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REGULATION BY 1,4-DIAMINES OF THE ORNITHINE DECARBOXYLASE ACTIVITY INDUCED BY ORNITHINE IN PERIFUSED TUMOR CELLS

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Abstract—Ornithine decarboxylase (ODC) activity of Ehrlich carcinoma cells was increased more than 36-fold after being maintained for 3.5 hr *in vitro* in a special chamber which allowed continuous perifusion with 0.5 mM ornithine; if incubated *in vitro* without perifusion the ODC activity was, of course, only 9-fold by the same concentration of ornithine. Ornithine withdrawal from the perifusion medium resulted in a decay of enzyme activity observed after 90 min; this decay was prevented by addition of 55 μM pyridoxal to the medium. The 1,4-diamines putrescine, spermidine, spermine, agmatine, histamine, serotonin, tryptamine, chlorpheniramine and harmaline at 55 μM strongly suppressed ODC induction by 0.5 mM ornithine in perifused Ehrlich ascites cells. Methyl derivatives also behave as strong inhibitors of ODC induction. On the contrary, N-acetylation paralleled with a decrease in the inhibition capacity: 55 μM *N*-acetyl putrescine, *N*-acetyl serotonin or *N*-ω-acetylhistamine suppressed ODC induction by ornithine in 66, 64 and 19%, respectively. The addition to the perifusion medium of the same concentrations of 1,3-diamines (1,3-diaminopropane, 1,3-diamino-2-propanol or the alkaloid gramine) as well as 1,5-diamines (1,5-diaminopentane and the antihistaminic doxylamine or cimetidine) failed to suppress the induction of ODC activity by ornithine. Interestingly, 1,4-benzenediamine, which strongly inhibits ODC activity when the induced enzyme is assayed in its presence, did not suppress the induction of the enzyme when both 0.5 mM ornithine and 55 μM 1,4-benzenediamine were present in the perifusion medium. The inhibitory capacity in down-regulating ODC is not due to differences in the diamine uptake by the cells. The results suggest that the N—N distance (6 Å) and the charge of one amino group are important chemical characteristics for regulatory effects.

Ornithine decarboxylase (ODC) (EC 4.1.1.17) is the rate-limiting enzyme for polyamine biosynthesis [1]. The protein has a very rapid turnover [2] and is produced by cells that have been stimulated by a variety of trophic agents, drugs, amino acids, hormones and growth factors [3]. It is well-established that polyamines exert a feedback regulation on ODC levels [4–6]. An inverse relationship was found between cellular polyamine content and ODC synthesis [7]. The administration of putrescine *in vitro* by injections at 1-hr intervals suppresses stimulation of ODC after transplantation of freshly harvested Ehrlich ascites tumor cells [8]. It is noteworthy that an inhibitor protein of ODC (antizyme) is also induced by putrescine and other polyamines in rat hepatomas [9] and in Ehrlich ascites carcinoma [10]. Although the complex mechanism of ODC control by polyamines is still unknown, many reports agree that short-term control of ODC is primarily post-transcriptional, since the administration of polyamines reduces the ODC activity without affecting its mRNA content [1, 6, 11, 12].

On the other hand, Reinehart and co-workers [13, 14] report that the induction of ODC activity by insulin and growth factors is mediated by asparagine and other neutral amino acids in transformed cell cultures. Kanamoto *et al.* [15] have demonstrated that, in adult rat hepatocytes maintained in salts/glucose medium, the enzyme is stimulated by asparagine and glucagon in a synergistic manner. In a previous work, Matés *et al.* [16] reported that ornithine is the best inducer of ODC activity when using confined Ehrlich ascites tumor cells continuously perifused with saline buffer medium added with different amino acids at a final concentration of 0.5 mM. This ornithine concentration is very close to physiological conditions [17]. Low concentrations of histamine and serotonin in the perifusion medium strongly suppress the induction of ODC by ornithine. As reported in this paper, we studied the short-term effect of several natural and structurally related diamines on ODC induction by ornithine in perifused cells. With the exception of cyclic diamines, all assayed 1,4-diamines strongly inhibited the induction of ODC activity. In contrast, 1,3- and 1,5-diamines failed to suppress the ODC induction.

