Sodium-dependent co-transported analogues of glucose stimulate ornithine decarboxylase mRNA expression in LLC-PK₁ cells

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Non-metabolizable analogues of glucose, including 1-*O*-methyl α -D-glucopyranoside (α MDG), that are co-transported with Na⁺ increase the specific activity of ornithine decarboxylase (ODC) in LLC-PK₁ cells [Lundgren and Vacca (1990) *Am. J. Physiol.* **259**, C647–C653]. The present study examines the effect of α MDG on LLC-PK₁-cell ODC mRNA expression. The relative concentration of ODC mRNA in cells incubated in Earle's balanced salts solution minus glucose (EBSS–G) plus 3 mM α MDG was 5–6-fold higher than the concentration of ODC mRNA in cells incubated in either EBSS–G alone or in EBSS–G plus 3 mM 3-*O*-methyl-D-glucopyranose, a non-metabolizable analogue of glucose that is taken up by a passive carrier-mediated glucose transporter. Actinomycin D and cycloheximide completely blocked the increase in ODC activity induced by α MDG. Actinomycin D was also a potent inhibitor of ODC mRNA expression by α MDG. Cycloheximide had very

little effect on the ability of this sugar to increase ODC mRNA. The relative concentration of ODC mRNA increased as a function of the incubation time in EBSS–G plus α MDG. The amount of ODC mRNA also increased as a function of the concentration of α MDG in EBSS–G. The addition of phlorizin (100 μ M) to EBSS–G prevented α MDG from increasing ODC mRNA in LLC-PK₁ cells. Phlorizin did not prevent phorbol 12-myristate 13-acetate (PMA) from enhancing LLC-PK₁-cell ODC mRNA expression. The positive effect of both α MDG and TPA on ODC mRNA expression was suppressed when cells were incubated in hypertonic EBSS–G. From these results it is suggested that the uptake of Na⁺-dependent co-transported sugars increase ODC activity by enhancing ODC gene transcription and that this process may be dependent on cell volume expansion.

INTRODUCTION

It has been recognized for a number of years that the addition of amino acids to tissue-culture media increases the specific activity of ornithine decarboxylase (ODC) [1,2], a key enzyme in the regulation of polyamine biosynthesis (for review of this system see [3]). It is now well established that Na⁺-dependent co-transported amino acids, but not amino acids that are transported by passive carrier-mediated systems, enhance ODC expression in a wide variety of different types of cultured cells, including neuroblastoma [4,5], Chinese-hamster ovary [6], rat glioma [7], rat hepatocytes [8], monkey kidney [9] and pig kidney [10]. In the absence of Na⁺-dependent co-transported amino acids, glucagon [8] and nerve growth factor or epidermal growth factor [5] were reported to be incapable of inducing ODC in their respective target cells. Collectively, the above observations raised the possibility that the uptake of certain amino acids alter the concentration and/or availability of a signal transducer(s) that is required for expression of this enzyme. This suggestion is supported by the additional observation that non-metabolizable, as well as metabolizable, Na⁺-dependent co-transported amino acids increase ODC activity [5]. The dependency on certain amino acids for the induction of this enzyme by hormones and mitogens led to the suggestion that ODC activity may be regulated, at least in part, by the uptake of Na⁺-dependent co-transported amino acids [4].

It is now recognized, at least for some types of cells, that Na⁺-dependent co-transported amino acids are not an absolute requirement for the induction of ODC. For example, Vero-cell ODC activity was shown to be induced by glucose in Earle's

balanced salts solution (EBSS) in the absence of any amino acids [9]. In the same study it was shown that the ability of glucose to increase Vero-cell ODC activity is dependent on the extracellular concentration of Na⁺, suggesting that this ion could have a role in regulating the activity of this enzyme. More recently, our laboratory examined the effect of non-metabolizable analogues of glucose on ODC activity in LLC-PK₁ cells [10], a well-characterized cell line that has both a Na⁺-dependent glucose co-transport system as well as the more prevalent passive carrier-mediated glucose-transport system (for review see [11,12]). The addition of non-metabolizable Na⁺-dependent co-transported sugars to EBSS minus glucose (EBSS–G) induced LLC-PK₁-cell ODC activity to high levels. In contrast, non-metabolizable sugars that are transported by the passive carrier-mediated glucose transport system had very little effect on enzyme activity. The above observations raised the possibility that an increase in cytosolic Na⁺, or perhaps an increase in cell volume resulting from the uptake of Na⁺-coupled co-transported substrates, might generate a signal that is required for the expression of this enzyme.

