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STUDIES ON THE ROLE OF PROTEIN SYNTHESIS AND OF SODIUM ON THE REGULATION OF ORNITHINE DECARBOXYLASE ACTIVITY

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The minimum requirements for eliciting or enhancing ornithine decarboxylase activity (EC. 4.1.1.17); L-ornithine carboxylase) in neuroblastoma cells incubated in salts-glucose solutions have been investigated. These incubation conditions permit the study of changes in ornithine decarboxylase activity independently of the growth-associated reactions that occur in cell culture media (Chen, K.Y. and Canellakis, E.S. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3791–3795). Ornithine decarboxylase activity can be elicited by a variety of asparagine and other amino acid analogs, including α-aminoisobutyric acid, that cannot participate in protein synthesis. Of the eleven asparagine analogs tested, α-N-CH_3-DL-asparagine is the most potent in eliciting ornithine decarboxylase activity and is equivalent to asparagine in this regard. Inclusion of polar groups into the asparagine molecule results in the loss of its ability to elicit ornithine decarboxylase activity. With the use of these analogs and of analogs of other amino acids it is shown that the rapid fall in ornithine decarboxylase activity that is noted following cycloheximide treatment may not be a consequence of the inhibition of protein synthesis. The rapid fall in ornithine decarboxylase activity is primarily due to the removal of the agent that elicits and stabilizes its activity. These results, the finding that α-aminoisobutyric acid stimulates ornithine decarboxylase activity and that sodium is required for the stimulation of ornithine decarboxylase activity are discussed in relation to the 'A' amino acid transport system.

Introduction

We initiated the use of buffered salts-glucose solutions in order to determine the detailed requirements for the enhancement of ornithine decarboxylase activity in neuroblastoma cells [1]. Prominent among these has been L-asparagine. We now find that a small number of asparagine and other amino acid analogs, with specific configurations will also elicit ornithine decarboxylase activity. We showed that although cAMP alone will not elicit ornithine decarboxylase activity, it lowers the requirement for L-asparagine [1]. We now find that Na^+ is also required to elicit ornithine decarboxylase activity.

Our working hypothesis is that the rise in ornithine decarboxylase activity that follows the addition of complete growth medium to cell cultures, occurs preparatory to the initiation of subsequent metabolic reactions that are required for growth. Following the initiation of these subsequent metabolic reactions, and presumably as the

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function of ornithine decarboxylase has been fulfilled, it is likely that its activity would fall. In buffered salts-glucose solutions however, the cells cannot proceed through the next steps in the growth process; consequently we may be seeing only the prolongation of the enhancement of the ornithine decarboxylase activity without its fulfillment by the subsequent metabolic reactions that are required for growth. In keeping with our hypothesis are the very different kinetics of ornithine decarboxylase enhancement under these different conditions, as shown in Fig. 4. Whereas the bulk of the ornithine decarboxylase activity has returned to normal by 6 h, when this enhancement has been elicited by growth medium, the activity of ornithine decarboxylase remains high for 16 h when it is elicited by L-aspargine in buffered salts-glucose solutions. The following experimental presentation should be seen as an attempt to dissect those reactions that are relevant to the first stage of ornithine decarboxylase enhancement.

The ability of L-asparagine to elicit ornithine decarboxylase activity has been shown with other cell lines, both in growth media and in buffered salts-glucose solutions [2–5]. In some cells, in addition to L-asparagine, cAMP or fetal bovine serum were required for maximal enhancement of ornithine decarboxylase activity [2]. We now show that ornithine decarboxylase activity can be elicited with amino acid analogs that cannot be incorporated into protein. We also show that the rapid decline in ornithine decarboxylase activity that can be caused by cycloheximide is not a necessary consequence of the inhibition of protein synthesis.

Our results also indicate that sodium is required for the stimulation of ornithine decarboxylase activity and that α-aminoisobutyric acid, the probe for the 'A' amino acid transport system also stimulates ornithine decarboxylase activity. These findings as well as our studies on the specificity of the amino acid analogs that stimulate ornithine decarboxylase activity suggest that fluctuations in ornithine decarboxylase activity may be related to the 'A' amino acid transport system.