MATERIALS AND METHODS

Ehrlich ascites cells. A hyperdiploid Lettré strain was maintained as reported previously by Quesada *et al.* [18]. Mice were inoculated and killed as described by Márquez *et al.* [17]. The cells were

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washed once with 0.9% NaCl, twice with phosphate saline buffer (6.16 mM KCl, 154 mM NaCl, 1.65 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, pH 7.4) and were centrifuged at 500 *g* for 5 min. The final cell suspension was 4.2×10^8 cell/mL.

Chemicals. Histamine dihydrochloride was from Fluka Biochemika (Switzerland). Ornithine, putrescine, cadaverine, 1,3-diaminopropane, 1,4-benzenediamine, spermidine, spermine, agmatine, serotonin, tryptamine, pyridoxamine, chlorpheniramine, harmine, harmaline, noreleagnine, aminoguanidine and the *N*-acetyl and methyl derivatives were purchased from the Sigma Chemical Co (St. Louis, MO, U.S.A.). HPLC reagents and chemicals were used as described by Márquez *et al.* [19].

Perifusion system. Continuous incubations were performed as described previously by Segura *et al.* [20]. A jacketed 10 mL cylindrical perifusion chamber was preferentially used. It was fitted with a 25 mm diameter cellulose nitrate filter of 8 μm pore size (Sartorious SM M 301) placed at the top of the chamber. The chamber had been filled previously with phosphate saline buffer to which the appropriate substrates and inhibitors were added. Buffer temperature was maintained at 37° by a thermostatically controlled water bath. Tumor cells were introduced rapidly into the chamber through the inlet tube to give a final concentration of 420×10^6 cells. The flow rate was kept at 0.18 mL/min by means of a Microperpex roller pump (Pharmacia, Uppsala, Sweden). After 3.5 hr of continuous perifusion, the erythrosine test revealed that cell integrity was more than 80%. To obtain the results shown in Fig. 1, cells were incubated for 2 hr in saline buffer with 0.5 mM ornithine. This was followed by a medium change and then perifusion for 90 min. The cells used were freshly harvested from 10-day-infected animals, when ODC activity is the lowest observed during the different growth phases of Ehrlich ascites tumor [17].

Ornithine decarboxylase assay. After the required perifusion time, cells were extraced from the chamber, centrifuged at 3000 *g* for 120 sec, frozen immediately and stored at −20° until use. Before ornithine decarboxylase assay, extracts were prepared as described previously by Matés *et al.* [16]. Ornithine decarboxylase activity was determined following the method reported by Mitchell *et al.* [21]. The final concentration of ornithine was 0.6 mM, including 18.5 kBq of L-[1-^{14}C]ornithine (sp. radioact. 2.22 GBq/mmol) added before each assay. $^{14}CO_2$ evolved in the decarboxylase reaction was then counted in an LKB Rack Beta (Sweden) scintillaton counter. All assays were carried out in duplicate.

Determination of free intracellular polyamines. Immediately after extraction from the perifusion chamber whole cells were centrifuged through 1 mL of a silicone–oil mixture, according to the method of Márquez *et al.* [22]. The calculated total aqueous volume of the cells was 0.60 ± 0.03 μL/10^6 cells [22]. Cells were frozen at −20° until use. Homogenization was carried out in cold 0.4 M perchloric acid (3×10^8 cells/mL) and 1 μmol/3×10^8 cells of hexanediamine or octanediamine was then added to the extracts as an internal standard.

