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DISTINCT MECHANISMS OF HYPOXANTHINE AND INOSINE TRANSPORT IN MEMBRANE VESICLES ISOLATED FROM CHINESE HAMSTER OVARY AND Balb 3T3 CELLS

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Summary

Both enzyme-mediated group translocation and facilitated diffusion have been proposed as mechanisms by which mammalian cells take up purine bases and nucleosides. We have investigated the mechanisms for hypoxanthine and inosine transport by using membrane vesicles from Chinese hamster ovary cells (CHO), Balb/c 3T3 and SV3T3 cells prepared by identical procedures. Uptake mechanisms were characterized by analyzing intravesicular contents, determining which substrates could exchange with the transport products, assaying for hypoxanthine phosphoribosyltransferase activity, and measuring the stimulation of uptake of hypoxanthine by phosphoribosyl pyrophosphate (*P*Rib-*PP*).

We found that the uptake of hypoxanthine in Balb 3T3 vesicles was stimulated 3—4-fold by *P*Rib-*PP*. The intravesicular product was predominantly IMP. The hypoxanthine phosphoribosyltransferase activity copurified with the vesicle preparation. These results suggest the possible involvement of this enzyme in hypoxanthine uptake in 3T3 vesicles. In contrast to the 3T3 vesicles, CHO vesicles prepared under identical procedures did not retain hypoxanthine phosphoribosyltransferase activity and did not demonstrate *P*Rib-*PP*-stimulated hypoxanthine uptake. The intravesicular product of hypoxanthine uptake in CHO vesicles was hypoxanthine. These results and data from our kinetic and exchange studies indicated that CHO vesicles transport hypoxanthine via facilitated diffusion. An analogous situation was observed for inosine uptake; CHO vesicles accumulated inosine via a facilitated diffusion mechanism, while in the same experiments SV3T3 vesicles exhibited a purine nucleoside phosphorylase-dependent translocation of the ribose moiety of inosine.

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Vesicles prepared from a CHO cell line temperature-sensitive for hypoxanthine uptake (Aza^{r}ts) showed a temperature-sensitivity in K_m for uptake parallel to that of the intact cells. This suggests that the defect in Aza^{r}ts may be caused by a missense mutation in the gene coding for the hypoxanthine transport carrier.

Introduction

We previously reported that the uptake of hypoxanthine in membrane vesicles isolated from Balb 3T3 cells [1,2] was stimulated 7—8-fold by *P*Rib-*PP*. In these vesicles the intravesicular uptake products in the presence of *P*Rib-*PP* were 80—90% IMP and 10—20% hypoxanthine. Hypoxanthine uptake was not stimulated by *P*Rib-*PP* in a mutant deficient in hypoxanthine phosphoribosyltransferase activity, indicating that the *P*Rib-*PP* stimulation was dependent on the presence of hypoxanthine phosphoribosyltransferase activity. It was, therefore, interpreted that hypoxanthine may cross the membrane by both hypoxanthine phosphoribosyltransferase-mediated and non-hypoxanthine phosphoribosyltransferase-mediated processes, as was found in *Salmonella typhimurium* [3]. Other laboratories, however, using rat hepatoma and Chinese hamster ovary or lung cells, found evidence for facilitated diffusion [4—7] and suggested it as the major mechanism of hypoxanthine uptake in the cells they studied; i.e. hypoxanthine was transported prior to its metabolic conversion.

Studies of inosine uptake by membrane vesicles from mouse fibroblast cells indicated that the uptake of inosine involved a concomitant cleavage to ribose-1-phosphate (Rib1*P*) and hypoxanthine [8]. This mechanism involved a membrane-localized purine nucleoside phosphorylase acting in a reaction during which hypoxanthine was released on the exterior membrane surface while the ribose moiety was phosphorylated in the process of being transported across the membrane. In membrane vesicles from polyoma-transformed hamster kidney cells two distinct mechanisms for inosine uptake were found; carrier-mediated diffusion as well as group translocation [9]. Cohen and Martin [10] also observed the existence of both purine nucleoside phosphorylase-dependent and -independent transport systems for human fibroblasts. That laboratory also found that another cell type, a mouse lymphoma line (S49), possessed only a broad spectrum carrier system for purine nucleosides and bases independent of any phosphorylase or transferase action, thus indicating that distinct mechanisms occur in different cell lines [11]. A similar single saturable carrier system had been previously described for human erythrocytes and rabbit polymorphonuclear leukocytes [12,13].

In order to investigate whether the above-described differences in the uptake of purines are attributable only to the specific cell types studied or also to the experimental methods employed for such studies, we have compared transport of hypoxanthine and inosine in membrane vesicles from CHO and Balb 3T3 and SV3T3 cells. In the present study, we have repeated our earlier observations indicating a close involvement (e.g. group translocation) of purine nucleoside phosphorylase and hypoxanthine phosphoribosyltransferase in the uptake of inosine and hypoxanthine, respectively, by 3T3 membrane vesicles and, un-

der identical experimental conditions, have observed that vesicles from CHO cells take up these substrates only via a carrier-mediated diffusion process. Since this observation with CHO cell vesicles is in complete accord with the mechanism postulated by others for that cell line we conclude that cell type differences are responsible for the different mechanisms of hypoxanthine and inosine uptake reported in various laboratories [4—7].