The site(s) at which the uptake of Na⁺-dependent co-transported sugars act to increase ODC activity remained to be identified. The present study examines the effect of non-metabolizable analogues of glucose on ODC mRNA levels in LLC-PK₁ cells. Results in the present paper demonstrate that the Na⁺-dependent co-transported sugar 1-*O*-methyl α -D-glucopyranoside (α MDG) increases the concentration of ODC mRNA in LLC-PK₁ cells. On the basis of results in this paper, it is suggested that the increase in ODC activity associated with compounds that are co-transported by Na⁺-coupled systems are

Abbreviations used: ODC, ornithine decarboxylase; α MDG, 1-*O*-methyl α -D-glucopyranoside; 3OMG, 3-*O*-methyl-D-glucopyranose; EBSS–G, Earle's balanced salts solution minus glucose; PMA, phorbol 12-myristate 13-acetate; SSC, 0.015 M sodium citrate/0.15 M NaCl, pH 7.0.

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dependent on an increase in ODC gene transcription, which in turn may be dependent on cell volume expansion.

MATERIALS AND METHODS

Treatment of LLC-PK₁ cells

The chemically defined tissue-culture medium, designated as DF10, was prepared as previously described [10]. For each experiment, confluent LLC-PK₁-cell stock cultures were subcultured from a Falcon T75 tissue-culture flask into 60 mm × 15 mm Falcon culture dishes (5.0 × 10⁵ cells/dish) containing 5.0 ml of DF10. Cultures were incubated at 37 °C in humidified air containing 5% CO₂ and fed with DF10 at 48 h intervals. Under these culture conditions LLC-PK₁ cells reach stationary-phase growth between 7 and 8 days [10].

Cells cultured for 10 days in DF10 were washed with 2.0 ml of EBSS-G. After the initial wash, 5.0 ml of ODC induction medium, as defined in the Results section, was added to dishes and cultures were incubated at 37 °C in humidified air containing 5% CO₂. Cells were then harvested for analysis of ODC enzyme activity and/or preparation of RNA.

ODC enzyme activity

At specified times, ODC induction media were removed from dishes and cells were rapidly washed with 2.0 ml of ice-cold phosphate-buffered saline. Each dish received 1.5 ml of ice-cold ODC buffer (50 mM Tris, 0.4 mM EDTA, 100 μM pyridoxal phosphate, 5 mM dithiothreitol, pH 7.4). Cells were removed with a rubber policeman, and then stored at -70 °C until assayed.

Frozen cell suspensions were thawed and cell homogenates were prepared as previously described [9]. A sample (0.6 ml) of cell supernatant was added to 0.35 ml of ODC buffer and preincubated for 10 min at 37 °C in a shaking water bath. The reaction was started by addition of 50 μl of L-ornithine (2.1 mM) containing 0.2 μCi of L-[1-¹⁴C]ornithine (58.6 μCi/mmol; Amersham, Arlington Heights, IL, U.S.A.). The assay was stopped after 60 min by addition of 0.5 ml of citric acid. Released ¹⁴CO₂ was trapped in Hyamine hydroxide, and radioactivity was determined as previously described [9].

Preparation of RNA

ODC induction media were removed from dishes, and 2.5 ml of RNA lysis buffer (4.0 M guanidine isothiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, and 0.1 M 2-mercapto-ethanol) was added to cell cultures. Cultures were left in the lysis buffer for 5 min at room temperature and then mixed repeatedly by pipette. Cell lysates were transferred to Falcon no. 2059 test tubes and frozen at -70 °C until preparation of total cellular RNA.

Frozen cell lysates were thawed and 2.0 ml was layered on a 2.5 μl pad of 5.7 M CsCl containing 0.1 M EDTA. The samples were centrifuged at 35000 rev./min for 15 h at 18 °C in a Beckman SW 50.1 rotor. RNA pellets were resuspended in water. A sample from each RNA sample was diluted into TE buffer (10 mM Tris/0.1 mM EDTA, pH 7.4), and the concentration of RNA was estimated spectrophotometrically as previously described [13]. Specific volumes from each sample were transferred to Microfuge tubes, followed by sufficient water to provide the same RNA concentrations throughout all tubes. RNA precipitation solution (0.1 vol. of 3 M sodium acetate, pH 5.0,

followed by 2.5 vol. of 100% ethanol) was added to each sample, which were then stored at -70 °C until used for either Northern-blot or RNA dot-blot assays.