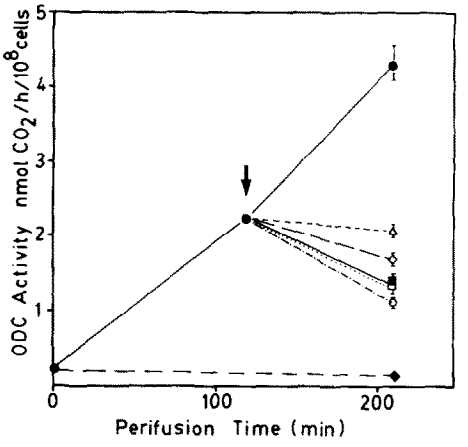


Fig. 1. Short-term effect of changing the perifusion medium. After 120 min of continuous perifusion with 0.5 mM ornithine, the medium was changed and the perifusion followed for 90 min. Saline buffer added with 55 μM pyridoxal (△); saline buffer added with 0.5 mM ornithine and 55 μM histamine (◇); saline buffer only (■); saline buffer added with 55 μM pyridoxal and 55 μM histamine (□) and saline buffer added with 55 μM histamine only (○). Controls: ODC activity during continuous perifusion with 0.5 mM ornithine (●) and after 3.5 hr of continuous perifusion with saline buffer only (◆). Arrows indicate change of substrate. Values are means of 3 different perifusions of cells obtained from different inoculated animals ± SEM.

The Dansyl-derivatives of the polyamines were obtained as described by Smith and Davies [23] with minor modifications: the speed-vacuum dried Dansyl-derivatives were dissolved in 0.5 mL of acetonitrile: water (70:30), filtered through HVLP 013 Millipore membranes (0.45 μm pore; Bedford, MA, U.S.A.) and assayed immediately or stored at −20°. Frozen samples were stable for at least a week. A Beckman HPLC system (San Ramon, CA, U.S.A.) was used, equipped with a 421-A Controller and a 110-B Solvent Delivery Module (with a 20 μL sample loop). A 420-AC Waters Millipore (Milford, MA, U.S.A.) fluorescence detector (excitation filter at 338 nm and emission cutoff filter at 425 nm) was used with an electronic control unit. Continuous on-line quantification of chromatographic peaked was performed using a Spectra Physics Model SP 4290 computing integrator, Separation of Dansyl-derivatives was performed on a 250 × 4 mm LiChrosorb® RP18 (5 μ) reversed phase column (Merck, Darmstadt, F.R.G.) at a constant room temperature of 22 ± 1°. Two mobile phases were used: (a) water:acetonitrile:methanol (5:3:2) and (b) acetonitrile:methanol (3:2). A sigmoidal-like gradient was used (Matés *et al.*, unpublished) and recovery was estimated to be more than 90% for all the assayed polyamines. Under these conditions each polyamine showed distinct R_f values.

Computerized molecular mechanic calculation. N—N interatomic distances were calculated using two different programs, Alchemy from Tripos

Table 1. Short-term effect of 1,4-diamines on the induction of ODC activity by ornithine in perifused Ehrlich ascites tumor cells

Perifusion conditions	Ornithine decarboxylase activity (nmol CO_2/hr/10^8 cells)	(% inhibition)	N—N Distance (Å)
0.5 mM Ornithine*	4.32 ± 0.25	—	—
0.5 mM Orn + 55 μM putrescine	0.54 ± 0.05	90	6.27
0.5 mM Orn + 55 μM spermidine	0.81 ± 0.10	83	6.22
0.5 mM Orn + 55 μM spermine	0.74 ± 0.04	85	6.22
0.5 mM Orn + 55 μM agmatine	0.80 ± 0.07	83	6.20
0.5 mM Orn + 55 μM histamine†	0.49 ± 0.03	91	6.01
0.5 mM Orn + 55 μM serotonin†	0.47 ± 0.01	91	5.91
0.5 mM Orn + 55 μM tryptamine	1.12 ± 0.05	76	5.91
0.5 mM Orn + 55 μM pyridoxamine	2.46 ± 0.04	43	5.17
0.5 mM Orn + 55 μM chlorpheniramine	0.32 ± 0.03	95	5.90
0.5 mM Orn + 55 μM 1,4-benzenediamine	3.86 ± 0.09	9	5.63

* Control is the value of ODC activity induced in cells by perifusion with 0.5 mM ornithine only.