Materials and Methods

Growth of cells. Chinese hamster ovary K1 cells [14] (abbreviated CHO), the CHO mutant $Aza^r ts$ [25], the A31 clone of Balb/c 3T3 [15] and SV813, an SV40 virus-transformed line of Balb/c 3T3 A31 [16], were grown in 720-cm^2 roller bottles at 37°C in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine and 10% calf serum (final concentration). The medium used for CHO cells was also supplemented with 0.01% proline.

Harvesting of cells. Confluent cultures of cells were harvested as described by Hochstadt et al. [17]. Cells were removed from roller bottles with a rubber scraper into phosphate-buffered saline (50 mM phosphate buffer (pH 7.5)/1 mM $MgCl_2$/5 mM KCl/0.15 M NaCl). Cell harvesting and subsequent centrifugation steps were performed at 2—4°C. The method of membrane vesicle preparation was essentially the same as that described earlier [8,17]. The purity of the plasma membrane fraction was assessed by marker enzyme assays. The mixed population of vesicles (used in uptake studies) for both CHO and Balb 3T3 were found to be free from the mitochondrial enzyme succinic dehydrogenase and had 6—7-fold more 5'-nucleotidase specific activity (plasma membrane marker) than the intact cells, typical of our preparations described previously in detail [8,17].

Transport assay. Transport assays were performed essentially as described earlier [8,17]. The 100-μl reaction mixtures contained 100—150 μg of membrane protein. Details of various additions in the transport assays are described in figure and table legends. In a typical transport assay 100 mM sucrose, 50 mM potassium phosphate buffer (pH 7.5) and membrane vesicles were preincubated at 37°C for 5—10 min. Originally phosphate was included merely as a physiological buffering agent [8]. It was then discovered that it was necessary to permit intravesicular accumulation of radioactivity from inosine in SV3T3 [8]; it was then added in all subsequent experiments to permit operation of the nucleoside phosphorylase system where and when present. Prior experiments [8,34] have already shown that when we inhibit inosine phosphorylase in the medium by reversing the reaction in the direction of the nucleoside synthesis, by addition of ribose-1-*P* no effect on the uptake was noted. The reaction was initiated by the addition of labelled substrate as indicated in the legends. Reactions were terminated at indicated times by dilution with 20 vol. 0.8 M NaCl (2—4°C) and the reaction mixture was then immediately poured over nitrocellulose filters, washed twice with cold 0.8 M NaCl. Membrane vesicles were collected on 0.3-μm Millipore filter discs. The total filtration and washing procedure for an individual sample did not take more than 10—15 s. The washing procedure was slightly modified from that previously reported [8,18] since we found that radioactivity was best retained when the 0.8 M NaCl stop solution was at

2—4°C. The radioactivity retained on the filter discs was measured in a liquid scintillation counter. In modifying the filtration technique we inquired into the several parameters of temperature, time of filtration, salt concentration, etc. While these inquiries indicated that as much as 25% of vesicle contents could have been lost by the original protocol, we could not detect any significant alterations in the ratio of vesicle contents to each other. Thus, while the new method improves total values to possibly indicate rates increased by up to 25% no basic interpretation is altered on this basis.

Chromatographic analysis of vesicle contents. The nitrocellulose filters from a transport assay were treated with boiling water as described previously [17, 19] to elute the intravesicular radioactivity. It had been previously determined that the boiling water did not alter product composition and that almost all the radioactivity (75—95%) was recovered from filters. The eluted intravesicular transport products were separated chromatographically on Eastman 13254 cellulose thin-layer sheets using solvent systems previously described [17].

Assay for hypoxanthine phosphoribosyltransferase activity. Hypoxanthine phosphoribosyltransferase activity in membrane vesicles from CHO and Balb 3T3 cells was assayed by a previously described procedure [20]. Briefly, this method involved incubation of [^{14}C]hypoxanthine with the membrane vesicles in the presence of *P*Rib-*PP* and Mg^{2+}. The reaction was terminated by the addition of K · EDTA to a final concentration of 100 mM or by boiling. Conversion of hypoxanthine to IMP or inosine was monitored by chromatographic separation as described above.

Protein determination. Protein concentration was determined by the method of Lowry et al. [21], using bovine serum albumin as a standard.

Results

Hypoxanthine uptake in CHO and Balb 3T3 membrane vesicles. Membrane vesicles isolated from Balb 3T3 and CHO cells by identical procedures were found to transport hypoxanthine. However, both the extent of accumulation and the intracellular products differed in the membrane vesicle preparations from these two cell types. The level of hypoxanthine in CHO vesicles at steady-state distribution was about 330 pmol/mg membrane protein, as compared to 65 pmol/mg membrane protein in the vesicles from Balb 3T3 cells (Fig. 1). This would represent an intravesicular concentration of 80 μM and 20 μM for CHO and 3T3, respectively, in contrast to 84 μM in the medium initially, if lumen volume is within the range observed for all prior rodent cell lines [8,9,34]. The low rate in 3T3, a growth-controlled cell line may reflect that most vesicles from confluent cells were not transporting. Since *P*Rib-*PP* has been shown to enhance hypoxanthine uptake in both bacterial membrane vesicles [3,22] and Balb 3T3 membrane vesicles [1,2], the uptake of hypoxanthine was studied in the presence of *P*Rib-*PP* in these vesicles. It was found that there was a minimum of a 3-fold stimulation in hypoxanthine uptake in Balb 3T3 vesicles (200 pmol/mg protein) and a small decrease in hypoxanthine accumulation with *P*Rib-*PP* in CHO membrane vesicles (Fig. 1A and B).