Northern blots and dot-blots

RNA samples were pelleted from the precipitation solution by centrifugation (12000 g for 15 min at 5 °C) and then dried by vacuum. For Northern-blot analysis, as previously described in detail [14], RNA pellets were dissolved into 10 μl of denaturing solution containing ethidium bromide. Samples were electrophoresed in formaldehyde/agarose gels and RNA was transferred by capillary action to Nytran. As previously demonstrated [15], utilization of ethidium bromide in the denaturing solution provided direct evidence in these studies that: (a) approximately equal amounts of RNA from different cell cultures were applied to gels for electrophoresis, (b) RNA integrity was maintained during sample preparation and electrophoresis, and (c) the transfer of RNA from gel to filter was complete. RNA dot-blots were performed with a vacuum manifold as previously published [14]. Filters from both Northern blots and dot-blots were then incubated for 2 h at 42 °C in prehybridization buffer as previously reported [14].

The ³²P-labelled probe for ODC mRNA was generated with a random-primed labelling kit (United States Biochemical Corp., Cleveland, OH, U.S.A.). The substrate was the 0.9 kb ODC cDNA insert purified from plasmid pOD48. After the prehybridization was complete, the ³²P-labelled ODC probe was denatured with NaOH as previously described [16] and then added directly to the prehybridization buffer (1.0 × 10⁶ d.p.m./ml). Filters were incubated overnight at 42 °C. They were then washed twice in 6 × SSC buffer (SSC = 0.015 M sodium citrate/0.15 M NaCl) plus 0.5% SDS and once in 1 × SSC plus 0.1% SDS for 15 min at room temperature. The final wash was carried out in 1 × SSC plus 0.1% SDS for 30 min at 56 °C.

Autoradiography and ODC mRNA analysis

Northern and dot-blot Nytran filters were exposed to Kodak X-Omat RP film at -70 °C by using Lighting Plus intensifying screens. Autoradiographs of ³²P-labelled hybridized RNA dot-blots were scanned with a Microtek MSF-300GS Image Scanner (Microtek, Torrance, CA, U.S.A.) that was linked to a Mackintosh IISI computer. The image generated by the Microtek greyscale scanner was captured by Image Studio (software by Letraset, Paramus, NJ, U.S.A.) and then analysed for intensity of greys relative to background by Scan Analysis (software by Biosoft, Milltown, NJ, U.S.A.). Quantification of image intensities on film was carried out at less than maximal densities. As previously documented [14], the greyscale intensity of exposed film (i.e. relative ODC mRNA levels) was proportional to the amount of RNA blotted provided that between 2 and 8 μg of RNA/well was dot-blotted and/or the exposure time was stopped before reaching a density of 1.6 units relative to the background. When images from a given dot-blot were determined to be over-exposed, autoradiography was repeated by using shorter exposure times.

DNA assays and cDNA probe

Cellular DNA was quantified fluorimetrically as previously described [17] with bisbenzimidazole (Hoechst 33258), with calf thymus DNA as standard. The plasmid pOD48, which contains mouse ODC cDNA [18], was generously given by Dr. Philip Coffino.

RESULTS

Northern analysis of ODC mRNA levels in LLC-PK₁ cells

A previous study from this laboratory [10] demonstrated that the specific activity of ODC in LLC-PK₁ cells is increased by α MDG, a non-metabolizable analogue of glucose that is taken up by the Na⁺-dependent co-transport system present in this cell line [11,12]. To determine if Na⁺-dependent co-transported sugars influence the concentration of ODC mRNA in LLC-PK₁ cells, the relative amounts of ODC mRNA were examined by Northern-blot analysis in cells incubated for 6 h in EBSS-G versus EBSS-G plus 3 mM α MDG (Figure 1). The concentration of ODC mRNA from cells incubated in EBSS-G

Figure 1 Northern-blot analysis of ODC mRNA levels from LLC-PK₁ cells incubated in EBSS-G, EBSS-G plus α MDG, and EBSS-G plus 3OMG