Materials and Methods

Materials. Dulbecco's modified Eagle's minimal medium, fetal bovine serum and L-asparagine were from GIBCO, Grand Island, N.Y. Except for α-*N*-methyl-DL-asparagine [6], the asparagine analogs were synthesized [7] and generously provided by Drs. P.K. Chang and R.E. Handschumacher of this department. The α-amino acids and their α-*N*-methylated analogs were obtained from Sigma Chemical Comp., St. Louis, MO, except for α-*N*-methyl-L-valine (Cyclo Chemical, Los Angeles, CA). α-Aminoisobutyric acid was obtained from Calbiochem La Jolla, CA., cycloheximide from Sigma, St. Louis, MO.; DL-[1-^{14}C]ornithine (55 mCi/mmole) from Moravek Biochemicals, City of Industry, CA. L-[U-^{14}C]Leucine (270 mCi/mmole, [2-^{14}C]uridine (50 mCi/mmol) and [*methyl*-^{3}H]thymidine (2 Ci/mmol) from New England Nuclear, Boston, MA; salts-glucose solution was purchased from Flow Laboratories, Rockville, MD as Earle's balanced salts solution (1.2 g $CaCl_2$, 0.4 g KCL, 0.2 g $MgSO_4 \cdot 7\ H_2O$), 6.8 g NaCl, 2.2 g $NaHCO_3$, 0.14 g $NaH_2PO_4 \cdot H_2O$ and 1.0 g glucose per liter). Isotonic and hypotonic salts-glucose solution contained 116 mM and 58 mM NaCl, respectively. The Na^+-free salts-glucose solution was prepared by replacing the NaCl, $NaHCO_3$, and $NaH_2PO_4 \cdot H_{20}$ with iso-osmolar amounts of choline chloride, $KHCO_3$ and KH_2PO_4, respectively. Reagent grade chemicals were used throughout.

In experiments that we have performed but do not detail, it was found that either Ca^{2+} or Mg^{2+} are sufficient to elicit ornithine decarboxylase activity. The maintenance of ornithine decarboxylase activity over prolonged periods of time is optimal, however, in the presence of 1.8 mM Ca^{2+} and 0.16 mM Mg^{2+}. Earle's balanced salts solution, designated as salts-glucose solution, was used because it approximates the optimal Ca^{2+}, Mg^{2+} concentration.

Cell culture. Mouse C1300, clone N18 neuroblastoma cells ($8 \cdot 10^5$) [8] were subcultured into 60 × 15 mm Falcon tissue culture dishes containing 5 ml Dulbecco's modified Eagle's minimal medium supplemented with 10% fetal bovine serum and incubated at 37°C in 95% air/5% CO_2. Growth medium was removed, the cultures were washed twice with isotonic 37°C salts-glucose solution and then incubated with the appropriate 37°C solution. Confluent 4-day-old cultures were used for the ornithine decarboxylase activity studies unless

indicated otherwise. The cultures were periodically tested for the presence of mycoplasma and were found to be uncontaminated [9].

Cell viability. In the variety of experimental conditions that were used, cell viability was found to be better than 95% of control, as determined by trypan blue exclusion as well as by the rate of incorporation of radioactive leucine, uridine or thymidine.

Assays. Ornithine decarboxylase activity was determined at 37°C at saturating substrate concentration (0.55 mM ornithine) [1,10] during periods of linear increase in ornithine decarboxylase activity. 1 unit of ornithine decarboxylase activity corresponds to 1 nmol CO_2 released per h. Protein content was determined by the Coommassie blue method described Bradford [11] using bovine serum albumin as the standard.

Measurement of protein synthesis. Cells were exposed for 30 min to L-[U-^{14}C]-leucine (0.4 uCi/ml), washed twice with 5 ml ice-cold phosphate-buffered saline and resuspended in ornithine decarboxylase assay buffer (50 mM Tris-HCl, pH 7.2 at 37°C, 0.1 mM EDTA and 50 μM pyridoxal 5-phosphate, 5 mM dithiothreitol). Portions were removed for the determination of the acid-soluble and the acid-insoluble radioactivity by liquid scintillation counting [12].

Results

The ability of asparagine analogs to elicit ornithine decarboxylase activity

A structure activity study of the ability of asparagine analogs to elicit ornithine decarboxylase activity in neuroblastoma cells maintained in a salts-glucose solution (Table I), indicated that: (a) the L-configuration of the α-NH_2 group is important but not essential, D-asparagine is minimally active; (b) a free α-amino group is not essential, the α-*N*-methyl analog is as effective as asparagine; however, the α-*N*-acetyl and the α-*N*-dinitrophenyl analogs are inactive, (c) replacement of a C-3 hydrogen with a methyl group yields threo-β-methyl-DL-asparagine which retains some activity; however, the corresponding β-hydroxy and β-amino analogs are inactive; (d) the length of the carbon backbone can be extended by one methylene group, glutamine is active; (e) the γ-amide group cannot be replaced with a carboxyl group, aspartic acid is inactive; (f) a γ-amide hydrogen can be replaced with a methyl group (γ-*N*-methyl-L-asparagine is active); however, it cannot be replaced with an amino group (γ-hydrazino-L-asparagine is inactive).

