Biochimica et Biophysica Acta, 601 (1980) 630—639

BBA 78937

AMINO ACID AND GLUCOSE TRANSPORT IN SARCOLEMMAL VESICLES FROM CHICK EMBRYO HEART

S. PARIS and G. AILHAUD

Centre de Biochimie, Faculté des Sciences, Parc Valrose, 06034 Nice Cédex (France)

(Received January 11th, 1980)

Key words: Muscarinic receptor; Amino acid transport; Glucose transport; (Cardiac plasma membrane)

Summary

Purified plasma membranes from chick embryo heart are shown to retain several functional properties of intact cardiac cells.

(1) Muscarinic cholinergic receptors copurify with previously used cell-surface markers, i.e., (K^+-dependent) *p*-nitrophenylphosphatase and insulin receptors (Paris, S., Fosset, M., Samuel, D. and Ailhaud, G. (1977) J. Mol. Cell. Cardiol. 9, 161—174).

(2) Neutral amino acids (L-alanine and α-aminoisobutyric acid) are actively transported into these osmotically active vesicles when an Na^+ electrochemical gradient is imposed. The affinity and specificity for amino acids are similar to those described for intact cardiac cells.

(3) D-Glucose is taken up more rapidly than L-glucose. The stereospecific transport system is saturable and Na^+-independent. The K_m value is close to that observed in intact cells with glucose analogues.

Introduction

A procedure for the purification of cardiac plasma membranes from chick embryo has been recently reported from this laboratory [1]. These membranes were at least 10-fold enriched relative to the homogenate with respect to K^+-dependent *p*-nitrophenylphosphatase and insulin binding. They appeared as closed vesicles under electron microscopy. The present work describes functional properties of these sarcolemmal vesicles that are similar to those of intact cells: firstly, they are enriched in muscarinic cholinergic receptors and, secondly, they have the ability to transport actively neutral amino acids and to mediate a facilitated diffusion for D-glucose.

Materials and Methods

Isolation of plasma membranes. Plasma membranes were prepared from freeze-thawed hearts of 14-day-old chick embryos as previously described [1]. The 70 000 × *g* pellet (P_3) was washed and recentrifuged at 70 000 × *g* (pellet P_4) before being laid on a saccharose gradient (previously described as gradient 1). Purification was determined using ouabain-sensitive, K^+-stimulated *p*-nitrophenylphosphatase activity as marker [2].

Plasma membranes were suspended in 0.25 M sucrose, 20 mM Tris-HCl, pH 7.4, and either kept at 0—4°C or frozen and stored in liquid N_2, with similar results in the transport assays.

Binding of [^{3}H]quinuclidinyl benzilate. Aliquots from each subcellular fraction (50 μg protein) were incubated for 1 h at room temperature with $1.3 \cdot 10^{-8}$ M [^{3}H]quinuclidinyl benzilate (97 700 dpm/pmol), either with or without $1 \cdot 10^{-5}$ M atropine. The reaction mixture contained 0.25 M sucrose and 20 mM Tris-HCl, pH 7.4, in a final volume of 100 μl. Following incubation, 90-μl aliquots were passed through Whatman glass fibre GF/B filters, and filters were washed three times with 2 ml of ice-cold Earle's balanced-salt solution. Radioactivity retained on the filters was measured by liquid scintillation spectrometry in 8 ml Picofluor 30 (Packard).

Specific binding was calculated from the difference between total binding (without atropine) and aspecific binding (in the presence of saturating atropine). Aspecific binding (including binding to the filter) was identical for all fractions; it represented 45% of total binding for the homogenate, and 7% for the purified membranes.

No K^+-dependent *p*-nitrophenylphosphatase activity could be detected in the filtrate, indicating quantitative retention of the membranes on GF/B filters. Specific binding was proportional to the amount of protein up to 50 μg in all fractions.

Amino acid and glucose uptake measurements. Transport assays on purified membrane vesicles were carried out for varying periods at room temperature with additions as described. Unless otherwise stated, the incubation medium (100 μl) contained 20 mM Tris-HCl, pH 7.4, and sucrose to give a final osmolarity of 0.34 osmol (referred to as Tris-HCl/sucrose medium). Uptake was initiated by addition of membrane suspensions. Following incubation, 80-μl aliquots were passed through Millipore GSWP filters (0.22 μm); filters were washed four times with 2 ml of ice-cold 10 mM Tris-HCl, pH 7.4, containing 0.8 M NaCl. Under these conditions efflux is very low during the washing procedure, as has been demonstrated with fibroblast membrane vesicles [3]. Radioactivity retained on the filters was measured as described above.

