Biochimica et Biophysica Acta 856 (1986) 428–436
Elsevier

BBA 73040

Maintenance of glucagon-stimulated system A amino acid transport activity in rat liver plasma membrane vesicles

Mark A. Schenerman and Michael S. Kilberg *

Department of Biochemistry and Molecular Biology, University of Florida School of Medicine, J. Hillis Miller Health Center, Gainesville, FL 32610 (U.S.A.)

(Received October 30th, 1985)

Key words: Amino-acid transport; Membrane vesicle; Transport system A; Glucagon; Adaptive regulation; (Rat liver)

Plasma membrane vesicles prepared from intact rat liver or isolated hepatocytes retain transport activity by systems A, ASC, N, and Gly. Selective substrates for these systems showed a Na^+-dependent overshoot indicative of energy-dependent transport, in this instance, driven by an artificially-imposed Na^+ gradient. Greater than 85% of Na^+-dependent 2-aminoisobutyric acid (AIB) uptake was blocked by an excess of 2-(methylamino)isobutyric acid (MeAIB) with an apparent K_i of 0.6 mM. Intact hepatocytes obtained from glucagon-treated rats exhibited a stimulation of system A activity and plasma membrane vesicles isolated from those same cells partially retained the elevated activity. Transport activity induced by substrate starvation of cultured hepatocytes was also evident in membrane vesicles prepared from those cells. The membrane-bound glucagon-stimulated system A activity decays rapidly during incubation of vesicles at 4°C ($t_{1/2}$ = 13 h), but not at −75°C. Several different inhibitors of proteolysis were ineffective in blocking the decay of transport activity. Hepatic system N transport activity was also elevated in plasma membrane vesicles from glucagon-treated rats, whereas system ASC was essentially unchanged. The results indicate that both glucagon and adaptive regulation cause an induction of amino acid transport through a plasma membrane-associated protein.

Introduction

Amino acid transport in rat liver has been studied extensively and the general features have been reviewed [1]. Four distinct transport processes, Systems A, ASC, N and Gly, mediate the Na^+-dependent uptake of neutral amino acids. System A, first described by Oxender and Christensen in Ehrlich cells [2] has been well characterized in rat hepatocytes [3]. For hepatocytes maintained in monolayer culture, the Na^+-dependent uptake of 2-aminoisobutyrate (AIB) is a relatively specific test for system A activity. However, if the Na^+-dependent uptake of AIB is mediated by more than one system in a particular tissue or cell type, the *N*-monomethylated derivative 2-(methylamino)isobutyrate (MeAIB) can be used to increase the selectiveness of the test for system A activity.

Hepatic system A is regulated by both hormones and substrate availability [1,4]. This modulation of transport activity is RNA- and protein synthesis-dependent and is thought to result from increased expression of a gene coding for a plasma membrane-associated glycoprotein [5]. However, studies on the regulation of amino acid transport in intact hepatocytes are complicated by the contribution of metabolism. One way to circumvent this

* To whom correspondence should be addressed.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; AIB, 2-aminoisobutyric acid; MeAIB, 2-(methylamino)isobutyric acid.

problem is through the use of isolated plasma membrane vesicles. Furthermore, one can test whether the enhanced transport following treatment with hormones or amino acid starvation is actually dependent on a newly synthesized plasma membrane-bound protein by monitoring the system A activity in isolated membrane vesicles. Many laboratories have studied amino acid transport in membrane vesicles [6], but only a few have investigated vesicles from rat liver [7–11]. Quinlan et al. [10], showed that when hepatocytes obtained from 24-h fasted rats were used to prepare plasma membrane vesicles, they exhibited a 3–4-fold stimulation of system A transport activity when compared to vesicles from a fed animal. Samson and Fehlmann [7] treated hepatocytes with dibutyryl cAMP prior to isolation of plasma membrane vesicles and found that these membranes contained increased Na^+-dependent alanine transport activity, a portion of which was inhibited by the system A-specific analog MeAIB.

The purpose of the present study was: (1) to determine whether all of the Na^+-dependent neutral amino acid transport systems survive plasma membrane isolation: (2) to use plasma membrane vesicles isolated from glucagon-injected rats or from cultured hepatocytes following amino acid-starvation (adaptive regulation) to demonstrate that the enhanced system A activity is retained in isolated plasma membranes; and (3) to compare the level of glucagon-induced system A activity in intact cells and membrane vesicles isolated from those same cells. Our results suggest that the system A transport activity in rat liver vesicles is labile during storage at 4°C, but that the activity induced either by glucagon or by amino acid starvation is partially retained in the isolated plasma membranes.