Values are means of 3 different perifusions at 37° of cells obtained from different inoculated animals. The N—N distance was calculated with the Alchemy program (IBM) and with the MMP2 (± 0.1 Å), at the minimum potential energy state.

† From Ref. 16.

Table 2. Short-term effect of *N*-acetyl and methyl derivatives of 1,4-diamines on the induction of ornithine decarboxylase activity by ornithine in perifused ascites tumor cells

Perifusion conditions	Ornithine decarboxylase activity (nmol CO_2/hr/10^8 cells)	(% inhibition)	N–N Distance (Å)
0.5 mM Orn + 55 μM *N*-acetylputrescine	1.52 ± 0.07	66	6.22
0.5 mM Orn + 55 μM *N*-ω-acetylhistamine	3.45 ± 0.01	19	5.98
0.5 mM Orn + 55 μM *N*-acetylserotonin	1.61 ± 0.10	64	5.91
0.5 mM Orn + 55 μM *N*-methylhistamine	0.45 ± 0.02	92	6.03
0.5 mM Orn + 55 μM 5-methyltryptamine	1.14 ± 0.08	75	5.91
0.5 mM Orn + 55 μM *N,N,N',N'*-tetramethyl-1,4-benzenediamine	3.80 ± 0.13	11	5.63

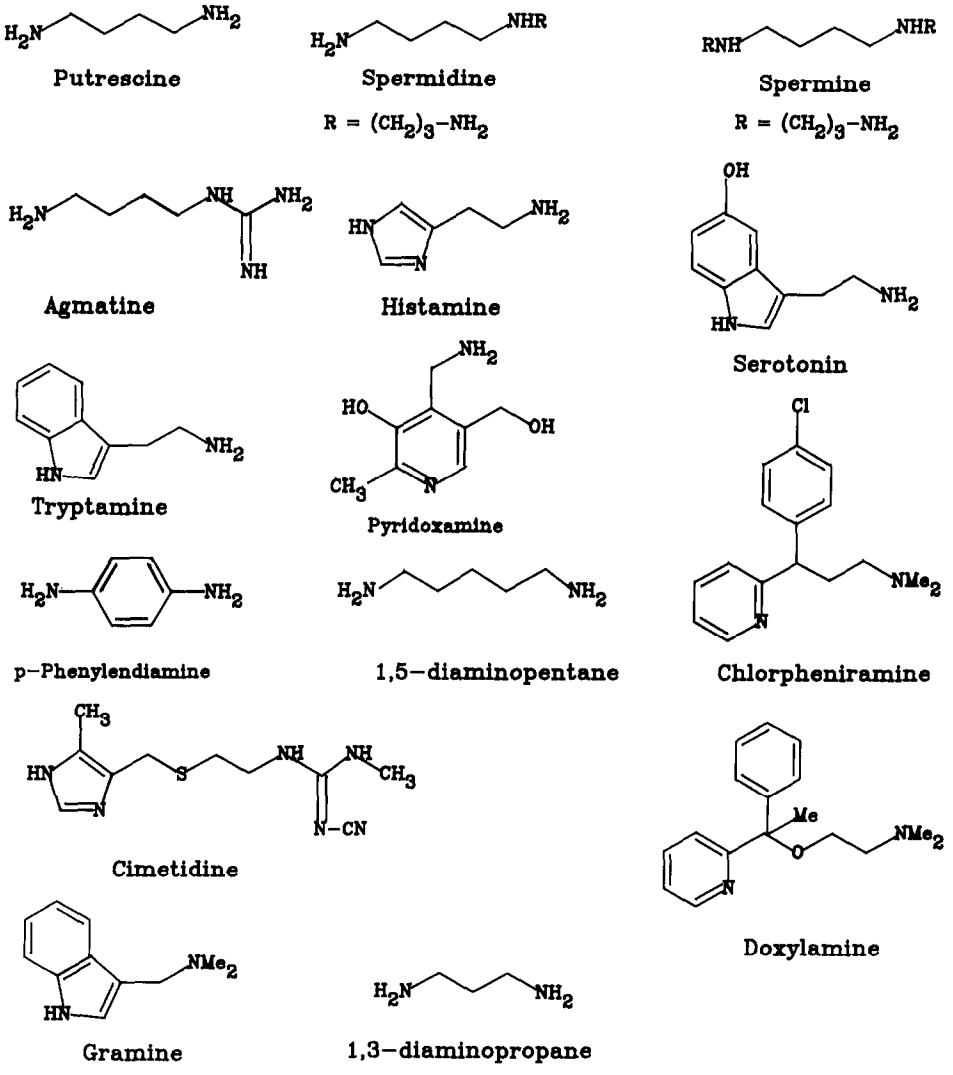
Experimental conditions as described in Table 1.