Zero-time uptake values were determined by adding membrane suspensions after addition of 2 ml of wash buffer to the incubation medium, and filtering and washing immediately. Alternatively, 80-μl aliquots of the incubation medium, pre-equilibrated at 0—4°C, were filtered immediately following the addition of membranes. Aspecific absorption, measured by either method, was independent of protein concentration and equal to approx. 0.04% of the total radioactivity present in the assay.

The initial rates of amino acid and glucose uptake were proportional to the

amount of vesicular protein in the range used.

Complete retention of the membranes on the filter was controlled as described above.

Due to the small amount of available biological material, for all uptake measurements described in the different figures, the reproducibility could only be assessed at two points of each kinetic analysis: standard errors did not exceed more than ±6% of the mean.

Determination of intravesicular volume. Membrane vesicles (5 mg protein) were incubated with poly([^{14}C]ethylene glycol), as impermeant marker, in 1 ml of 0.25 M sucrose, 20 mM Tris-HCl, pH 7.4. After repeated mixing, the suspension was centrifuged for 60 min at 70 000 × *g*. Radioactivity was measured in the supernatant (590 dpm/μl). The pellet was weighed before and after drying, to constant weight under P_2O_5, to determine total water (84 μl). Extravesicular water was determined from the radioactivity trapped in the pellet. The difference between the two values indicated an intravesicular volume of 7.5 μl/mg protein for the most purified fraction (fraction 17—24% of the sucrose gradient).

Definition of the tracer space. Transport activity is expressed as μl equivalents of medium taken up per mg protein. This is a convenient way of normalizing the data from different experiments in which different concentrations of labelled solute were used.

Materials. Radioactive isotopes were purchased from the following suppliers: [3-^{3}H]quinuclidinyl benzilate, Radiochemical Centre, Amersham; poly([1,2-^{14}C]ethylene glycol), α-amino[1-^{14}C]isobutyric acid and L-[1-^{3}H(n)]glucose, New England Nuclear; L-[U-^{14}C]alanine and D-[6-^{3}H]glucose, Commissariat à l'Energie Atomique.

Results

Muscarinic cholinergic receptors as markers of cardiac plasma membranes. Specific binding of [^{3}H]quinuclidinyl benzilate, a potent muscarinic antagonist, has been recently described in a number of tissues, including rat brain [4], rat heart [5] and chick embryo cardiac cells [6,7]. In homogenates of embryonic chick heart, half-saturation of muscarinic receptors was observed at 0.3—0.4 nM [^{3}H]quinuclidinyl benzilate, irrespective of embryo age [6,7].

We analysed the distribution of these receptors throughout the purification procedure of cardiac plasma vesicles. A large excess of unlabelled atropine, another potent muscarinic antagonist, was used to inhibit specific binding of [^{3}H]quinuclidinyl benzilate. The data in Table I clearly indicate a copurification of the muscarinic receptors with the ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity.

Active amino acid transport in cardiac plasma membrane vesicles. Fig. 1 shows the time course of uptake of L-[^{14}C]alanine in cardiac membrane vesicles. The uptake rate in sucrose medium was identical to that in the presence of 100 mM KSCN, whereas it was 3-fold higher in the presence of 100 mM NaSCN. The same equilibration level was reached under all conditions after 1—2 h of incubation.

The Na^+ effect was also observed with α-amino[^{14}C]isobutyric acid (Fig. 2).

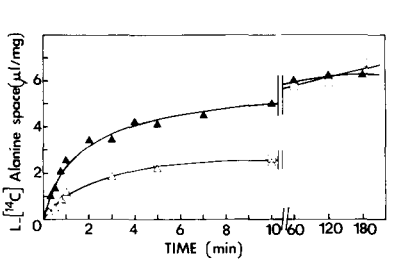
TABLE I

COPURIFICATION OF OUABAIN SENSITIVE *p*-NITROPHENYLPHOSPHATASE AND MUSCARINIC CHOLINERGIC RECEPTORS

Each value is the mean of two determinations not different by more than ±5%. See Materials and Methods for details. The enrichment factor (relative to homogenate) is given in parentheses for each marker.