Cells were grown for 10 days, washed with EBSS-G, and then incubated for 6 h in their respective media. Lanes 1, 4 and 7 received 10 μ g of RNA prepared from individual cultures incubated in EBSS-G. Lanes 2, 5 and 8 received 10 μ g of RNA prepared from individual cultures incubated in EBSS-G + α MDG (3 mM). Lanes 3, 6 and 9 received 10 μ g of RNA prepared from individual cultures incubated in EBSS-G + 3OMG (3 mM). ODC mRNA was run with RNA size markers ranging from 1.4 to 9.5 kb. Preparation, fractionation and visual detection of RNA were performed as described in the Materials and methods section.

Table 1 Effect of α MDG on LLC-PK₁ ODC activity and ODC mRNA concentrations with and without cycloheximide and actinomycin D

Cells were cultured for 10 days, washed with EBSS-G and then, as specified below, preincubated for 30 min in either EBSS-G or EBSS-G plus inhibitor. At zero time, preincubation media were removed from the plates and the cells were incubated for 6 h in the following media; EBSS-G (preincubated in EBSS-G) was incubated in EBSS-G; MDG (preincubated in EBSS-G) was incubated in EBSS-G plus 3 mM α MDG; MDG + ACT (preincubated in EBSS-G plus 0.8 μ g/ml actinomycin D) was incubated in EBSS-G plus 3 mM α MDG plus 0.8 μ g/ml of actinomycin D; and MDG + CYCLO (preincubated in EBSS-G plus 4.5 μ g/ml cycloheximide) was incubated in EBSS-G plus 3 mM α MDG plus 4.5 μ g/ml cycloheximide. Cells were harvested and assayed for ODC activity and for ODC mRNA after dot-blotting 5 μ g of RNA as described in the Materials and methods section. Specific activity is expressed in pmol of CO₂ formed/h per μ g of DNA (trace, less than 10 pmol/h per μ g of DNA; ND, not detected).

Medium	Specific activity	Relative ODC mRNA concentration
EBSS-G	76 $\pm$ 46	0.43 $\pm$ 0.01
MDG	435 $\pm$ 138	2.04 $\pm$ 0.16
MDG + ACT	trace	ND
MDG + CYCLO	trace	1.83 $\pm$ 0.15

Figure 2 Changes in ODC mRNA concentrations as a function of increasing concentrations of α MDG

Cells were cultured for 10 days, washed with EBSS-G, and then incubated for 6 h in EBSS-G containing the specified concentration of α MDG. Cells were harvested and assayed for ODC mRNA after dot-blotting 5 μ g of RNA as described in the Materials and methods section. Each point represents the mean $\pm$ S.D. of three replicate plates of cells.

containing α MDG (Figure 1, lanes 2, 5 and 8) was markedly higher than the ODC mRNA level characteristic of cells incubated in only EBSS-G (Figure 1, lanes 1, 4 and 7). In the same experiment, an additional set of cells was incubated for 6 h in EBSS-G plus 3 mM 3-O-methyl-D-glucopyranose (3OMG) (Figure 1, lanes 3, 6 and 9). This sugar is a non-metabolizable analogue of glucose that is transported into LLC-PK₁ cells primarily by a passive carrier-mediated glucose transporter [12]. In contrast with the positive effect of α MDG on ODC mRNA expression, the relative concentration of ODC mRNA in cells incubated with 3OMG appeared to be approximately equal to the concentration of ODC mRNA in cells incubated in only EBSS-G (Figure 1, lanes 1, 4 and 7 versus lanes 3, 6 and 9).

Effect of actinomycin D and cycloheximide on ODC mRNA levels

The observation that ODC mRNA is elevated in cells incubated with α MDG raised the possibility that the uptake of Na⁺-dependent co-transported sugars might influence the rate of ODC gene transcription. To test the above possibility, the specific activity of ODC and the relative concentrations of ODC mRNA were determined in cells incubated in EBSS-G plus α MDG with or without actinomycin D or cycloheximide (Table 1). Only trace amounts of enzyme activity were detected in cells incubated in EBSS-G plus α MDG containing either actinomycin D or cycloheximide. Similarly, actinomycin D completely prevented the increase in ODC mRNA by α MDG (Table 1). In contrast with its effect on enzyme activity, cycloheximide had very little effect on the level of ODC mRNA that results from incubating cells in EBSS-G plus α MDG.

