Charles, unpublished results).

Cellular ATP content was depressed by monensin in both fresh and Ca^{2+}-depeleted cells. This is most easily explained on the basis of increased futile cycling of Na^+ and Ca^{2+}: monensin-induced Na^+ influx leads to acceleration of energy-consuming Na^+ pumping in the plasma membrane [7]. As well, if mitochondrial efflux of Ca^{2+} in these cells occurs by Na^+-Ca^{2+} exchange as is the case in muscle [16], elevation of internal Na^+ should shift Ca^{2+} from the mitochondria to the cytoplasm, and this, in turn should activate the energy-consuming processes of mitochondrial uptake of Ca^{2+} and its extrusion at the plasma membrane. The observation that ATP levels were

higher in the Ca^{2+}-depeleted cells than in controls is consistent with this concept and suggests that futile cycling of Ca^{2+} is decreased in the Ca^{2+}-depleted cells.

Stimulation of hexose transport in nucleated red cells is usually accompanied both by metabolic depletion as reflected by decreased ATP levels and by increased cytoplasmic Ca^{2+}. These two effects are interrelated, as metabolic depletion will lead to Ca^{2+} release from internal stores, and high cytoplasmic Ca^{2+} levels will enhance metabolic depletion, as discussed above. It remains a matter of controversy [3,4,15] if metabolic depletion stimulates hexose transport directly or if the effect is due to the resulting alterations in Ca^{2+} distribution. The present data show that hexose transport was strongly stimulated by monensin even when the ATP content was only moderately decreased (28%). On the other hand, in Ca^{2+}-depleted cells monensin did not alter hexose transport although it did cause a significant drop in ATP content. As well, 3-*O*-methyl-D-glucose transport returned to the low basal rate following treatment with A23187 in the presence of Ca^{2+} and subsequent removal of the ionophore, although ATP levels at that time were severely depressed [4]. The above observations represent indirect evidence favouring a direct link of hexose transport regulation to internal Ca^{2+} rather than to metabolic depletion.

The experiments in Table III were done to determine under what conditions hexose transport in pigeon erthyrocytes is inhibited by the specific hexose transport inhibitor, cytochalasin B. It has been argued by Simons [15] that a saturable, cytochalasin B-sensitive transport pathway is virtually absent in fresh, untreated cells but is activated by cyanide and by A23187 $+Ca^{2+}$. Contrary to this view, it was found that cytochalasin B inhibits to the same extent 3-*O*-methyl-D-glucose transport in the basal state and when stimulated by monensin, A23187 or cyanide. Since the metabolic status of the cells varies from experiment to experiment one cannot strictly exclude the possibility that any inhibition by cytochalasin B might reflect the operation of the stimulated, saturable transport pathway. To overcome this uncertainty the cells were preincubated and incubated with a mixture of oxidative metabolic substrates (adenine, inosine, fumarate; see Materials and Methods) which has been shown [8,10] to maintain high cellular ATP levels. Under such conditions there should be no stimulation of hexose transport due to metabolic depletion. We find that even in the presence of the metabolic substrates cytochalasin B inhibited hexose transport in untreated cells almost as much as in those stimulated by monensin. There was, nevertheless, a cytochalasin B-insensitive component of 3-*O*-methyl-D-glucose uptake, under both basal and stimulated conditions. Its nature is not apparent from the present experiments but it is clearly additional to the 'nonspecific' uptake exhibited by L-glucose which is already corrected for in the calculation of results. Regen and Morgan [17] have described a similar diffusional component of 3-*O*-methyl-D-glucose uptake in rabbit erythrocytes, and they have pointed out that the increased permeability of this compound may reflect greater lipid solubility which is related to alkyl substitution at C-3 of the glucose molecule and that it may be observed only in cells where specific, mediated transport is slow. Thus, the cytochalasin B-insensitive uptake component may not represent a physiological transport pathway but rather a peculiarity of 3-*O*-methyl-D-glucose. Under some conditions this monosaccharide may not be the ideal D-glucose analog.

Acknowledgements

We thank Mrs. B. Cook for excellent technical assistance. This work was supported by grants from the Medical Research Council of Canada and the Manitoba Heart Foundation. I.B. is a Career Investigator of the MRC.

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