Materials and Methods

Hepatocyte isolation and transport assay

Hepatocytes were isolated from male Sprague-Dawley rats (100–200 g) as described previously [12]. Both the control and the hormone-treated rats were fasted overnight prior to cell isolation. The experimental animals were injected with 1 mg of glucagon per 100 g body weight 4 h before surgery. Small portions of both control and hormone-treated hepatocytes were suspended in Na^+-containing Krebs-Ringer bicarbonate buffer (NaKR buffer) with 0.1 mM cycloheximide and were then placed in monolayer culture [12]. Following a 2-h culture period, the activity of system A was determined by assaying the Na^+-dependent transport of 50 μM AIB for 1 min at 37°C. The remaining cells were resuspended in 40 ml of ice-cold buffer A (0.25 M sucrose/0.2 mM $MgCl_2$/10 mM Hepes KOH (pH 7.5)) for preparation of a plasma membrane-enriched subcellular fraction.

Plasma membrane isolation

Both the control and glucagon-treated hepatocytes were homogenized using 25 strokes of a Potter-Elvehjem homogenizer with a motor driven, tight-fitting teflon pestle. Plasma membrane vesicles were prepared as described by Van Amelsvoort et al. [11]. The membranes were resuspended by vortex mixing and diluted to a final concentration of approx. 10 mg protein per ml in buffer A. The overall yield of the procedure was about 1.5 mg protein per g liver (wet weight).

When membrane vesicles were prepared from cultured cells following substrate starvation, freshly isolated hepatocytes were placed in 150 mm collagen-coated dishes in NaKR buffer (amino acid-free medium) or NaKR buffer containing 20 mM asparagine (amino acid-supplemented medium) at a density of 27 million viable cells per dish [12]. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air for 6 h and then each dish was rinsed with 10 ml of phosphate-buffered saline (0.9% NaCl/10 mM Na_2HPO_4 (pH 7.5)). The cells were scraped into 5 ml of buffer A and homogenized using 25 strokes with the Potter-Elvehjem homogenizer. Membrane vesicles were isolated as described by Van Amelsvoort et al. [11]. The total yield from 10 dishes of cultured cells was approx. 3 mg of membrane protein.

Vesicle transport assay

Just prior to use, the membrane vesicles were diluted with buffer A to a final concentration of 2.5 mg protein per ml and then incubated at 22°C for 15 min. To initiate amino acid uptake, 20 μl (50 μg protein) of the vesicle suspension was

added to 20 μl of buffer A supplemented with 10 mM $MgCl_2$, 120 mM of either NaSCN or KSCN, and 200 μM radioactively-labelled amino acid. These two solutions will be referred to as Na^+- and K^+-uptake buffers, respectively. Where indicated in the figure legends, 60 mM Na_2SO_4 or K_2SO_4 was used to replace the corresponding thiocyanate salts. Uptake was terminated by the addition of 1 ml of ice-cold buffer A containing 100 mM NaCl (stop-buffer). The mixture was immediately passed over a 0.45 μm nitrocellulose filter. The filter was washed with another 3 ml of ice-cold stop-buffer and then analyzed for trapped radioactivity in 5 ml of Bray's scintillation cocktail [13].

Enzyme marker assays

The activities of 5'-nucleotidase [14], glucose-6-phosphatase [15], succinate:cytochrome *c* reductase [16], and cytochrome oxidase [16] were assayed by previously described methods. Inorganic phosphate was determined by the method of Fiske and SubbaRow [17]. Fluoride-stimulated (10 mM NaF) adenylate cyclase activity was measured by a modification of the procedure described by Wisher and Evans [18]. The cAMP produced was detected by a protein binding assay supplied as a kit by the Amersham Corp. Tests for contamination of the final membrane fraction by intracellular membranes showed a similar profile to that obtained by Van Amelsvoort et al. [11]. Although glucose-6-phosphatase activity, a marker for microsomal membranes, was enriched about 3-fold (homogenate = 10.2 ± 1.5, vesicles = 31.9 ± 1.6 μmol P_i/mg protein per h), there was a 10-fold reduction in the level of mitochondrial enzyme marker activities. For example, the succinate:cytochrome *c* reductase activities in the homogenate and final vesicle preparation were 14.02 ± 0.31 and 1.32 ± 0.09 nmol cytochrome *c* reduced per mg protein per min, respectively.

Protein determination

Vesicle protein was determined by a modification of the method of Bensadoun and Weinstein [19]. Approximately 0.01 to 0.05 mg of membrane protein was suspended in 1 ml of 0.1% sodium dodecyl sulfate. Following a 10 min incubation at 22°C, the protein was precipitated by adding 750 μl of ice-cold 24% (w/v) trichloroacetic acid and then pelleted by centrifugation at 12000 × *g* for 20 min. The protein content of the pellet was measured by a modification of the Lowry technique as described previously [12]. Bovine serum albumin (5 to 100 μg) was used as the standard.