The apparent K_m and V values for hypoxanthine uptake were measured in CHO and Balb 3T3 vesicles. The average values obtained in 3 experiments in CHO

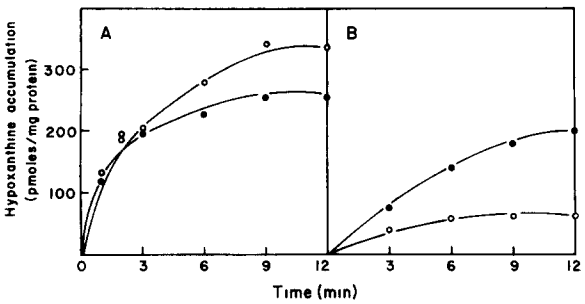

Fig. 1. Hypoxanthine uptake in CHO and Balb 3T3 membrane vesicles. The 100 μl transport assay mixtures contained 150 mM sucrose/50 mM potassium phosphate buffer (pH 7.5), and 100—150 μg membrane protein. To some assay tubes, *P*Rib-*PP* was added (500 μM) prior to preincubation. The reaction was initiated by the addition of 84 μM [8-^{14}C]hypoxanthine (59.5 mCi/mmol). ○, hypoxanthine uptake without *P*Rib-*PP*; ●, hypoxanthine uptake with *P*Rib-*PP*. (A) CHO membrane vesicles, (B) Balb 3T3 membrane vesicles.

were K_m 25 μM; *V* 333 μmol/min per mg protein; in Balb 3T3, in the presence of 0.5 mM *P*Rib-*PP* K_m 18 μM, *V* 357 μmol/min per mg protein (Fig. 3A, B). mM *P*Rib-*PP* K_m 18 μM, *V* 357 μmol/min per mg protein (Fig. 2A, B). The intravesicular content of hypoxanthine accumulated by CHO vesicles did not exceed the external concentration of hypoxanthine. In the course of three separate experiments using Balb 3T3 without adding *P*Rib-*PP* it was not possible to determine a K_m value.

Intravesicular products of hypoxanthine uptake. Various intravesicular products were identified during hypoxanthine uptake as described in Materials and Methods. In CHO vesicles the only intravesicular product which accumulated during hypoxanthine uptake in the presence or absence of *P*Rib-*PP* was hypoxanthine (Fig. 3A). In contrast, in the Balb 3T3 vesicles, the main intravesicular product of hypoxanthine uptake in the presence of *P*Rib-*PP* was identified as IMP (Fig. 3B). Intravesicular hypoxanthine was always also present. Thus, it appears that only the *P*Rib-*PP* stimulated increment of hypoxanthine taken up

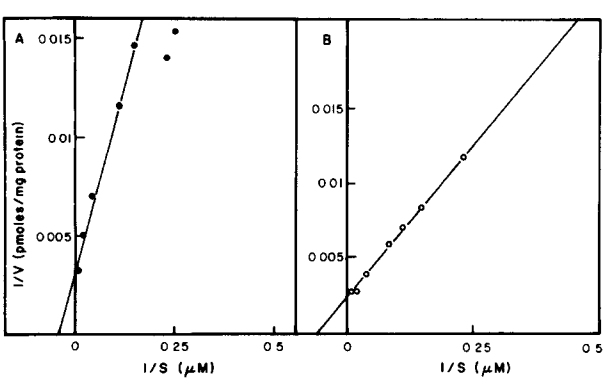

Fig. 2. Kinetics of hypoxanthine uptake. The reaction mixture composition was the same as described for Fig. 1. The reactions were initiated with [8-^{14}C]hypoxanthine (59.5 mCi/mmol) at concentrations from 2 to 50 μM. Control and experimental reactions were terminated at 0 and 1 min, respectively; membranes were collected and retained radioactivity was counted as described in Materials and Methods. The figure represents a double reciprocal plot of uptake as a function of hypoxanthine concentration. (A) CHO vesicles, ●; (B) Balb 3T3 vesicles in the presence of 500 μM *P*Rib-*PP*, ○.