Fraction	Ouabain-sensitive K^+-stimulated *p*-nitrophenylphosphatase (nmol/min per mg)	[^{3}H]Quinuclidinyl benzilate specific binding (pmol/mg)
Homogenate S_0	8.6 (1)	0.31 (1)
1200 × *g* supernatant S_1	7.1 (0.88)	0.27 (0.87)
12 000 × *g* supernatant S_2	6.7 (0.78)	0.28 (0.90)
70 000 × *g* pellet P_4	26 (3)	1.43 (4.6)
Sucrose gradient		
Fraction 17—24%	115.5 (13.4)	3.32 (10.7)
Fraction 24—27%	65.7 (7.6)	2.37 (7.6)

The stimulation factor depended upon the nature of the accompanying anion, since NaSCN was more effective than NaCl (Fig. 2A). In the presence of sodium, α-aminoisobutyric acid uptake did not vary significantly after 1 h and remained constant up to 20 h (Fig. 2B). The value for accessible space observed at the plateau was consistent with the intravesicular water volume determined with poly[^{14}C]ethylene glycol (7.5 μl/mg protein). When sodium was replaced by choline, the same space was accessible to the amino acid analogue, but a prolonged incubation was usually necessary (Fig. 2B). With some membrane preparations, equilibration could be reached within 1 h in choline chloride, which was similar to the time course observed with [^{14}C]alanine in sucrose (Fig. 1). The osmotic sensitivity of α-aminoisobutyric acid uptake (Fig. 3) indicated that amino acids were transported across the membrane into the intravesicular space rather than merely being bound to the membrane. The influence



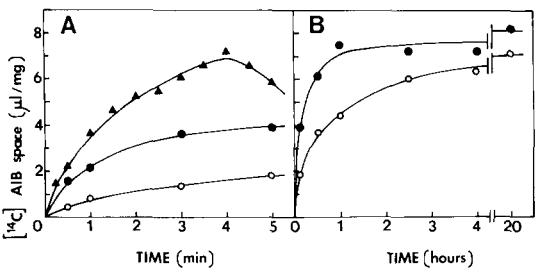
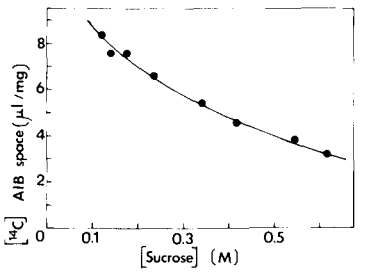


Fig. 1. Effect of a Na^+ gradient on uptake of L-[^{14}C]alanine by cardiac membrane vesicles. Membrane vesicles (40 μg protein) were incubated, for the times indicated, in the standard assay medium with 0.13 mM L-[^{14}C]alanine (63 000 dpm/nmol) in the presence of 100 mM NaSCN (▲), 100 mM KSCN (△), or in 0.25 M sucrose without any salt (□).

Fig. 2. Time course of α-amino[^{14}C]isobutyric acid (AIB) uptake with and without an Na^+ gradient. A. Membrane vesicles (27 μg protein) were incubated in Tris Cl/sucrose with 98 μM amino[^{14}C]isobutyrate (56 000 dpm/nmol) and 100 mM choline chloride (○), 100 mM NaCl (●) or 100 mM NaSCN (▲). B. Membrane vesicles (40 μg protein) were incubated with 0.13 mM amino[^{14}C]isobutyrate (40 000 dpm/nmol) and 100 mM NaCl (●) or 100 mM choline chloride (○).



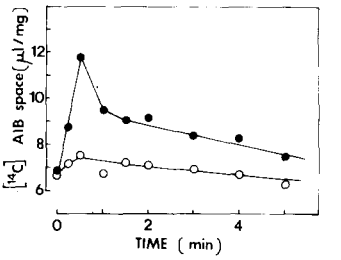


Fig. 3. Osmotic sensitivity of α-amino[^{14}C]isobutyric acid (AIB). Membrane vesicles (40 μg protein) were incubated for 15 h at room temperature with 0.22 mM amino[^{14}C]isobutyrate (40 000 dpm/nmol) and the indicated concentrations of sucrose, in 20 mM Tris-HCl, pH 7.4.