In general we note that a number of changes can be made in the structure of L-asparagine that permit it to retain at least a portion of its ability to enhance ornithine decarboxylase activity. Invariably all these changes entail replacement of hydrogen atoms with methyl groups; none of the hydrogen atoms that can be replaced with methyl groups

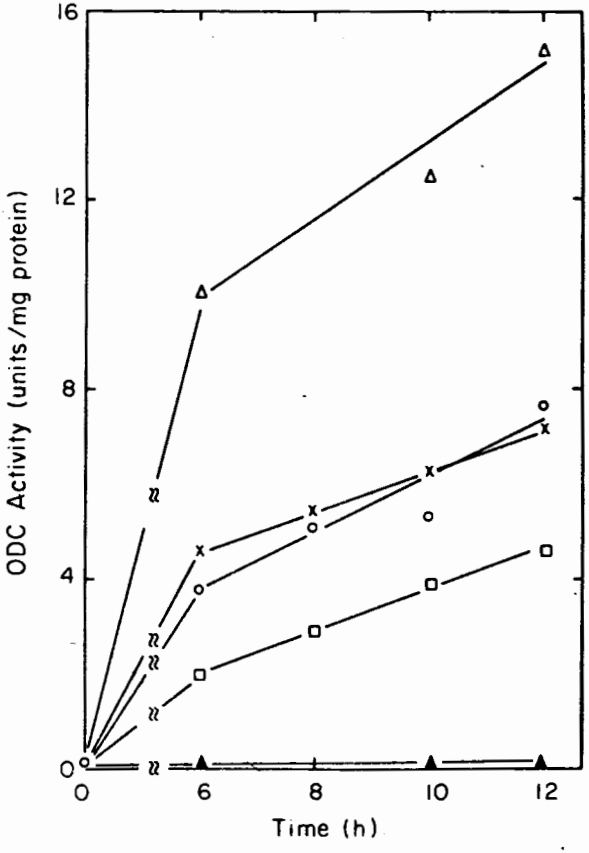


Fig. 1. Effect of asparagine analogs in eliciting ornithine decarboxylase (ODC) activity in neuroblastoma cell cultures. Cell cultures were washed free of growth medium and incubated in isotonic salts-glucose solution without additions (▲) or containing 10 mM L-asparagine (△), 10 mM γ-*N*-methyl-L-asparagine (○——○), 10 mM α-methyl (or 2-methyl)-DL-asparagine (□), or 10 mM threo-β-methyl-DL-asparagine (×——×) for the indicated times.

TABLE I

EFFECTS OF ASPARAGINE ANALOGS ON ORNITHINE DECARBOXYLASE ACTIVITY, PROTEIN SYNTHESIS AND ACID-SOLUBLE [^{14}C]LEUCINE

Neuroblastoma cell cultures were incubated with the indicated analog (10 mM in isotonic salts-glucose solution for 8 h. Analysis indicated a progressive increase of ornithine decarboxylase activity during this time period (see Methods), comparable results were obtained at lower analog concentrations.

Analog	Parameters (%)			Analog	Parameter (%)		
	Ornithine decarboxylase [a]	Protein [b] synthesis	Acid-soluble [c] leucine		Ornithine decarboxylase [a]	Protein [b] synthesis	Acid-soluble leucine
L-Asparagine	100	100	100	β-hydroxy-DL-asparagine	1	–	–
None	0	160	96	β-amino-DL-asparagine	0	13	143
D-Asparagine	28	22	75	L-glutamine	50	173	75
α-*N*-Methyl-DL-asparagine	100	120	104	L-aspartic acid	0	239	217
α-*N*-Acetyl-L-asparagine	0	–	–	γ-*N*-methyl-L-asparagine	47	–	–
α-*N*-Dinitrophenyl-L-asparagine	0	–	–	γ-*N*-hydrazine-L-asparagine	0	30	157
α-Methyl-DL-asparagine	25	101	104	γ-*N*-*p*-hydroxyanilino-L-asparagine	0	–	–
Threo-β-methyl-DL-asparagine	47	78	156				

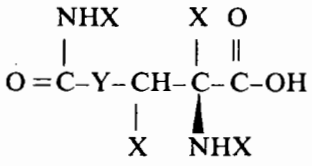
[a] 100% represents 20 units/mg protein; 0% represents undetectable activity.

[b] Protein synthesis as defined by incorporation of [^{14}C]leucine into protein during a 30-min pulse at 6 h. L-Asparagine = 100% = 29 700 cpm/mg protein. (—) represents 'not determined'.

[c] Intracellular acid-soluble pool of [^{14}C]leucine during protein synthesis (b). L-Asparagine = 100% = 8500 cpm/mg protein.

appear to be replaceable with the polar hydroxy or amino groups.