Materials

The radioisotopes used were: 2-amino[*methyl*-^{3}H]isobutyric acid (AIB), ICN Pharmaceuticals; L-[^{3}H]cystine, Schwarz/Mann; L-[2,5-^{3}H]histidine, [2-^{3}H]glycine, L-[2,3-^{3}H]alanine, and 3-*O*-methyl-D-[U-^{14}C]glucose, Amersham. Filters used for transport assays were either Millipore type HAWP (0.45 μm) or Gelman type GN-6 (0.45 μm). Highly purified glucagon was a generous gift from Dr. Mary Root of Lilly Laboratories. Rats were from a colony maintained by the University of Florida Animal Resources facility.

Results

MeAIB inhibition of AIB uptake

Fig. 1 illustrates the inhibition of Na^+-dependent AIB uptake by MeAIB, a system A-specific analog [20]. The inhibition was concentration dependent yielding an apparent K_i of 0.6 ± 0.2 mM. This value is in good agreement with data indicating that the K_m for the high-affinity Na^+-dependent component of MeAIB uptake by isolated hepatocytes is about 0.3 mM (unpublished results). Although an excess of MeAIB does not completely block AIB uptake, it is clear that most (> 85%) of the Na^+-dependent AIB uptake occurs by the MeAIB-inhibitable route (i.e., system A). These data support the use of AIB as a relatively selective substrate for system A in isolated rat liver plasma membrane vesicles.

The Na^+-dependent uptake of AIB was proportional to the amount of membrane protein present in the incubation mixture and varied inversely with extravesicular osmolarity (data not shown). These observations indicate that the amino acid uptake is protein-mediated and that the amino acid is trapped in an osmotically-sensitive space. The intravesicular volume was determined to be 1.2 μl/mg protein by the method of Kletzien et al. [21].

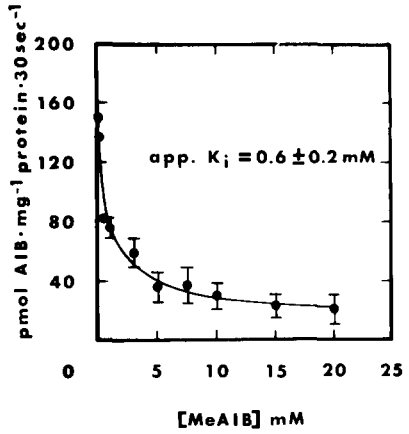

Fig. 1. MeAIB inhibition of Na^+-dependent AIB uptake by isolated membrane vesicles. Rat liver lasma membranes were assayed for Na^+-dependent AIB transport in the presence of the system A-specific probe, MeAIB, at the indicated concentrations. The Na^+-dependent uptake of 200 μM AIB was measured as described in Materials and Methods. Computer analysis of the data yielded an estimated K_i of 0.6 ± 0.2 mM for MeAIB. The results are reported as the averages ± S.D. of assays in triplicate.

Na^+-dependent transport of naturally-occurring amino acids

The uptake of four individual amino acids was measured to obtain evidence for the presence of systems A, ASC, N, and Gly in the isolated membrane vesicles. The rate of alanine transport in the presence of a Na^+ gradient was considerably greater than that in the absence of Na^+ (Fig. 2A). The time-course of alanine uptake showed a rapid Na^+-dependent overshoot, about 50% of which was not inhibitable by an excess of MeAIB. These data, in agreement with results from intact hepatocytes [22] and isolated liver membranes [7,9], confirm that the hepatic Na^+-dependent uptake of alanine, at a substrate concentration of 200 μM, is about equally divided between systems A and ASC.

Additional evidence for the presence of system ASC was obtained by examining cysteine transport. As reported previously for isolated hepatocytes [22], the Na^+-dependent transport of cysteine by the membrane vesicles was not inhibited by an excess of MeAIB (Fig. 2B). The Na^+-dependent uptake rate for cysteine was considerably less than that seen for alanine (e.g., 150 versus 450 pmol · (mg protein)$^{-1}$ · 15 s^{-1}).

The Na^+-dependent transport of glutamine and histidine by intact hepatocytes is mediated to a large extent by system N [23]. In the absence of MeAIB, histidine uptake by the vesicles (Fig. 2C) showed a rapid Na^+-dependent overshoot that decayed at a slow rate. When Na^+-dependent histidine was assayed in the presence of an excess of MeAIB, conditions that provide a specific test for system N activity [23], a rapid overshoot with an accumulation of greater than 400 pmol · (mg protein)$^{-1}$ · 15 s^{-1} was observed. These results suggest the presence of system N activity in the isolated vesicles, but also indicate that histidine transport in this membrane preparation is not restricted entirely to system N.