Fig. 4. Effect of SCN^- on α-amino[^{14}C]isobutyric acid (AIB) uptake. Membrane vesicles were preincubated for 1 h at room temperature with amino[^{14}C]isobutyrate and 100 mM NaCl (•) or 100 mM choline chloride (○) under the conditions described for Fig. 2A. At zero time, 100 mM KSCN was added; uptake was measured at the indicated times.

of osmolarity could be also demonstrated in 15-min uptakes, with 100 mM NaCl in the medium (data not shown).

These data suggest the existence in these vesicles of an Na^+-dependent transport system for neutral amino acids. The driving force would be provided by an Na^+ electrochemical gradient, since making the interior negative with the permeant anion, SCN^-, stimulated amino acid uptake. With an NaSCN gradient, a small 'overshoot' could be observed with some membrane preparations (Fig. 2A). This phenomenon was more pronounced when the vesicles were pre-equilibrated with α-amino[^{14}C]isobutyric acid, in the presence of NaCl, before imposing an interior negative membrane potential with KSCN (Fig. 4). Under these conditions, there was a transient but significant accumulation of the labelled amino acid above the equilibrium level. No significant accumulation occurred when sodium was replaced by choline. The electrical potential due to the rapid diffusion of SCN^- was very quickly dissipated (within 1 min). This probably explains why the overshoot was not apparent in all experiments, depending on the relative importance, in the membrane preparation, of the amino acid carrier activity and the passive permeability to ions (e.g., see Fig. 1).

Competition experiments were carried out between L-[^{14}C]alanine and different unlabelled amino acids, in the presence of an Na^+ gradient (Table II). The data clearly show that L-alanine and α-aminoisobutyric acid are transported by the same system, which has a higher affinity for alanine than for α-aminoisobutyric acid. In a number of cells, these two amino acids have been shown to share a common carrier, designated as 'A system' in the classification of Christensen et al. [8]. A small overlap was observed with the 'ASC system' represented by L-threonine, whereas inhibition by L-leucine or L-phenylalanine ('L-system') was very low even when the acids were present in great excess.

In the presence of an Na^+ gradient, uptake of L-[^{14}C]alanine by cardiac plasma membranes was a saturable process with respect to amino acid concentration, with an apparent K_m of 0.35 mM and a V of 1.4 nmol/min per mg protein (Fig. 5).

Specific D-glucose transport in cardiac plasma membrane vesicles. D-[^{3}H]-

TABLE II

SPECIFICITY OF Na^+-GRADIENT-STIMULATED AMINO ACID UPTAKE

The incubation medium contained, in 100 μl Tris-HCl/sucrose: 0.114 mM L-[^{14}C]alanine (63 000 dpm/nmol), 100 mM NaSCN, unlabelled amino acids at the indicated concentrations and 100 μg membrane protein. The initial rate of uptake was measured after 30 s. Corrections were made for aspecific absorption. 100% represented 0.74 nmol L-[^{14}C]alanine taken up per min per mg protein. Results were averaged from duplicate assays with a maximal range of ±6%.

Unlabelled amino acid	Initial rate of uptake of L-[^{14}C]alanine (% of control)
No addition	100
L-Alanine	
1 mM	47
5 mM	21
α-Aminoisobutyrate	
1 mM	67
5 mM	35
L-Threonine	
1 mM	92
5 mM	64
L-Leucine	
1 mM	101
5 mM	80
L-Phenylalanine	
1 mM	108
5 mM	86

Glucose was taken up by cardiac plasma vesicles more rapidly than was L-[^{3}H]-glucose (Fig. 6A). This uptake showed a marked response to medium osmolarity (Fig. 6B). An inverse proportionality was observed and extrapolation to infinite medium osmolarity indicated that no uptake would take place. Thus,