Kinetics of ODC mRNA expression

LLC-PK₁ ODC mRNA levels were determined as a function of the concentration of α MDG in EBSS-G (Figure 2). The relative concentration of ODC mRNA increased with increasing concentrations of α MDG, with maximal expression occurring between 0.75 and 1.5 mM α MDG.

The relative concentration of ODC mRNA was also examined as a function of the incubation time in EBSS-G plus 3 mM α MDG (Figure 3). Maximal expression of ODC mRNA under these assay conditions appears to occur between 4 and 6 h.

Figure 3 Time course of appearance of ODC mRNA as a function of incubation time in EBSS—G plus α MDG

Cells were cultured for 10 days, washed with EBSS—G, and then incubated for the specified time in EBSS—G + α MDG (3 mM). Cells were harvested and assayed for ODC mRNA after dot-blotting 5 μ g of RNA as described in the Materials and methods section. Each point represents the mean $\pm$ S.D. of three replicate plates of cells.

ODC mRNA concentrations did not decline after reaching maximal levels, but remained elevated for at least 24 h, the longest time period examined.

Requirement of a functional Na^+ -dependent glucose co-transport system

Phlorizin binds to the Na^+ -dependent glucose co-transport system and prevents the uptake of Na^+ -dependent co-transported sugars [11,12]. The addition of 100 μ M phlorizin to cells incubated in EBSS—G plus 3 mM α MDG prevented the induction of ODC mRNA by this sugar (Table 2). The ability of phlorizin to prevent the increase in ODC mRNA by α MDG suggests that the effect of α MDG on ODC mRNA requires a functional Na^+ -dependent co-transport system. However, the possibility exists that the inhibitory effect of phlorizin on ODC mRNA is non-specific.

The tumour promoter phorbol 12-myristate 13-acetate (PMA) has been shown to enhance ODC mRNA synthesis in a number of biological systems [19,20]. In contrast with the inhibitory effect of phlorizin on ODC mRNA levels resulting from α MDG, the increase in ODC mRNA that results from incubating cells in EBSS—G plus PMA was not decreased by 100 μ M phlorizin (Table 2). This result supports the suggestion that a functional Na^+ -dependent glucose co-transport system is necessary for increased ODC gene transcription by α MDG.

Effect of increased extracellular osmolarity on ODC mRNA expression

The mechanism by which α MDG increases the relative concentration of ODC mRNA remains to be identified. However, the uptake of Na^+ -dependent co-transported amino acids [21] and sugars [22] has been shown to produce a transient increase in cell volume in various types of cells, followed by a regulatory volume decrease. On the basis of previous studies from this laboratory, it was suggested that ODC activity may be regulated, at least in part, by a signal transducer(s) that is generated in response to changes in cell volume [10,14]. If the increase in ODC mRNA by α MDG is dependent on an increase in cell volume, then incubation of LLC-PK₁ cells in hypertonic EBSS—G plus α MDG, as opposed to isotonic EBSS—G plus α MDG, should prevent α MDG from increasing the concentration of ODC

Table 2 Effect of phlorizin on ODC mRNA expression induced by α MDG compared with PMA

Cells were cultured for 10 days, washed with EBSS—G, and then incubated for 6 h in specified media: EBSS—G (EBSS—G); EBSS—G plus 3 mM α MDG (MDG); EBSS—G plus 3 mM α MDG plus 100 μ g/ml phlorizin (MDG + PHL); EBSS—G plus 10 μ g/ml PMA (PMA); and EBSS—G plus 10 μ g/ml PMA plus 100 μ g/ml phlorizin (PMA + PHL). Cells were harvested and assayed for ODC mRNA after dot-blotting 5 μ g of RNA as described in the Materials and methods section. Each assay represents the mean $\pm$ S.D. of three replicate plates of cells.