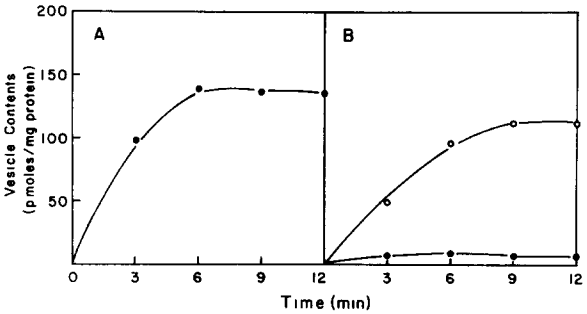

Fig. 3. Intravesicular products of hypoxanthine uptake by CHO and Balb 3T3 membrane vesicles. Intravesicular products during hypoxanthine uptake were identified chromatographically as described in Materials and Methods. (A) CHO membrane vesicles. (B) Balb 3T3 membrane vesicles. •, radioactivity accumulated as hypoxanthine with *P*Rib-*PP*; ○, radioactivity accumulated as inosine monophosphate with *P*Rib-*PP*.

accumulates as IMP. The concentration of IMP was found to increase with time (Fig. 3B). The difference in uptake level between Fig. 1 and Fig. 3 is reflected in that both 3T3 and CHO reactions contained *P*Rib-*PP* and thus the CHO level is to be compared with the lower curve on Fig. 1. Further recovery of material from the filter was 75% in the experiment. However, this has been found to be representative of vesicle contents (see Materials and Methods and Refs. 8, 9 and 34).

Exchange of intravesicular products. In CHO vesicles, the exchange of accumulated hypoxanthine can be induced by the addition of external unlabelled hypoxanthine (1 mM) to the vesicles. Almost all of the accumulated labelled hypoxanthine (85%) can be exchanged with the exogenous hypoxanthine, if added during the steady-state phase of hypoxanthine uptake (Fig. 4A). The

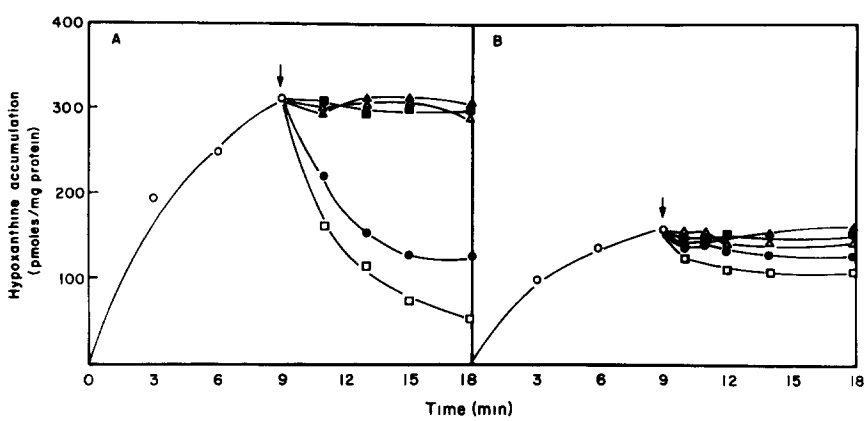

Fig. 4. Exchange of intravesicular products during hypoxanthine uptake by CHO and Balb 3T3 vesicles. The reaction mixture was the same as that described for Fig. 1. The reaction was initiated with 84 μM [8-^{14}C]hypoxanthine (59.5 mCi/mmol). At the time indicated by the arrow, 1 mM hypoxanthine, adenine, guanine, uridine or inosine was added to the reaction mixtures. At the indicated times reactions were terminated, membranes collected and the retained radioactivity was counted as described in Materials and Methods. (A) CHO vesicles, (B) Balb 3T3 vesicles; □, hypoxanthine; △, adenine; •, guanine; ■, inosine; ▲, uridine.

addition of guanine (1 mM) during hypoxanthine uptake could also result in an exchange with the accumulated hypoxanthine which was nearly the same in extent as that obtained with nonradioactive hypoxanthine (Fig. 4A). Addition of the other bases and nucleosides, e.g. adenine, inosine and uridine, during hypoxanthine uptake did not significantly effect the total hypoxanthine accumulation in these vesicles (Fig. 4A). These results suggest that a common carrier may transport hypoxanthine and guanine, but the specificity of this carrier does not extend to all bases and nucleosides in CHO cells as has been suggested from work on hepatoma cell lines [23,24] and a mouse lymphoma line [11].

In contrast to CHO vesicles, the addition of unlabelled hypoxanthine (1 mM) to the reaction mixture containing Balb 3T3 membrane vesicles at a time when the steady-state level of accumulation would otherwise be observed led to an exchange of only 25—30% of the accumulated hypoxanthine (Fig. 4B). Other nucleosides and bases tested showed even less capacity to exchange with the products of hypoxanthine accumulation inosine, 4%; uridine, 2%; guanine, 18%; adenine, 12%. These findings are consistent with our intravesicular product results which indicated that Balb 3T3 vesicles contained primarily IMP while CHO vesicles contained only hypoxanthine. The extent of exchange is also consistent with the amount of hypoxanthine that accumulates in the absence of *P*Rib-*PP* (Fig. 1B). The observation that exchangeable hypoxanthine does not become 'trapped' as IMP even in the presence of an operant hypoxanthine phosphoribosyltransferase and *P*Rib-*PP* suggests that this internal hypoxanthine is not accessible to the phosphoribosyltransferase in 3T3. This also suggests that the *P*Rib-*PP*-stimulated increment of hypoxanthine uptake may represent hypoxanthine that is converted to IMP during entry.