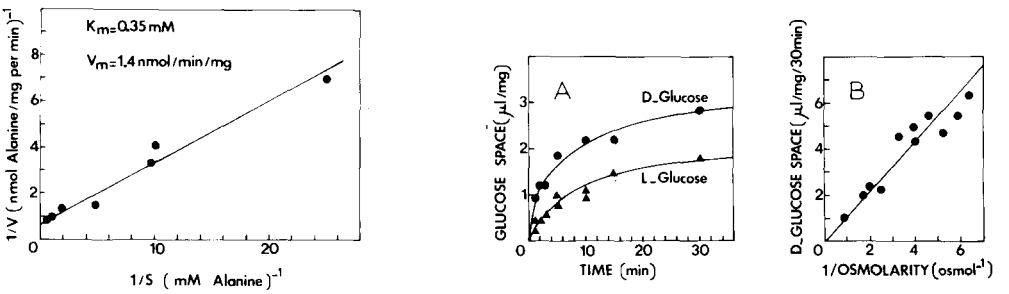
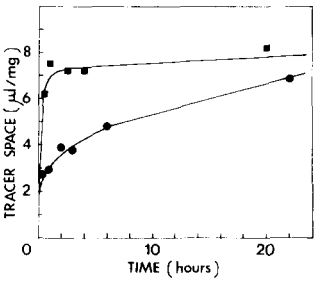


Fig. 5. Concentration dependence of the initial rate of L-[^{14}C]alanine uptake. Membrane vesicles (54 μg protein) were incubated in Tris-HCl/sucrose medium with 100 mM NaSCN and various concentrations of L-[^{14}C]alanine for 0.1, 0.2 and 0.3 min, to determine the initial rate of uptake. Attempts were made to determine Na^+-independent uptake, using 100 mM KSCN instead of NaSCN, however the initial rates were too low to be measured accurately. Therefore, the initial velocities of total uptake were reported in the Lineweaver-Burk plot.

Fig. 6. A. Time course of D- and L-glucose uptake. Membrane vesicles (40 μg protein) were incubated for the indicated times in the standard assay medium (Tris-HCl/sucrose) with 0.4 mM D-[^{3}H]glucose, 74 000 dpm/nmol (●) or with 0.36 mM L-[^{3}H]glucose, 96 000 dpm/nmol (▲). B. Osmotic sensitivity of D-[^{3}H]-glucose uptake. Membrane vesicles (38 μg protein) were incubated with 2.8 mM D-[^{3}H]glucose (11 200 dpm/nmol), 20 mM Tris-HCl, pH 7.4, and sufficient sucrose to give the indicated osmolarity. The uptake was measured after 30 min and corrected for zero-time value which was independent of osmolarity.



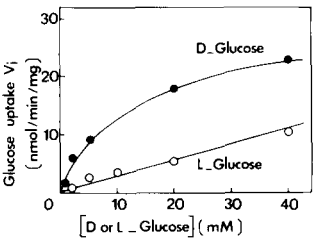


Fig. 7. Comparative time course of vesicles equilibration with α-amino[^{14}C]isobutyric acid and D-[^{3}H]-glucose. Uptake of D-[^{3}H]glucose was measured under the conditions described for Fig. 6A (●). The curve representing uptake of α-amino[^{14}C]isobutyric acid was taken from Fig. 2B (■).

Fig. 8. Concentration dependence of the initial rate of D- and L-[^{3}H]glucose uptake. Each assay contained 90 µg membrane protein and 0.5 µCi D- or L-[^{3}H]glucose at the indicated concentrations. The initial rates of uptake were calculated from values obtained at 15 s and 30 s.

all glucose uptake could be accounted for by transport into membrane vesicles, without any significant binding to the membrane surface.

A 30 min incubation was not sufficient to complete D-glucose equilibration, since the accessible volume slowly increased after prolonged incubation (Fig. 7). After 20 h it came to within 10% of the α-aminoisobutyric acid space, which suggests that the same intravesicular space was accessible to both substrates.

L-Glucose uptake most likely reflects passive diffusion of sugar since, like the diffusion process, it was a linear function of concentration. In contrast, D-glucose uptake was saturable (Fig. 8), which supports the notion that D-glucose uptake occurs via two systems: one carrier-mediated and specific for the D-isomer, and the other a non-specific leak pathway. Therefore, uptake of D-glucose was corrected for L-glucose diffusion to determine kinetic parameters of the carrier-mediated transport system. A K_m of 5 mM and a V of 15 nmol/min per mg were obtained from a Lineweaver-Burk plot of the data of Fig. 8 (not shown).

No sodium dependence was observed for D-glucose uptake: the time course of uptake was identical in sucrose, in 100 mM choline chloride/sucrose, and in 100 mM NaCl/sucrose. Accordingly, a facilitated diffusion system is most likely to be responsible for specific D-glucose transport in these cardiac plasma membranes.