The following general structure summarizes the changes in asparagine that can be made and permit it to retain, or partially retain, its ability to elicit ornithine decarboxylase activity:



(X represents a methyl group, Y represents a methylene group while ▲ represents the configuration of carbon 2).

The kinetics of enhancement of ornithine decarboxylase activity by some of these asparagine analogs is shown in Fig. 1. While ornithine decarboxylase activity increased with time of exposure of the neuroblastoma cells to γ-*N*-methyl-L-asparagine, to 2-methyl-DL-asparagine, or to threo-β-methyl-DL-asparagine, the response was always lower than that elicited by L-asparagine. Exposure of neuroblastoma cells to α-*N*-methyl-DL-asparagine (Fig. 2) enhanced ornithine decarboxylase activity to levels that were similar to those that could be elicited by its unmethylated parent compound L-asparagine (Table I).

The structural requirements for asparagine that have been described and the kinetics of the enhancement of ornithine decarboxylase activity by asparagine analogs lead us to the conclusion that ornithine decarboxylase activity can be elicited in cells by asparagine analogs that cannot be incorporated into proteins or glycoproteins.

The ability of amino acids and their analogs to elicit ornithine decarboxylase activity

α-N-Methylamino acids. The observation that α-*N*-methyl-DL-asparagine was as effective as L-asparagine in eliciting ornithine decarboxylase activity, prompted a similar study of various amino acids and of their α-*N*-methyl analogs. The amino acid analogs could be grouped into three classes with regard to their ability to enhance ornithine decarboxylase activity.

α-Amino-DL-isobutyric acid elicited ornithine decarboxylase activity in a time- and concentration-dependent fashion, similar to that of L-asparagine and α-*N*-methyl-DL-asparagine (Figs. 2 and 3). This property of α-amino-DL-isobutyric acid, was reduced in the α-*N*-methyl-DL-isobutyric acid (Table II). In contrast, replacement of a hydrogen of the α-NH_2 group of valine by a methyl

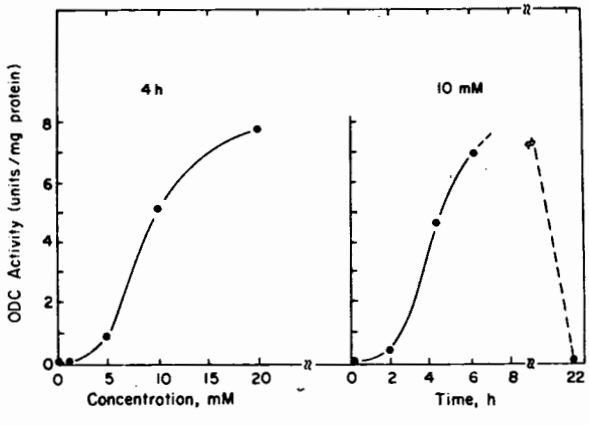


Fig. 2. Effect of the concentration and time of exposure to α-*N*-methyl-DL-asparagine on ornithine decarboxylase activity. Confluent cell cultures were washed free of growth medium and incubated with α-*N*-methyl-DL-asparagine in isotonic salts-glucose solution; the time of incubation (4 h) and the concentration (10 mM) are indicated.

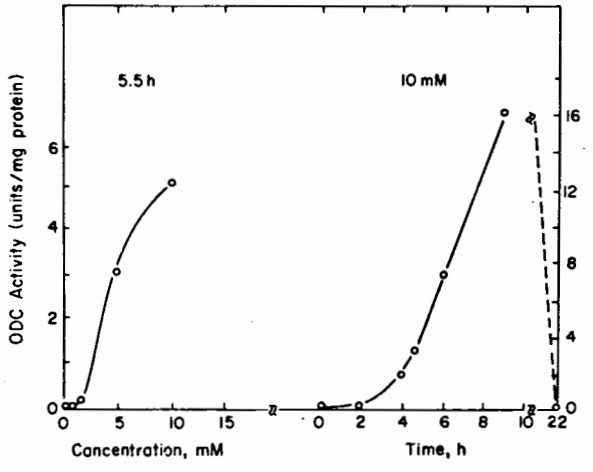


Fig. 3. Effect of the concentration and time of exposure to α-amino-DL-isobutyric acid on ornithine decarboxylase activity. Confluent cell cultures were washed free of growth medium and incubated with α-amino-DL-isobutyric acid in isotonic salts-glucose solution. Time of incubation (5.5 h) and concentration (10 mM) used are indicated.