Hypoxanthine uptake in a CHO mutant, temperature-sensitive for azaguanine sensitivity. Harris and Whitmore [25] isolated a CHO mutant cell line (Aza^{r}ts) temperature-sensitive for the uptake of hypoxanthine and other purine bases. Hypoxanthine phosphoribosyltransferase activity in extracts of these cells did not exhibit temperature-sensitive behavior. On the basis of the above data, the authors concluded that hypoxanthine phosphoribosyltransferase did not mediate a group translocation of hypoxanthine. Further, the authors [25] determined that in these cells the K_m for hypoxanthine uptake varied with the temperature. Since the data we presented earlier in this paper for CHO vesicles (Figs. 1—4) confirm the hypothesis proposed by the intact cell studies of others [23,24] with respect to hypoxanthine uptake mechanism, we wished to ascertain whether the vesicles and the cell assay would concur in a more subtle aspect, the temperature-sensitive K_m change in Aza^{r}ts. Membrane vesicles were isolated from the Aza^{r}ts cells grown at permissive and nonpermissive temperatures. Experiments similar to those presented in Figs. 1—4 indicated that both vesicle preparations demonstrated only carrier-mediated diffusion of hypoxanthine. When the hypoxanthine-uptake kinetics of vesicles prepared from Aza^{r}ts grown at the two temperatures were compared, it was found that the temperature-sensitive change in K_m value for hypoxanthine uptake observed in intact cells could also be demonstrated in these membrane vesicles (Fig. 5A and B). This demonstration that the characteristics of a temperature-sensitive mutant are observed in its membrane vesicles indicates that Aza^{r}ts cells may have a mis-

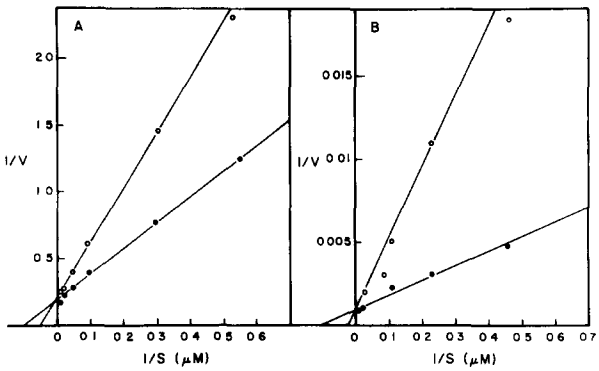

Fig. 5. Lineweaver-Burk plot of hypoxanthine uptake by Aza^{r}ts cells and its membrane vesicles at 33°C (●) and 37°C (○). The cells were grown at different temperatures (33°C and 37°C) and membrane vesicles were isolated from them. Cells and the vesicles isolated from them were assayed at the growth temperature of the cells. The reaction mixture and assay procedure were the same as described for Figs. 1 and 2. (A) CHO cells grown at the two temperatures, (B) vesicles isolated from cells grown at the two temperatures.

sense mutation in the hypoxanthine carrier and may be a particularly useful cell line for further analysis and isolation of this carrier. It further indicates that the vesicle system is a useful tool in transport mechanism studies.

Uptake of inosine in CHO membrane vesicles. Fig. 6A shows the course of uptake when inosine was used as substrate for CHO membrane vesicles. The

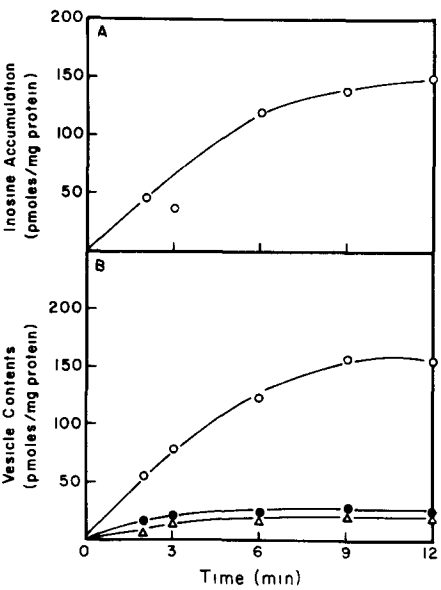

Fig. 6. Transport and intravesicular products of inosine uptake in CHO membrane vesicles. The reaction mixture was the same as that described for Fig. 1. The reaction was initiated with 40 μM of [U-^{14}C]-inosine (45.8 mCi/mmol). The intravesicular products were identified chromatographically, as described in Materials and Methods. (A) Uptake of inosine (○) (B) Intravesicular product(s) of inosine uptake: ●, hypoxanthine; △, Rib1*P*; ○, inosine.