Hepatic Na^+-dependent glycine transport is mediated by systems A, ASC, and Gly [24]. System Gly activity can be assayed selectively by measuring Na^+-dependent glycine uptake in the presence of an amino acid that can inhibit efficiently the other two systems. We have chosen threonine for this purpose because, in cultured hepatocytes, this amino acid is transported effectively by both systems A and ASC [3]. When Na^+-dependent glycine uptake was measured in the vesicles, approx. 40% escaped inhibition by threonine (Fig. 2D). Although these data show that a significant portion of glycine uptake occurs by systems A and ASC, they also demonstrate that system Gly activity can be measured readily in isolated liver plasma membrane vesicles.

Effect of glucagon-treatment and amino acid-starvation on system A activity in plasma membrane vesicles

It is well documented that glucagon-treatment, either in vivo [4] or in vitro [25,26], causes a protein synthesis-dependent increase in hepatic system A transport activity. Our laboratory has provided evidence that the hormone-induced molecule responsible for the stimulation of system A activity is a glycoprotein [5]. The induction by glucagon is generally considered to result from de novo synthesis of carrier-associated molecules and their subsequent insertion into the plasma membrane. To determine whether the system A-associated glycoprotein induced by glucagon is plasma membrane-bound, the system A activity was assayed in membrane vesicles from hepatocytes

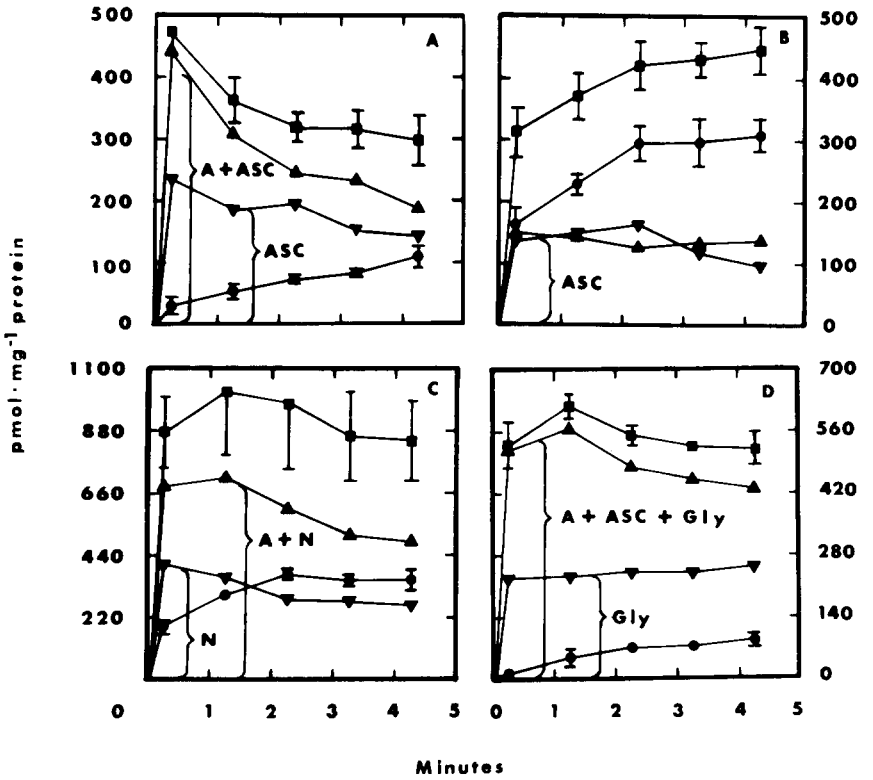

Fig. 2. Alanine, cysteine, histidine and glycine transport by rat liver plasma membranes. Membrane vesicles were used to measure the transport of the indicated substrate. The transport of alanine (A), cysteine (plus 1 mM dithiothreitol) (B), or histidine (C) was tested in the presence or absence of 5 mM MeAIB. Glycine (D) uptake was assayed in the presence or absence of 5 mM threonine. The substrate concentration in each case was 200 μM and the transport was measured at 22°C. The difference between uptake in Na^+ (■) and that in K^+ (●) was taken as the total Na^+-dependent transport (▲). The Na^+-dependent component in the presence of the inhibitor (MeAIB or threonine) is also shown (▼). See the text for interpretation of these data as evidence for the specific transport systems indicated. The results are presented as the averages ± S.D. of three determinations.

taken from either normal or glucagon-treated rats. When the transport in vesicles isolated from glucagon-treated rats was first tested using thiocyanate as the counter-anion in the uptake buffer, the Na^+-dependent overshoot observed was too rapid to measure (data not shown). In order to demonstrate the peak of Na^+-dependent AIB uptake, sulfate was used to replace thiocyanate as the counter anion in the uptake buffers. The lower lipophilicity of the sulfate anion slowed the Na^+-dependent uptake so that the peak of the Na^+-dependent overshoot could be measured more accurately.