TABLE II

EFFECTS OF AMINO ACIDS AND THEIR ANALOGS ON ORNITHINE DECARBOXYLASE ACTIVITY, PROTEIN SYNTHESIS AND ACID-SOLUBLE POOL

Confluent neuroblastoma cell cultures were exposed to the indicated amino acid or its analog (10 mM) in isotonic salts-glucose solution for 6 h.

Amino acid	Parameters %								
	Unsubstituted amino acid			α-*N*-Methylated amino acid			α-Amino acid amide		
	Ornithine decarboxylase [a]	Protein synthesis [b]	Acid-soluble leucine [c]	Ornithine decarboxylase [a]	Protein synthesis [b]	Acid-soluble leucine [c]	Ornithine decarboxylase [a]	Protein synthesis [b]	Acid-soluble leucine [c]
L-Asparagine	100	100	100	–	–	–	–	–	–
α-Amino-DL-isobutyric acid	54	50	111	0	99	114	–	–	–
L-Alanine	0	–	–	0	–	–	–	–	–
L-Valine	0	135	64	11	170	117	0	135	297
L-Leucine	0	–	–	0	–	–	–	–	–
L-Serine	27	60	54	–	–	–	0	197	191
L-Glutamic acid	0	263	213	0	–	–	–	–	–
L-Methionine	0	60	60	–	–	–	0	42	29
L-Lysine	0	72	101	0	–	–	–	–	–
L-Proline	10	–	–	–	–	–	0	–	–

[a] 100% represents 16 units/mg protein; 0% represents undetectable activity; (—) represents not tested.

[b] Protein synthesis as defined by incorporation of [^{14}C]leucine into protein during a 30 min pulse that was made at 6 h. L-Asparagine = 100% = 29700 cpm/mg protein.

[c] Intracellular acid-soluble pool of [^{14}C]leucine during (c). L-Asparagine = 100% = 8500 cpm/mg protein.

group, to form α-*N*-methyl-L-valine, bestowed some activity to the inactive L-valine (Table II).

L-Alanine, L-leucine, L-lysine and L-glutamic acid form a category of amino acids that do not elicit ornithine decarboxylase activity, either in their free form or as α-*N*-methylamino acids (Table II).

Therefore, the replacement of a hydrogen atom of the α-amino group by a methyl group appears to have a variety of effects. Some α-*N*-methyl analogs of amino acids elicit ornithine decarboxylase activity whereas the parent amino acid is incapable of eliciting ornithine decarboxylase activity (L-valine), others can retain the full ability of the parent amino acids to elicit ornithine decarboxylase activity (L-asparagine), while a third category of α-*N*-methylated amino acids is less effective in eliciting ornithine decarboxylase activity than the parent amino acids.

Amino acid amides. The amino acid amides, L-asparagine and L-glutamine elicit ornithine decarboxylase activity while L-asparagine acid and L-glutamic acid are inactive (Table II and cf. Ref. 1). None of the amides of the monocarboxylic amino acids that we have tested elicit ornithine decarboxylase activity (Table II). As with the asparagine analogs, we may derive the general conclusion that ornithine decarboxylase activity can be elicited by amino acid analogs that cannot participate in protein synthesis.

The relationship of macromolecular synthesis to the enhancement of ornithine decarboxylase activity

It has been demonstrated that α-aminoisobutyric acid is not incorporated into protein [13–14]; furthermore the requirement of a free amino group for peptide bond synthesis precludes the extensive promotion of protein synthesis by incorporation of the α-*N*-methylamino acids into proteins.

It was however necessary to establish whether these unnatural amino acids or even their normal counterparts may exert their effect on ornithine decarboxylase activity indirectly by promoting protein synthesis, i.e., by simulating step-up conditions. We have therefore determined leucine incorporation into proteins as well as the transport of radioactive leucine into cells in the presence of many of these amino acids. Some of these amino acids that do not elicit, or marginally elicit, ornithine decarboxylase activity, greatly enhance the uptake of radioactive leucine into the acid soluble pool and/or protein synthesis (Tables I and II) (α-methyl- (or 2-methyl-)-DL-asparagine, L-aspartic acid, L-glutamic acid, L-valine, α-*N*-methyl-valine, L-valine amide, L-serine amide, α-*N*-methyl-DL-isobutyric acid). On the other hand, amino acids that elicit approx. 50% or more of ornithine decarboxylase activity, may: (a) slightly stimulate protein synthesis (α-*N*-methyl-DL-asparagine), (b) enhance protein synthesis (L-glutamine) or, (c) inhibit protein synthesis (L-asparagine, threo-β-methyl-DL-asparagine). Consequently, there does not appear to be a direct relationship between the overall enhancement of cellular protein synthesis and the ability of amino acids to elicit ornithine decarboxylase activity.