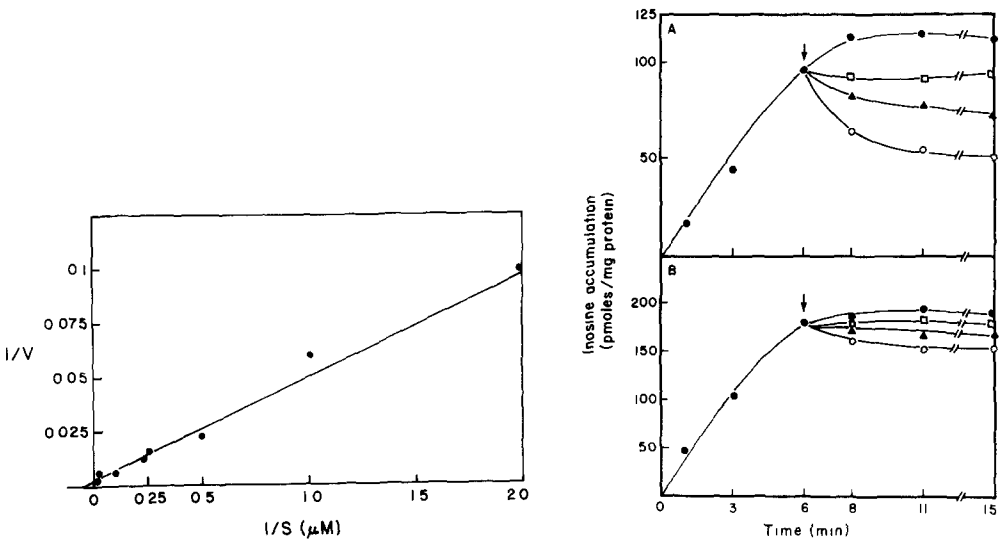

Fig. 7. Lineweaver-Burk plot of inosine uptake in CHO membrane vesicles. The reaction mixture composition was the same as that described for Fig. 1. The reactions were initiated with [U-^{14}C]inosine (45.8 mCi/mmol) at the concentrations indicated.

Fig. 8. Exchange of intravesicular products during inosine uptake by the membrane vesicles. The reaction mixture was the same as that described for Fig. 1. The reaction was initiated with 40 μM [U-^{14}C]-inosine (45.8 mCi/mmol). At the time indicated by the arrow, 1 mM of (○) inosine, (▲) uridine, (□) adenine or (●) nothing was added to the reaction mixtures. At the indicated times the reactions were terminated, membranes collected and radioactivity counted. (A) CHO vesicles; (B) SV 3T3 vesicles.

total accumulation was about 150 pmol/mg protein. The uptake of inosine in CHO vesicles demonstrated Michaelis-Menten kinetics. K_m (for ionosine) = 18 μM and V = 400 pmol/min per mg membrane protein (Fig. 7). When membrane vesicle contents were analyzed for their intravesicular products during inosine uptake, it was observed that virtually all of the accumulated inosine could be recovered as free inosine. There were only very low intravesicular levels of hypoxanthine or Rib1*P* (Fig. 6B). These results with CHO vesicles were totally different from our observations for inosine uptake in vesicles from several mouse fibroblast cell lines [8,18]. Membrane vesicles from SV3T3 cells showed intravesicular accumulation of Rib1*P* and a very small amount of free hypoxanthine, with no detectable inosine [8]. When experiments with SV3T3 vesicles were performed in parallel to the above CHO experiments in order to control all parameters of the assay, the divergent results described above were again obtained (data not shown). These experiments show that under identical experimental conditions vesicles from CHO cells take up inosine without metabolic conversion before, during or after transport while SV3T3 vesicles exhibit purine nucleoside phosphorylase-coupled inosine uptake.

Exchange of intravesicular transport product(s) of inosine uptake in CHO membrane vesicles. The exchange of accumulated inosine from CHO membrane vesicles could be induced by extravesicular inosine (1 mM) but not by adenine. About 50% of the intravesicular inosine could be exchanged with unlabelled inosine added during the course of uptake (Fig. 8A). Still more exchange could

be demonstrated if 2 mM inosine was used (data not shown). Uridine has earlier been shown to exchange with the inosine taken up by human fibroblasts [10]. In CHO vesicles uridine could induce exchange with intravesicular inosine, however, the extent of efflux was only 25—35%. These exchange results were very different for SV3T3 vesicles, in which unlabelled inosine did not exchange with the uptake products of labelled inosine (Fig. 8B). Uridine and adenine also did not affect the intravesicular accumulation of inosine in SV3T3 vesicles (Fig. 8B).

These results again indicate that CHO and SV3T3 differ in inosine-uptake mechanism. Our exchange data further demonstrate that the carrier for inosine in CHO vesicles appears to have slightly less specificity than the purine nucleoside phosphorylase mechanism which accepts only inosine, which, while it would not be expected to exchange, has been shown in our prior studies not to utilize other substrates [8,18,34].

Discussion

Some controversy has arisen over the mechanism of purine nucleoside and base transport in animal cells. Three mechanisms have been suggested: (1) Non-mediated diffusion [26]. (2) Facilitated diffusion (mediated) [2,6,27]. (3) Group translocation [2,8,10]. Several cell lines have been studied using experimental techniques that have included measurement of uptake by membrane vesicles [2,8,17,28—30] and by intact cells, both with [23,24] and without [4,5] rapid sampling procedures.