Fig. 3 illustrates the time course for the Na^+-dependent uptake of AIB in normal or glucagon-treated vesicles *. The AIB transport peaks at approximately 3 min in the control membranes, whereas the maximal uptake occurs at only 1 min in the glucagon-treated vesicles. All subsequent assays of AIB transport that were designed to make comparisons between control and hormone-treated vesicles were performed for 15 s.

The system A activity in primary cultures of rat hepatocytes is also induced if the cells are incubated in an amino acid-free medium [27]. This process, referred to as adaptive regulation, is also

* For brevity, the vesicles prepared from the hepatocytes that were isolated from the glucagon-injected rats will be referred to as 'glucagon-treated vesicles'.

thought to result from increased synthesis of a system A-associated glycoprotein located in the plasma membrane [5]. When hepatocytes were incubated for 6 h in amino acid-free medium (NaKR buffer) or NaKR buffer supplemented with 20 mM asparagine and then membrane vesicles were prepared from those cells, the system A activity was enhanced nearly 6-fold in the vesicles from starved cells. The rate of AIB uptake in the vesicles from starved (no amino acid) and fed (20 mM asparagine) cells was 189 ± 14 and 33 ± 6 pmol · (mg protein)$^{-1}$ · min^{-1}, respectively. The degree of induction for system A transport activity measured in intact cells is generally 5–10-fold.

After several membrane preparations had been tested for the level of glucagon-dependent stimulation of Na^+-dependent AIB transport, it appeared as though the degree of hormone-induced activity for intact cells was not fully represented in the isolated membrane vesicles. To monitor the degree of hormone stimulation, hepatocytes from glucagon-injected rats were assayed for system A activity and then plasma membrane vesicles were isolated from the same preparation of cells. The data shown in Table I are representative of many experiments. In this instance, the intact cells showed a 30-fold increase in system A transport following glucagon treatment, whereas the membrane vesicles showed a 13-fold change.

One possible explanation for the incomplete retention of stimulated system A activity in the membrane vesicles compared to the intact cells is that the composition of the control and glucagon-treated vesicles is different with respect to the three domains known to exist on the hepatocyte surface [18,28]. It has been reported that Na^+-dependent alanine transport activity is unequally distributed on the cell surface [29,30]. Plasma membrane marker enzymes were assayed to determine if the amount of canalicular and basolateral membrane in the control and glucagon-treated vesicles was similar. The control vesicles showed an enrichment of 6-fold for 5′-nucleotidase (canalicular) and 2-fold for fluoride-stimulated adenylate cyclase (basolateral) when compared to the homogenate, whereas the vesicles from glucagon-treated rats showed a 9-fold enrichment of 5′-nucleotidase activity and a 5-fold enrichment of fluoride-stimulated adenylate cyclase

TABLE I

GLUCAGON STIMULATION OF SYSTEM A IN RAT HEPATOCYTES IS RETAINED IN ISOLATED PLASMA MEMBRANE VESICLES

Rats were injected with 1 mg of glucagon per 100 g body weight 4 h prior to hepatocyte isolation. Hepatocytes and the corresponding membrane vesicles from the same preparation of cells were isolated and analyzed for system A activity as described in Materials and Methods. The velocities given are for Na^+-dependent AIB uptake and are the averages ± S.D. of three individual assays. Note that the length of the assays in the whole cells was 1 min, whereas the assays employing membrane vesicles were for 15 s. Values shown in the last column indicate the degree of the glucagon-dependent stimulation that was determined by calculating the ratio of the velocities (glucagon-treated/control).

Preparation	pmol AIB · (mg protein)$^{-1}$ · (unit time)$^{-1}$		Glucagon-dependent ratio
	Control	Glucagon-treated	
Intact cells	28.9 ± 4.9	872 ± 64	30.2
Membrane vesicles	45.4 ± 22.0	571 ± 5.4	12.6

TABLE II

ENZYME ACTIVITIES IN MEMBRANES FROM CONTROL AND GLUCAGON-TREATED HEPATOCYTES

Membrane vesicles (100 μg) were analyzed for 5′-nucleotidase and adenylate cyclase activity as described in Materials and Methods. Values for 5′-nucleotidase activity are expressed as μmol P_i formed per mg protein per h, whereas those for adenylate cyclase activity are expressed as nmol cAMP formed per mg protein per h. Adenylate cyclase was stimulated using 10 mM NaF, the non-stimulated level of adenylate cyclase ranged from 0.02 to 0.05 and 0.13 to 0.29 in the homogenate and membrane vesicles, respectively, and has been subtracted from the results to yield the data shown. The results are given as the averages of three determinations; the standard deviations were less than 15%.