Contrasting effects produced by the imposition of step-up growth conditions

The above experiments indicate that ornithine decarboxylase activity can be elicited and stabilized under conditions that do not promote protein synthesis. We have addressed the converse question. Does the promotion of step-up or growth conditions necessarily promote or stabilize ornithine decarboxylase activity?

It is generally accepted that exposure of confluent neuroblastoma cells to step-up conditions by the addition of Dulbecco's growth medium plus 10% fetal bovine serum results in an increase in ornithine decarboxylase activity [15, cf. also Refs. 16 and 17). In fact, Fig. 4 shows that ornithine decarboxylase activity rises rapidly if Dulbecco's growth medium plus 10% fetal bovine serum is added to cells that have been maintained in salts-glucose solution for 12 h. However, Fig. 4 shows that such step-up conditions can also produce the opposite effect. If Dulbecco's growth medium plus 10% fetal bovine serum is added to cells that already have a high ornithine decarboxylase activity, a very rapid decline in ornithine decarboxylase activity occurs. In such a case, step-up conditions neither raise nor do they even stabilize the existing level of ornithine decarboxylase activity. Consequently, depending upon the state of the cell, the initiation of cellular growth, can have opposing effects on the cellular level of ornithine decarboxylase activity.

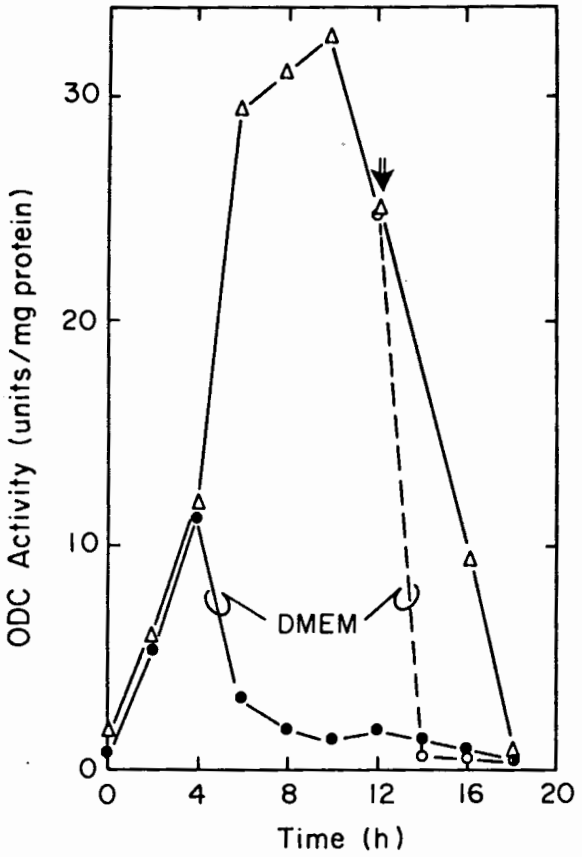


Fig. 4. Opposing effects of step-up or growth conditions on ornithine decarboxylase activity. A. Stimulation of ornithine decarboxylase activity by Dulbecco's growth medium (DMEM) plus 10% fetal bovine serum (●——●) or by 10 mM L-asparagine in salts-glucose solution (△——△). B. Decrease in ornithine decarboxylase activity subsequent to the addition of Dulbecco's growth medium plus 10% fetal bovine serum at arrow (○——○) to the asparagine-induced cells (△——△).

The $T_{1/2}$ of Ornithine decarboxylase activity is independent of the inhibition of protein synthesis

In Table III, ornithine decarboxylase is elicited in neuroblastoma cells by a variety of means, i.e., by adding L-asparagine or α-*N*-methyl-DL-asparagine to cells maintained in isotonic salts-glucose or by converting from isotonic salts-glucose to hypotonic salts-glucose solution (see below and Table IV).

When the respective inducers are removed, ornithine decarboxylase activity falls with a $T_{1/2}$ of 12–50 min. The same $T_{1/2}$ is obtained if the inducer is removed and cycloheximide is added. Consequently, cycloheximide does not decrease the $T_{1/2}$ of ornithine decarboxylase activity in the absence of the inducer. On the other hand, if the inducer is retained and cycloheximide is added, the $T_{1/2}$ of ornithine decarboxylase is greatly prolonged to a range of 150–400 min; a half-life that is commensurate with the overall existence of ornithine decarboxylase activity under optimal conditions of ornithine decarboxylase induction (see Fig. 4). It therefore appears that the $T_{1/2}$ of ornithine decarboxylase is primarily defined by the presence or absence of the inducer and not by the inhibition of protein synthesis.

This result is consistent with the results shown in Tables I and II which indicate that the enhancement of ornithine decarboxylase activity can occur with unnatural amino acids and is not necessarily related to the overall rate of protein synthesis.