The interpretation of the results of studies of transport by intact cells is often complicated by the possibilities of subsequent intracellular metabolism of transport substrates. Experiments utilizing rapid sampling techniques minimize this problem, but present technical difficulties with cell lines that require attachment to a substratum. Studies with transport-competent membrane vesicles avoid the problem of post-transport metabolism, but the ability of these vesicles to accurately portray in vivo processes has been questioned [31]. The results presented in this paper indicate: (a) that membrane vesicle transport studies provide data that concurs with that obtained in studies of intact cells of the same cell line; and (b) that the mechanisms of purine base and nucleoside transport are cell-line-specific.

Support for the validity of the use of vesicles as a probe for transport mechanism derives from the concordance of our finding that CHO cell membrane vesicles transport inosine and hypoxanthine via facilitated diffusion, with the results of others who have reported the same mechanism for intact CHO cells on the basis of rapid sampling experiments [6,7]. Furthermore, our observation that membrane vesicles prepared from Aza^rts cells display temperature-sensitive changes in the kinetic constants of hypoxanthine transport similar to those observed in the intact cells also strongly suggests that transport in membrane vesicles accurately reflects in vivo behavior. Finally, an additional observation relevant to this question was made in studies with the TGN-1 cell line, a thioguanine-resistant mutant cell line with an electrophoretically altered hypoxanthine phosphoribosyltransferase that was derived from an SV813 cell line [16]. TGN-1 cells are deficient in hypoxanthine uptake, and we have found that this

deficiency is also demonstrable in membrane vesicles prepared from them (Hochstadt, J. et al., unpublished results).

On the question of transport mechanisms, we had earlier reported a hypoxanthine phosphoribosyltransferase-dependent hypoxanthine transport system in the membrane vesicles of Balb 3T3, possibly suggesting a group translocation mechanism. Several reports have appeared in which hypoxanthine phosphoribosyltransferase-deficient cell lines failed to exhibit saturable hypoxanthine uptake, thus corroborating this mechanism for purine base uptake [32,33]. However, in recent reports, carrier-mediated facilitated diffusion was indicated as the only mechanism of purine base uptake in Chinese hamster ovary and lung cells and in Novikoff hepatoma cells [4—7]. The results we report here confirm and extend our earlier observations indicating a hypoxanthine phosphoribosyltransferase-mediated involvement, possibly a group translocation, transport mechanism for hypoxanthine in Balb 3T3 vesicles; it further demonstrates that this base enters CHO cell vesicles only via facilitated diffusion. Thus, any apparent discrepancy between our earlier results and those of others [6,7] appears to resolve when the same cell lines are studied. We, thus, conclude that different cell lines have different mechanisms for hypoxanthine uptake.

In our studies of mechanisms of hypoxanthine uptake in Balb 3T3, it is possible that differential trapping of hypoxanthine phosphoribosyltransferase within the vesicles during their preparation may account for the *P*Rib-*PP*-mediated stimulation. Extracts of Balb 3T3 cells and CHO cells both have hypoxanthine phosphoribosyltransferase activity (4.8 nmol/mg per min in CHO, 6.7 nmol/mg per min in 3T3) but this activity is measurable in membrane vesicles only in the Balb 3T3 preparation (less than 0.1 nmol/mg per min in CHO, 4.4 nmol/mg per min in 3T3). It is unlikely, however, that its presence is due to trapping, because the preparation procedure used lyses and reseals these vesicles twice in large volumes of buffer, and was identical for both cell lines. Furthermore, our data indicate that the presence of hypoxanthine phosphoribosyltransferase in membrane vesicles is not a sufficient condition to cause *P*Rib-*PP*-stimulatable hypoxanthine uptake. Vesicles prepared from L cells [34], SV3T3 cells [8,18], and Balb 3T3 cells [1,2] contain comparable hypoxanthine phosphoribosyltransferase activity, yet only the Balb 3T3 vesicles demonstrate hypoxanthine phosphoribosyltransferase-mediated uptake of hypoxanthine. The hypoxanthine phosphoribosyltransferase activity associated with purified L cell and 3T3 membranes (Balb/c and SV813) appears not to be merely contaminating activity since it remained with the membranes; uridine kinase [8,18,34], on the other hand, which we monitored as a cytoplasmic enzyme marker, was absent from the membrane preparations [19]. Further, the exchange data presented for 3T3 vesicles in this paper indicates that the portion of hypoxanthine taken up in the presence of *P*Rib-*PP* that was exchangeable (25—30%, Fig. 4B) is equal to the amount of hypoxanthine taken up and recovered as free hypoxanthine (30% of the *P*Rib-*PP*-stimulated rate, Fig. 1). Thus, even with an operant mechanism to convert internal hypoxanthine to IMP, that fraction remains exchangeable. The observation that the hypoxanthine phosphoribosyltransferase enzyme activity is much in excess of the transport rate may reflect both presence of unsealed vesicles as well as nonvectorial processes [2,34] demonstrated for bacteria [35].