Donor rat Enzyme tested	Homogenate	Membrane vesicles	Enrichment
Control			
5′-Nucleotidase	2.1	12.0	5.7
Fluoride-stimulated adenylate cyclase	0.26	0.60	2.3
Glucagon-treated			
5′-Nucleotidase	1.9	17.3	9.1
Fluoride-stimulated adenylate cyclase	0.22	1.11	5.1

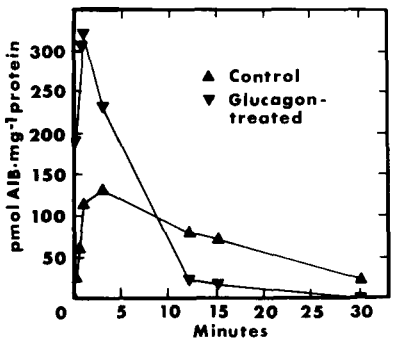

Fig. 3. System A activity in plasma membrane vesicles from control or glucagon-injected rats. Membrane vesicles were prepared from control rats (▲) or rats injected with 1 mg glucagon per 100 g body weight (▼). The freshly isolated vesicles were immediately tested for Na^+-dependent AIB transport as described in Materials and Methods using either 60 mM Na_2SO_4 or K_2SO_4 uptake buffer. The time of uptake at 22°C was varied from 10 s to 30 min. The results are expressed as the averages of triplicate determinations and the standard deviations, omitted for clarity, were generally less than 10%.

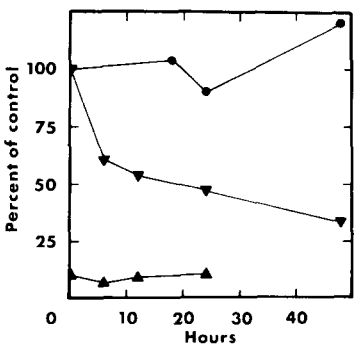

Fig. 4. Decay of system A activity in membrane vesicles incubated at 4°C or −75°C. Isolated membrane vesicles either from glucagon-treated (●, ▼) or control rats (▲) were incubated at 4°C (▼, ▲) or −75°C (●) for the indicated times. Vesicles stored at −75°C were in aliquots and were thawed only once prior to assay. At the indicated times, Na^+-dependent AIB uptake was measured as described in Materials and Methods using either 60 mM Na_2SO_4 or K_2SO_4 uptake buffer. The results are expressed as a percent of the initial Na^+-dependent uptake in the freshly isolated vesicles from glucagon-treated rats (423 ± 42 pmol AIB·(mg protein)$^{-1}$·15 s^{-1}). The data are the averages of triplicate determinations and the standard deviations were typically less than 20%.

(Table II). These data indicate that there are differences in the content of canalicular and basolateral membrane between the two vesicle populations, but the glucagon-treated vesicles actually show a higher degree of enrichment for both markers. The canalicular/basolateral membrane ratio appears to be similar for both preparations (control = 2.5, glucagon-treated = 1.8). Hence, a difference in the composition with respect to membrane domains is probably not responsible for the decreased glucagon-dependent stimulation reported in Table II.

Inactivation of glucagon-stimulated system A activity

The decreased retention of stimulated system A activity in membrane vesicles might also be explained if the carrier complex is irreversibly

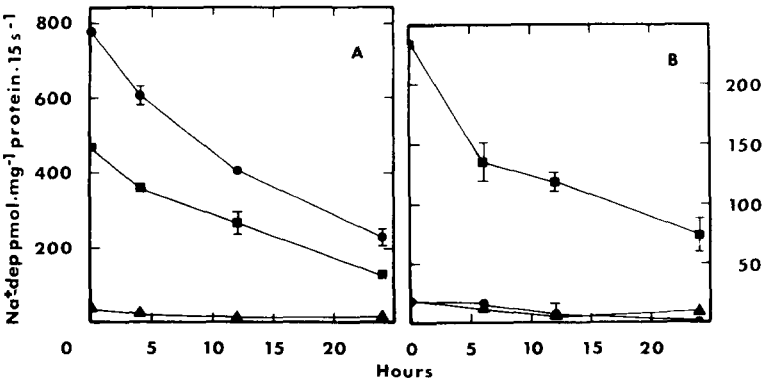

Fig. 5. Decay of systems A, N, and ASC in glucagon-treated and normal vesicles. Membrane vesicles from glucagon-treated (A) or control rats (B) were incubated at 4°C for the indicated times. After each period of incubation, system A (●), system N (■), and system ASC (▲) were tested as described in Fig. 2. The results are expressed as the mean ± S.D. of triplicate determinations of the Na^+-dependent uptake per mg protein per 15 s. Where not indicated, the error bars are contained within the symbols.

inactivated during the isolation procedure. In order to test this hypothesis, freshly isolated membrane vesicles from normal or glucagon-treated rats were incubated at 4°C for 48 h. At specific intervals, the system A activity was assayed in both vesicle preparations. The results indicate that at 4°C there is a time-dependent loss of system A activity in the glucagon-treated vesicles (Fig. 4). In contrast, maintenance of the vesicles at −75°C for 48 h protected greater than 85% of the initial activity (Fig. 4).