Isotonic salts-glucose solutions: Na^+ is required for eliciting ornithine decarboxylase activity by L-asparagine

The above results strongly suggest that parameters other than protein synthesis may be involved in the enhancement of ornithine decarboxylase activity.

We have indicated [11] that the increase in ornithine decarboxylase activity that occurs during the G1 stage of the cell cycle or when stationary cells are exposed to growth conditions such as Dulbecco's growth medium plus 10% fetal bovine serum (Fig. 4) correlates with the change in ion flux that occurs during these times [18–21]. In the following we show that addition of 10 mM L-asparagine to neuroblastoma cells maintained in isotonic salts-glucose solution elicits ornithine decarboxylase activity only in the presence of Na^+, (Table IV, conditions 1b and 2b). Ornithine decarboxylase activity is not elicited in the absence of Na^+. However, as much as 50% of the Na^+ can be replaced with an iso-osmolar equivalent of choline, sucrose or mannitol (Table IV, conditions 2b and 3b) without affecting the enhancement of ornithine decarboxylase activity by L-asparagine. It appears therefore, that Na^+ is required for the enhancement of ornithine decarboxylase activity by L-asparagine in isotonic salts-glucose solution.

TABLE III

COMPARATIVE EFFECT OF CYCLOHEXIMIDE AND OF THE INDUCER UPON THE $T_{1/2}$ OF ORNITHINE DECARBOXYLASE

Ornithine decarboxylase activity was induced for 4 h in isotonic salts-glucose by L-asparagine or by α-*N*-methyl-DL-asparagine. It was also induced by hypotonic salts-glucose alone or in combination with L-asparagine. After 4 h, either (A) cycloheximide was added; or (B) the inducers (L-asparagine, α-*N*-methyl-DL-asparagine, the hypotonicity or the hypotonicity plus L-asparagine) were removed and replaced with isotonic salts-glucose, with or without cycloheximide. The activity or ornithine decarboxylase was then determined. (Amino acid concentration = 10 mM, cycloheximide concentration = 50 μg/ml). The cycloheximide inhibited protein synthesis by more than 95% within 5 min, as determined by [^{14}C]leucine incorporation into protein. Values represent half-life of ornithine decarboxylase in minutes.

Ornithine decarboxylase activity induced by	(A) Inducer removed		(B) Inducer retained (Cycloheximide added)
	No additions	Cycloheximide added	
L-Asparagine	12–24	12–24	180–400
α-*N*-Methyl-DL-asparagine	50	48	240
Hypotonicity	15–18	15–18	150
Hypotonicity plus L-asparagine	10	11	240

TABLE IV

EFFECT OF TONICITY AND OF Na^+ ON ORNITHINE DECARBOXYLASE ACTIVITY IN NEUROBLASTOMA CELLS

Confluent neuroblastoma cell cultures were incubated in the indicated salts-glucose solutions for 6 h; they were then harvested for ornithine decarboxylase activity determinations. Earlier times gave lower values, indicating continuously progressive time curves.

Condition	Molarity		L-Asparagine	Ornithine decarboxylase activity
	NaCl (mM)	Choline-Cl (mM)	(10 mM)	Units/mg protein)
A. Isotonic salts-glucose solution				
Ia	116	0	−	0.0
1b	116	0	+	13.4
2a	0	116	−	0.0
2b	0	116	+	0.0
3a	58	58 (or 116 mM sucrose or mannitol)	−	0
3b	58	58 (or 116 mM sucrose or mannitol)	+	13.5 *to* 14.0
B. Hypotonic salts-glucose solution				
4a	58	0	−	2.9
4b	58	0	+	14.8
5a	0	58	−	15.6
5b	0	58	+	66.8

Hypotonic salts-glucose solutions: Na^+ inhibits the enhancement of ornithine decarboxylase activity

It is known that hypotonicity results in a small but reproducible enhancement of ornithine decarboxylase activity [22–24] (see also Table IV, condition 4a vs. 1a or 3a). We find that this enhancement of ornithine decarboxylase activity in hypotonic media is inhibited by Na^+ (Table IV, condition 5a vs. 4a). Maximal enhancement of ornithine decarboxylase activity in hypotonic salts-glucose solution occurs in the presence of L-asparagine and in the absence of Na^+ (Table IV, condition 5b vs. 4b). Na^+ prevents this maximal enhancement of ornithine decarboxylase activity by L-asparagine in hypotonic media (Table IV, condition 4b vs. 5b).