Medium	Relative ODC mRNA concentrations
EBSS—G	0.18 $\pm$ 0.01
MDG	1.29 $\pm$ 0.03
MDG + PHL	0.23 $\pm$ 0.23
PMA	1.15 $\pm$ 0.08
PMA + PHL	1.09 $\pm$ 0.12

Table 3 Effect of hypertonic medium on ODC mRNA expression induced by α MDG compared with PMA

Cells were cultured for 10 days, washed with EBSS—G, and then incubated for 6 h in the designated media: isotonic EBSS—G (ISO); hypertonic EBSS—G (HYPER); isotonic EBSS—G plus 3 mM α MDG (ISO + MDG); hypertonic EBSS—G plus 3 mM α MDG (HYPER + MDG); isotonic EBSS—G plus 10 μ g/ml PMA (ISO + PMA); and hypertonic EBSS—G plus 10 μ g/ml PMA (HYPER + PMA). Hypertonic media were made by addition of 58 mM sucrose to EBSS—G. Cells were harvested and assayed for ODC mRNA after dot-blotting 5 μ g of RNA as described in the Materials and methods section. Each assay represents the mean $\pm$ S.D. of three replicate plates of cells.

Medium	Relative ODC mRNA concentrations
ISO	0.11 $\pm$ 0.03
HYPER	0.08 $\pm$ 0.02
ISO + MDG	0.74 $\pm$ 0.03
HYPER + MDG	0.24 $\pm$ 0.09
ISO + PMA	0.91 $\pm$ 0.05
HYPER + PMA	0.19 $\pm$ 0.02

mRNA. The relative concentration of ODC mRNA in cells incubated in hypertonic EBSS—G plus α MDG remained at near-basal levels (Table 3). In the same experiment, the effect of hypertonic media was examined on ODC mRNA expression due to PMA. Hypertonic EBSS—G also prevented PMA from increasing ODC mRNA in LLC-PK₁ cells. These observations raise the possibility that the positive effect of both α MDG and PMA on ODC gene transcription may be dependent on cell volume expansion.

DISCUSSION

The central finding in this study is that α MDG, a non-metabolizable analogue of glucose, increases ODC mRNA expression in LLC-PK₁ cells. Furthermore, this study establishes that the previously reported positive effect of α MDG on LLC-PK₁ ODC activity [10] is dependent on increased ODC mRNA synthesis. It has been recognized for several years that amino acids that are co-transported with Na^+ increase the specific activity of ODC in a wide variety of different cell lines [4–10]. Our laboratory recently demonstrated that Na^+ -dependent co-transported sugars, when presented to the appropriate cell type, are as effective as amino acids in elevating the activity of this

enzyme [10]. The site(s) and the mechanism(s) by which Na^+ -dependent co-transported sugars act to increase ODC activity remained to be identified. Results in the present study suggest that the uptake of Na^+ -dependent co-transported sugars increase LLC-PK₁ ODC activity by influencing the rate of ODC gene transcription.

The glucose Na^+ -coupled co-transport system and the glucose passive carrier-mediated transport system of LLC-PK₁ cells have been studied in considerable detail [11,12]: α MDG is transported into LLC-PK₁ cells almost exclusively by the Na^+ -dependent co-transport system, the carrier-mediated transport system having very little affinity for this sugar. In contrast with α MDG, the nonmetabolizable sugar 3OMG, at the concentration used in the present study (3 mM), has been shown to be taken up primarily by a passive carrier-mediated glucose transport system. Thus this cell line can be used to differentiate between the effect on ODC activity of general substrate influx by a passive carrier-mediated system versus the more specific effect on ODC activity of substrates that are taken up by Na^+ -dependent co-transported systems. Northern-blot analysis of RNA isolated from LLC-PK₁ cells incubated in EBSS-G plus the above sugars revealed that α MDG, but not 3OMG, increases ODC mRNA above basal concentrations (Figure 1). The observation that phlorizin, a potent inhibitor of the glucose Na^+ -dependent co-transport system [11,12], prevented α MDG, but not PMA, from increasing the concentration of ODC mRNA (Table 2), demonstrates that a functional Na^+ -dependent co-transport system is required. This result is compatible with the previous report [10] that LLC-PK₁-cell ODC activity is enhanced by various Na^+ -dependent co-transported sugars, but not by sugars such as 3OMG that are transported by passive carrier-mediated systems.