The addition of 2 mM phenylmethylsulfonyl fluoride, 2 mM ethylenediaminetetraacetic acid, trasylol (30 trypsin inhibitor units per ml), 0.1 mM leupeptin, 0.5 mM bacitracin, or 1 mM dithiothreitol to the vesicle suspension did not slow the decay of system A activity in the membrane vesicles maintained at 4°C. Similar results were obtained if the vesicles were subjected to a freeze-thaw cycle in the presence of the inhibitors to trap them inside the intravesicular space.

To determine if the degradative process observed was specific for system A, we tested the activity of systems N and ASC in control and glucagon-treated vesicles during incubation at 4°C. The glucagon-treated vesicles show similar patterns of decay for systems A, N, and ASC (Fig. 5). Transport by system A and system N was enhanced in the glucagon-treated vesicles when compared to the control membranes. Interestingly, these data are consistent with earlier observations in cultured hepatocytes that suggested that system N is stimulated by glucagon in vivo [5]. The half-life of decay of systems A, N, and ASC is approx. 13 h. Membrane vesicles isolated from control hepatocytes also exhibit decay of systems A, N, and ASC (Fig. 5B). However, in the case of systems A and ASC, these measurements are imprecise due to the low level of activity present.

Discussion

The data presented demonstrate that isolated rat liver plasma membrane vesicles retain the ability to concentrate substrates of systems A, ASC, N, and Gly when energized with an artificially-imposed Na^+ gradient. Although not described in this report, additional studies with the isolated vesicles have shown that: (1) Na^+-dependent alanine transport is temperature-dependent and stereospecific; (2) Li^+ substitutes for Na^+ effectively with respect to system ASC, but rather poorly for system A; (3) Na^+-dependent AIB uptake is blocked by ionophores that collapse the trans-membrane Na^+ gradient; and (4) system ASC activity is relatively constant between pH 6.0 and 8.0, but system A-mediated uptake is inhibited by 66% at pH 6.0. Several of these basic characteristics have been reported by others [7–11] and are consistent with the known properties of system A in intact hepatocytes [3].

Glucagon is a potent stimulator of hepatic system A activity [4,25,26]. For example, hepatocytes isolated from glucagon-injected rats can exhibit an enhanced system A activity of 20–100-fold [4]. The hormone-induction is blocked by cycloheximide [25,26] and tunicamycin [5]; observations that suggest a role for a newly-synthesized membrane-bound glycoprotein. Like others [7,10], we observe that when plasma membrane vesicles are prepared from hepatocytes that contain stimulated system A activity, enhanced transport is detected in the experimental preparation of membranes when compared to control vesicles. However, none of the previous reports had documented the level of stimulation in the liver tissue or hepatocytes from which the membrane vesicles had been isolated. In the present study, the data show that glucagon-dependent stimulation of systems A and N in hepatocytes is retained in freshly isolated plasma membrane vesicles. Furthermore, we find that the degree of glucagon induction observed in the membrane vesicles is proportional to, but not equal to, that seen in the intact cell. We have also demonstrated that system A activity stimulated by amino acid starvation of cultured hepatocytes, is retained in isolated membrane vesicles. These results are supportive of the proposal that the increase in system A activity following glucagon treatment or substrate starvation of cells is caused by the insertion into the plasma membrane of a system A-associated glycoprotein.

In an attempt to explain why only a portion of the glucagon-stimulated activity appeared to be present in the membrane vesicles, each of the fractions of the entire membrane preparation was tested for Na^+-dependent AIB transport activity during the isolation of vesicles from hepatocytes

taken from either normal or glucagon-treated rats. Those experiments showed that approx. 10% of the total units of Na^+-dependent AIB uptake found in the homogenate of either the glucagon-treated or control cells was recovered in the final plasma membrane fraction. None of the fractions that were usually discarded during the membrane isolation procedure contained an enrichment of transport activity. Hence, although some of the glucagon-stimulated activity is lost in various fractions that are discarded during the membrane isolation procedure, a 50% reduction or more in the expected level of transport cannot be accounted for in this way.