Discussion

In a previous report [1], we showed that cardiac membranes obtained with our purification procedure were similarly enriched in K^+-stimulated *p*-nitrophenylphosphatase and insulin receptors. As indicated in this report, another cell-surface receptor copurified with these two markers — the muscarinic cholinergic receptor. This result if of interest for two reasons.

Firstly, it lends strong support to the muscular origin of our membrane preparation. Whereas ($Na^+ + K^+$)-ATPase (or K^+-stimulated *p*-nitrophenylphosphatase) is known to be rather widespread in all types of cell, and insulin bind-

ing had been demonstrated in cultured fibroblasts [9], the acetylcholine muscarinic receptors are much more specific to muscular cells of heart tissue; no specific [^{3}H]quinuclidinyl benzilate binding could be detected in cultures of chick embryo fibroblasts [10].

Secondly, it suggests the use of the muscarinic cholinergic receptor as a suitable marker for cardiac sarcolemma; [^{3}H]quinuclidinyl benzilate binding provides a simple and sensitive assay to check the purity and yield of the plasma membranes.

Since the description of our purification procedure, other methods have been described for the preparation of cardiac plasma membranes from different animal species. These papers reported the use of other membrane markers such as enzymatic radio-iodination and sialic acid content [11], labelled-ouabain binding [12], or concanavalin binding [13]. All of these markers are certainly suitable cell-surface markers but they are likely to be less specific to muscular tissue than muscarinic receptors.

Cardiac plasma membranes prepared by our method appeared as closed vesicles by electron microscopy [1]. This observation is supported by our current demonstration of the osmotic sensitivity of amino acid and glucose uptake by the membrane preparations. Intravesicular water space was determined to be 7.5 μl/mg protein. Values ranging from 1—2 μl/mg [3,14—16] to 7.4 μl/mg [17] have been reported for membrane vesicles of various origin. Obviously, many factors can influence this volume, such as vesicular size or the proportion of closed vesicles. It is noteable that in less purified cardiac membranes (fraction 24—27% of the sucrose gradient [1]), the intravesicular space was somewhat lower (3—4 μl/mg). In both fractions, this water space was fully accessible to amino acids and glucose, although several hours were necessary for glucose equilibration. Therefore, using the distribution volume of glucose or an analogue after 30 or 60 min, as is frequently done, could lead to underestimated values of the intravesicular volume. Under our conditions, neutral amino acids proved to be better solutes for fast equilibration (Fig. 7). The water space of cardiac vesicles was not influenced by the ionic strength of the incubation medium at constant osmotic pressure. In contrast, the observations of Cheng et al. [18] with sarcolemmal vesicles from rat skeletal muscle led them to conclude that salt solutions promoted sealing of vesicles.

The present results demonstrate that cardiac sarcolemmal membranes retain the basic transport properties of intact tissue with respect to neutral amino acids and glucose. Our purpose was not to study exhaustively the characteristics of these transport systems but rather to demonstrate that they were still functional in isolated membranes.

The present data show that neutral amino acids such as alanine, or the analogue, α-aminoisobutyric acid, were transported into the vesicles by a specific Na^+ electrochemical gradient-stimulated system. The specificity pattern and kinetic parameters compared well with those described by Gazzola et al. [19] on isolated chick cardiac cells in suspension. Similar results have also been obtained on membrane vesicles prepared from other types of cells (for review see Refs. 20 and 21).

Cardiac sarcolemmal vesicles also exhibited a specific carrier for D-glucose. Glucose transport, when occurring merely by facilitated diffusion, without

energy dependence, has proved difficult to measure accurately in membrane vesicles [15,16,18,22,23]. In our system also, determination of initial rates of uptake for D-glucose was much more difficult than that for amino acids.

Diffusion of L-glucose into cardiac vesicles appears to be more rapid than diffusion into intact cells in culture (Paris et al., unpublished results). However, the membranes did not seem to be dramatically leaky, since equilibration with L-glucose required a very long time (half-equilibration was not reached by 3 h). Moreover, as underlined by Hopfer [21], vesicles can be filled with solutes much faster than cells, even though the permeability of the membrane is identical, because of the lower volume-to-surface ratio.