Past studies of the effect of cycloheximide on ODC mRNA expression by different compounds have been variable and appear to depend on the cell type and/or the effector compound in question: Cycloheximide has been reported to accentuate ODC mRNA synthesis in some activated systems [23,24], to inhibit an increase in ODC mRNA synthesis in other types of cells [19], and to have a partial inhibitory effect on ODC mRNA synthesis in other activated biological systems [25]. In the present study cycloheximide completely prevented α MDG from increasing ODC activity (Table 1), suggesting that an elevation in enzyme activity by α MDG requires new protein synthesis, as opposed to decreasing the rate of ODC protein turnover. In contrast with inhibiting enzyme activity, cycloheximide had very little effect on the ability of α MDG to increase the concentration of LLC-PK₁-cell ODC mRNA. The latter result suggests that on-going protein synthesis is not a prerequisite for increased ODC gene transcription induced by α MDG, an observation that is consistent with the lack of an effect of cycloheximide on increased ODC mRNA synthesis that results in hypotonically stressed LLC-PK₁ cells [14]. Results in the present study are compatible with the suggestion that the uptake of Na^+ -dependent cotransported substrates elevate the specific activity of ODC primarily, if not totally, by increasing the concentration of ODC mRNA. However, an earlier report that examined the effect of asparagine, a Na^+ -dependent co-transported amino acid, on rat hepatocyte ODC activity provided evidence that the increase in ODC activity was due in large part to an increase in the translation of ODC mRNA, as opposed to an increased ODC gene transcription [8]. The earlier study apparently did not examine the effect of either cycloheximide or actinomycin D on the hepatocyte system, information which might allow a more direct comparison between the two biological systems.

The observation that actinomycin D completely prevents α MDG from increasing ODC mRNA (Table 1) suggests that the

uptake of this sugar enhances ODC gene transcription, as opposed to decreasing the rate of ODC mRNA turnover. Although the mechanism for elevation of ODC mRNA by α MDG remains to be characterized, it is unlikely that a non-metabolizable sugar increases the concentration of ODC mRNA directly by modifying the rate of ODC gene transcription. A more feasible explanation is that the uptake of this type of compound alters the concentration and/or availability of a recognized second messenger, and that this effector compound, directly or indirectly, enhances the transcription rate of this gene. Previous studies have shown that the concentration of a variety of second messengers is influenced by cell volume expansion (for review see [26]). It has also been reported that the uptake of Na^+ -dependent co-transported sugars such as α MDG produce a transient increase in the volume of LLC-PK₁ cells which is followed by marked changes in the content of inorganic ions related to regulatory volume decrease [22]. A recent report from our laboratory demonstrated that ODC mRNA concentrations are elevated in a variety of different types of cells, including LLC-PK₁ cells, when hypotonically stressed [14]; and, on the basis of the effect of hypotonic stress on these cells, it was suggested that a factor related to cell volume expansion amplifies ODC gene transcription. In a previous report it was demonstrated that the specific activity of ODC remained at basal levels when cells were incubated in hypertonic EBSS-G plus α MDG, even though the rate of α MDG uptake in hypertonically treated cells was comparable with the rate of α MDG uptake in isotonicity treated cells [10]. In the present study hypertonic stress prevented α MDG from increasing ODC mRNA. These observations suggest that the uptake of α MDG is not in and of itself sufficient to induce ODC, but cell volume expansion is necessary.

The suggestion that a given metabolic process may be regulated by a signal transducer(s) that is responsive to changes in cell volume is not a unique concept. Other studies, based on more direct evidence, suggests that several metabolic processes, including bile-acid-dependent bile flow and biliary taurocholate excretion in liver [27], and the control of hepatic proteolysis [21], may be regulated by cell volume expansion. It is now recognized that the uptake of Na^+ -dependent cotransported amino acids increases cell volume in intact tissue such as liver [21,27]. Interestingly, it has previously been reported that chicks fed with α -aminoisobutyric acid, a non-metabolizable Na^+ -dependent co-transported amino acid, are characterized by increased liver and renal ODC activity [28], an observation that raises the possibility of a relationship between cell volume expansion and ODC activity *in vivo*. It remains to be directly demonstrated that ODC activity is influenced by changes in cell volume. If this is eventually documented, the physiological significance, if any, will still remain to be identified. Nevertheless, it was recently reported that both cell viability and the rate of growth of cells incubated in hypotonic media is enhanced by increased concentrations of putrescine [29], the product of ornithine decarboxylation. Additional studies of the effect of cell volume on ODC activity may provide new insights into how such a variety of compounds with such diverse structures and functions, including over 60 different hormones and mitogens (for recent list of these factors see [30,31]), can influence the activity of this enzyme.

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