A plausible explanation for the apparent loss of hormone-stimulated system A activity is irreversible inactivation of the carrier protein. Several processes could account for the inactivation of the transport activity such as loss of a loosely-attached system A-associated membrane protein, a non-specific change in the degree of post-translational modification, a change in vesicle permeability, or a degradation of the carrier itself by proteolysis. The results showing similar inactivation rates for systems N and ASC suggest that the loss of transport by system A may not be a specific process.

A final possibility is that the isolated membranes respond differently than do the intact hepatocytes to the artificially imposed Na^+ electrochemical gradient. Whether the permeability to Na^+ or to the counter-anions (Cl^- or SCN^-) is different between cells and isolated vesicles is unknown and will require a more comprehensive investigation of the ion transport properties of membrane vesicles prepared in the manner described.

Acknowledgements

The authors would like to thank Tom Chiles and Donna Bracy for their assistance in the preparation of membrane vesicles. This research was supported by grants AM-28374 and AM-31580 from the National Institute for Arthritis, Diabetes, Digestive, and Kidney Diseases, The National Institutes of Health (U.S.A.).

References

1 Kilberg, M.S. (1982) J. Membrane Biol. 69, 1–12
2 Oxender, D.L. and Christensen, H.n. (1963) J. Biol. Chem. 238, 3686–3699
3 Kilberg, M.S., Barber, E.F. and Handlogten, M.E. (1985) Curr. Top. Cell. Reg. 25, 133–163
4 Handlogten, M.E. and Kilberg, M.S. (1984) J. Biol. Chem. 259, 3519–3525
5 Barber, E.F., Handlogten, M.E. and Kilberg, M.S. (1983) J. Biol. Chem. 258, 11851–11855
6 Lever, J.E. (1980) CRC Crit. Rev. Biochem. 7, 187–246
7 Samson, M. and Fehlmann, M. (1982) Biochim. Biophys. Acta 687, 35–41
8 Sips, H.J., Apitule, M.E.A. and Van Dam, K. (1980) Biochim. Biophys. Acta 600, 577–580
9 Sips, H.J., Van Amelsvoort, J.M.M. and Van Dam, K. (1980) Eur. J. Biochem. 105, 217–224
10 Quinlan, D.C., Todderud, C.G., Kelley, D.S. and Kletzien, R.F. (1982) Biochem. J. 208, 685–693
11 Van Amelsvoort, J.M.M., Sips, H.J. and Van Dam, K. (1978) Biochem. J. 174, 1083–1086
12 Kilberg, M.S., Vida, T.A. and Barber, E.F. (1983) J. Cell. Physiol. 114, 45–52
13 Bray, G.A. (1960) Anal. Biochem. 1, 279–285
14 Morre, D.J. (1971) Methods Enzymol. 22, 130–148
15 Swanson, M.A. (1955) Methods Enzymol. 2, 541–543
16 Kilberg, M.S. and Christensen, H.N. (1979) Biochemistry 18, 1525–1530
17 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
18 Wisher, M.H. and Evans, W.H. (1975) Biochem. J. 146, 375–388
19 Bensadoun, A. and Weinstein, D. (1976) Anal. Biochem. 70, 241–250
20 Kilberg, M.S., Handlogten, M.E. and Christensen, H.N. (1981) J. Biol. Chem. 256, 3304–3312
21 Kletzien, R.F., Pariza, M.W., Becker, J.E. and Potter, V.R. (1975) Anal. Biochem. 68, 537–544
22 Kilberg, M.S., Christensen, H.N. and Handlogten, M.E. (1979) Biochem. Biophys. Res. Commun. 88, 744–751
23 Kilberg, M.S., Handlogten, M.E. and Christensen, H.N. (1980) J. Biol. Chem. 255, 4011–4019
24 Christensen, H.N. and Handlogten, M.E. (1981) Biochem. Biophys. Res. Commun. 98, 102–107
25 LeCam, A. and Freychet, P. (1976) Biochem. Biophys. Res. Commun. 72, 893–901
26 Pariza, M.W., Butcher, F.R., Kletzien, R.F., Becker, J.E. and Potter, V.R. (1976) Proc. Natl. Acad. Sci. USA 73, 4511–4515
27 Kelley, D.S. and Potter, V.R. (1978) J. Biol. Chem. 53, 9009–9017
28 Hubbard, A.L., Wall, D.A. and Ma, A. (1983) J. Cell Biol. 96, 217–229
29 Van Amelsvoort, J.M.M., Sips, H.J., Apitule, M.E.A. and Van Dam, K. (1980) Biochim. Biophys. Acta 600, 950–960
30 Meier, P.J., St. Meier-Abt, A., Barrett, C. and Boyer, J.L. (1984) J. Biol. Chem. 259, 10614–10622