Our experiments with inosine also indicate cell-line-specificity in uptake mechanisms. We had previously reported [2,8] that in SV3T3 membrane vesicles inosine is transported via a group translocation process during which it is cleaved by purine nucleoside phosphorylase to Rib1*P* as it enters the vesicle, while hypoxanthine is released extravesicularly. Similar results were subsequently obtained with vesicles from L_{929} cells [34] and Balb 3T3 cells [1]. Membrane vesicles from BHK cells were found to transport inosine via both purine nucleoside phosphorylase-mediated and facilitated diffusion processes [9]. Cohen and Martin [10] demonstrated the same duality of mechanisms in intact human fibroblasts. That laboratory also studied a mouse cell line which exhibited a totally distinct broad spectrum-facilitated carrier mechanism independent of purine nucleoside phosphorylase action [11]. Facilitated diffusion was also the only nucleoside-uptake mechanism reported for intact CHO and Novikoff rat hepatoma cells in a study employing rapid sampling transport assays [6,7].

In the present study, in which membrane vesicles from CHO and SV3T3 cells were prepared and assayed under identical conditions, we observed different transport mechanisms in the two cell types: one of purine nucleoside phosphorylase-mediated translocation of inosine into SV3T3 vesicles; and a facilitated diffusion mechanism in vesicles from CHO cells. This latter observation is in agreement with the results of studies performed with intact CHO cells [23,24]. Thus, as with hypoxanthine, the mechanism of uptake of inosine varies with the cell line under study.

While these studies do not definitively rule out that some or perhaps even all of the phosphorylated compounds accumulating in 3T3 vesicles were metabolized after rather than during transport, they clearly indicate that very definite differences in the observations are due to the cell types involved and not merely the procedures used.

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References

1 Quinlan, D.C. and Hochstadt, J. (1974) Fed. Proc. 33, 772
2 Hochstadt, J. and Quinlan, D.C. (1976) J. Cell. Physiol. 89, 839—852
3 Jackman, E.L. and Hochstadt, J. (1976) J. Bacteriol. 126, 312—326
4 Berlin, R.D. (1973) J. Biol. Chem. 248, 4724—4730
5 Alford, B.L. and Barnes, E.M. (1976) J. Biol. Chem. 251, 4823—4827
6 Zylka, J.M. and Plagemann, P.G.W. (1975) J. Biol. Chem. 250, 5756—5762
7 Wohlhueter, R.M., Marz, R. and Plagemann, P.G.W. (1978) J. Membr. Biol. 42, 247—264
8 Quinlan, D.C. and Hochstadt, J. (1976) J. Biol. Chem. 251, 344—354
9 Dowd, D.J., Quinlan, D.C. and Hochstadt, J. (1977) Biochemistry 16, 4526—4532
10 Cohen, A. and Martin, D.W., Jr. (1977) J. Biol. Chem. 252, 4428—4430
11 Ullman, B., Gudas, J.L., Clift, M.S. and Martin, D.W., Jr. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1074—1078
12 Oliver, J.M. and Paterson, A.R.P. (1971) Can. J. Biochem. 49, 262—268

13 Taube, R.A. and Berlin, R.D. (1972) Biochim. Biophys. Acta 225, 6—18
14 Puck, T.T. and Kao, F.T. (1968) Proc. Natl. Acad. Sci. U.S.A. 60, 561—568
15 Aaronson, A.S. and Todaro, G.J. (1968) J. Cell Physiol. 72, 141—148
16 Jha, K.K. and Ozer, H.L. (1976) Somat. Cell Genet. 2, 215—223
17 Hochstadt, J., Quinlan, D.C., Rader, R., Li, C.C. and Dowd, D. (1975) in Methods in Membrane Biology (Korn, E.D., ed.), Vol. 5, pp. 117—162, Plenum Press, New York
18 Quinlan, D.C., Li, C.C. and Hochstadt, J. (1976) J. Supramol. Struct. 4, 427—439
19 Quinlan, D.C. and Hochstadt, J. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 5000—5003
20 Hochstadt-Ozer, J. and Stadtman, E.R. (1971) J. Biol. Chem. 240, 5294—5303
21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
22 Hochstadt-Ozer, J. and Stadtman, E.R. (1971) J. Biol. Chem. 246, 5304—5311
23 Plagemann, P.G.W., Martz, R. and Erbe, J. (1976) J. Cell Physiol. 89, 1—18
24 Plagemann, P.G.W., Martz, R. and Wohlhueter, R.M. (1978) J. Cell Physiol. 97, 49—72
25 Harris, J.F. and Whitemore, G.F. (1974) J. Cell Physiol. 83, 43—52
26 Jacques, J.A. and Ginsberg, F. (1960) Proc. Soc. Exp. Biol. Med. 105, 478—480
27 Berlin, R.D. (1970) Science 168, 1539—1545
28 Lever, J.E. (1976) J. Cell Physiol. 89, 779—787
29 Lever, J.E. (1977) J. Supramol. Strict. 6, 103—124
30 Lever, J.E. (1977) J. Biol. Chem. 252, 1990—1997
31 Koren, R., Shohami, E., Bibo, O. and Stein, W.D. (1978) FEBS Lett. 86, 71—75
32 Benke, D.J., Herrick, N. and Herbert, A. (1973) Biochem. Med. 8, 308—315
33 Epstein, J. and Littlefield, J.W. (1977) Exptl. Cell Res. 106, 247—251
34 Li, C.C. and Hochstadt, J. (1976) J. Biol. Chem. 251, 1181—1187
35 Kaback, H.R. (1970) Annu. Rev. Biochem. 39, 544