It is therefore apparent that the enhancement of ornithine decarboxylase activity in both isotonic as well as in hypotonic salts-glucose solutions is related to a sodium-sensitive step. In isotonic solutions Na^+ is essential for the enhancement of ornithine decarboxylase activity while in hypotonic salts-glucose solution, Na^+ prevents the maximal enhancement of ornithine decarboxylase activity. As will be discussed later, these characteristics are similar to those of the 'A' amino acid transport system, which is dependent upon Na^+ and whose function is altered by changes in osmolarity [25].

Discussion

In order to understand the essential role of asparagine in the enhancement of ornithine decarboxylase activity in mammalian cells [1–3] we decided to establish whether concurrent incorporation of asparagine into proteins or glycoproteins was necessary for the enhancement of ornithine decarboxylase activity. For this reason, we focused on the structure-activity relationship of asparagine as well as of other amino acid analogs.

The results show that the concentrations of asparagine which stimulate ornithine decarboxylase activity, inhibited the basal level of protein synthesis. Furthermore, some of the asparagine analogs that enhance ornithine decarboxylase activity, cannot be incorporated into proteins or glycoproteins. In addition, amino acid analogs, such as α-*N*-methyl valine, α-aminoisobutyric acid [13,14] which elicit ornithine decarboxylase activity cannot be incorporated into proteins. Similarly, we have noted that there is no direct relationship between the enhancement of cellular protein synthesis and the ability of amino acids to elicit ornithine decarboxylase activity. These results find additional support in the cycloheximide experiments. In hypotonic solutions or in the presence of L-asparagine or in the presence of α-*N*-methyl-DL-asparagine, the inhibition of protein synthesis by cycloheximide does not result in a rapid loss of ornithine decarboxylase activity. Only if the hypotonic solution is made isotonic or if L-asparagine or if α-*N*-methyl-DL-asparagine are removed, does a rapid loss in ornithine decarboxylase activity occur. This fall in ornithine decarboxylase occurs whether cycloheximide is present or absent.

Tomkins and co-workers [26–27] have shown that nutritional step-down conditions enhance tyrosine aminotransferase degradation and that this enhanced degradation is blocked by protein synthesis inhibitors such as cycloheximide. Our results in Table III, however, emphasize that the stabilization or destabilization of ornithine decarboxylase activity is the direct consequence of the respective presence or absence of the agent, or of the condition, that was used to stimulate ornithine decarboxylase activity and not the consequence of the inhibition of protein synthesis. It would therefore appear that in those cases in which cycloheximide causes a fall in ornithine decarboxylase activity [28], such an effect may be due to side effects of cycloheximide rather than due to the inhibition of protein synthesis by cycloheximide [11].

It is appropriate to note the very close correspondence that exists between the behavior of ornithine decarboxylase and the behavior of its antizyme [29–32] in response to the agents that elicit their activity. Antizyme activity is elicited by putrescine and falls only when putrescine is removed from the medium, regardless whether cycloheximide is present or not [33]. Both ornithine decarboxylase activity and antizyme activity are maintained by the presence of their respective eliciting agents and fall subsequent to the removal of the eliciting agents. Neither of these phenomena is affected by the presence or absence of cycloheximide [33].

These data support our earlier suggestion that the regulation of ornithine decarboxylase activity may also occur at the post translational level, through the interconversion of a latent from of ornithine decarboxylase to an active form. Such mechanisms could include the non-covalent association of ornithine decarboxylase [29] with antizyme or the phosphorylation or ornithine decarboxylase by the antizyme-like polyamine-dependent kinase [34].

In order to acquire some additional information on the factors that may regulate the fluctuations in ornithine decarboxylase activity, especially in relation to the asparagine and amino acid analogs, we have studied the role of sodium. We have shown that Na^+ is an absolute requirement for the enhancement of ornithine decarboxylase activity in isotonic salts-glucose solution. In hypotonic salts-glucose solution, however, Na^+ inhibits the enhancement of ornithine decarboxylase activity, both in the absence of L-asparagine (Table IV, 4a vs. 5a) and in the presence of L-asparagine (Table IV, 4b vs. 5b). These findings, and the fact that ornithine decarboxylase activity can be enhanced by α-aminoisobutyric acid, the probe for the 'A' amino acid transport system, suggest that the fluctuations in ornithine decarboxylase activity may be related to the 'A' amino acid transport system. The following similarities are apparent: The 'A' transport system is dependent on Na^+ and its functioning is altered by changes in osmolarity [25]. In general, users of the 'A' system are small nonpolar amino acids or analogs. α-Aminoisobutyric acid as well as amino acids with methylated α-amino groups appear to preferentially or exclusively utilize this transport system [25]. These characteristics are consistent with the 'tolerated' changes in the amino acid analogs tested in this study and with the fact that polar substituents in the asparagine molecule result in the loss of its ability to elicit ornithine decarboxylase activity.

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