Thus, our difficulty in accurately measuring initial rates of glucose transport activity did not arise from excessive leakiness of the vesicles, nor from an overly rapid equilibration. Rather, it resulted from a very low activity of the specific carrier, despite which uptake was not linear for more than 30 s. It is worth noting that in intact cardiac cells in culture, transport of D-gluclose and analogues has been shown to be extremely rapid (Paris et al., unpublished results). The reason for this discrepancy is not clear. Carrier molecules may have been removed or inactivated during the fractionation procedure; alternatively, the transport activity may in some manner be activated in the intact cell. This question has been recently discussed by Lever [15] from her observations on fibroblast membranes, although contradictory results have been obtained by Inui et al. [16].

Nevertheless, our data clearly demonstrate that a specific D-glucose carrier is still present and functional in cardiac plasma vesicles prepared by our purification procedure. These vesicles have therefore retained both the specific, Na^+-dependent, A-system, for transport of neutral amino acids, and the specific facilitation system for transport of sugars. Together with their enrichment in ($Na^+ + K^+$)-ATPase, insulin receptors and muscarinic cholinergic receptors, these properties suggest that these vesicles remain representative of the whole cell surface and not merely of some specialized area.

Acknowledgements

This work was supported by the Centre National de la Recherche Scientifique and the Commissariat à l'Energie Atomique. We wish to thank Professor Lazdunski's group for their helpful discussions during the course of [^{3}H]guinuclidinyl benzilate binding experiments, Drs. M. Murphy and J. Pouysségur for their critical reading of the manuscript, and Mrs. G. Oillaux for typing the manuscript.

References

1 Paris, S., Fosset, M., Samuel, D. and Ailhaud, G. (1977) J. Mol. Cell. Cardiol. 9, 161—174
2 Gache, C., Rossi, B. and Lazdunski, M. (1976) Eur. J. Biochem. 65, 293—306
3 Lever, J.E. (1977) J. Biol. Chem. 252, 1990—1997
4 Yamamura, H.I. and Snyder, S.H. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 1725—1729
5 Cavey, D., Vincent, J.P. and Lazdunski, M. (1977) FEBS Lett. 84, 110—114
6 Galper, J.B., Klein, W. and Catterall, W.A. (1977) J. Biol. Chem. 252, 8692—8699
7 Renaud, J.F., Barhanin, J., Cavey, D., Fosset, M. and Lazdunski, M. (1980) Dev. Biol., Vol. 77

8 Christensen, H.N., de Cespedes, C., Handlogten, M.E. and Ronquist, G. (1973) Biochim. Biophys. Acta 300, 487—522
9 Thomopoulos, P. and Roth, J. (1976) Cell 8, 417—423
10 Fosset, M., Renaud, J.F., Lenoir, M.C., Kamenka, J.M., Geneste, P. and Lazdunski, M. (1979) FEBS Lett. 103, 133—137
11 Bers, D.M. (1979) Biochim. Biophys. Acta 555, 131—146
12 Van Alstyne, E., Bartschat, D.K., Wellsmith, N.V., Poe, S.L., Schilling, W.P. and Lindenmayer, G.E. (1979) Biochim. Biophys. Acta 553, 388—395
13 Misselwitz, H.J., Will, H., Schulze, W., Will-Shahab, L. and Wollenberger, A. (1979) Biochim. Biophys. Acta 553, 197—212
14 Aronson, P.S. and Sacktor, B. (1975) J. Biol. Chem. 250, 6032—6039
15 Lever, J.E. (1979) J. Biol. Chem. 254, 2961—2967
16 Inui, K.I., Moller, D.E., Tillotson, L.G. and Isselbacher, K.J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 3972—3976
17 Kanner, B.I. (1978) Biochemistry 17, 1207—1211
18 Cheng, L.C., Rogus, E.M. and Zierler, K. (1978) Biochim. Biophys. Acta 513, 141—155
19 Gazzola, G.C., Franchi, R., Saibene, V., Ronchi, P. and Guidotti, G.G. (1972) Biochim. Biophys. Acta 266, 407—421
20 Lever, J.E. (1977) J. Supramol. Struct. 6, 103—124
21 Hopfer, U. (1978) Am. J. Physiol. 234, F89-F96
22 Carter, J.R., Avruch, J. and Martin, D.B. (1972) J. Biol. Chem. 247, 2682—2688
23 Bachmann, W. and Challoner. D. (1976) Biochim. Biophys. Acta 443, 254—266