Table 3. Short-term effect of 1,3 diamines and 1,5-diamines on the induction of ornithine decarboxylase activity by ornithine in perifused Ehrlich ascites tumor cells

Perfusion conditions	Ornithine decarboxylase activity (nmol CO_2/hr/10^8 cells)	(% inhibition)	N—N Distance (Å)
0.5 mM Orn + 55 μM 1,3-diaminopropane	3.98 ± 0.07	6	4.92
0.5 mM Orn + 55 μM 1,3-diamino-2-propanol	4.01 ± 0.04	5	4.92
0.5 mM Orn + 55 μM gramine	4.09 ± 0.09	4	4.92
0.5 mM Orn + 55 μM 1,5-diaminopentane	4.04 ± 0.08	5	5.40
0.5 mM Orn + 55 μM doxylamine	3.99 ± 0.08	6	6.31
0.5 mM Orn + 55 μM cimetidine	4.23 ± 0.11	2	6.25

Experimental conditions as described in Table 1.

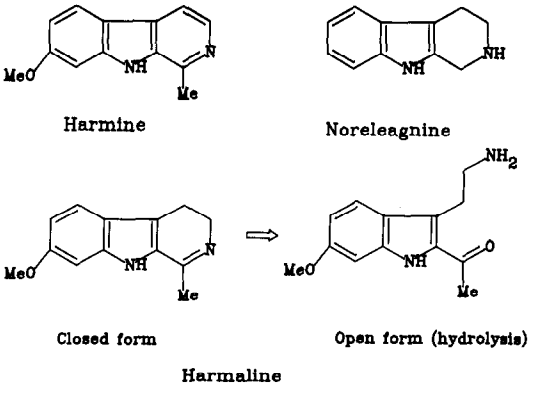
(Tripos Associationes, Inc.; 1988) and the MMP2 program by Prof. Allinger (QCPE 395; 1977). Input files were made using the MMP2 program. Several modifications were necessary to equate the kind of atom with the file output. In addition, some new non-implemented constants were introduced in the 1977 MMP2 version using dates from other versions such as MMPMI (K. E. Gilbert and J. J. Gajewski, Indiana University), MMX (K. E. Gilbert, Serena Software; 1988) and MM2 85 (QCPE 395; 1985).



Scheme 1. Structure of 1,4; 1,5 and 1,3 diamines tested on the regulation of ODC activity induced by 0.5 mM ornithine.

RESULTS

The perifusion technique allows continuous removal of products which can produce feedback inhibition of the process under study, as occurs in the case of ODC induction [1–3]. Actually, in cells incubated continuously in saline buffer with 0.5 mM ornithine (Fig. 1), the ODC activity was 36-fold higher than in cells perifused with saline buffer only, after 3.5 hr of perifusion. However, under the same initial conditions, in batch incubations only a 9-fold induction of ODC activity was detected. Asparagine was able to induce only an 8.5-fold increase in ODC activity after 3.5 hr of continuous perifusion. The induction of ODC activity was a temperature-dependent process; after 3.5 hr of perifusion at 22° and 47°, ODC activities were 3.79 and 4.56 nmol CO_2/hr/10^8 cells, the induction of ODC activity being 33- and 38-fold, respectively. The temperature was kept at 37° in all the experiments presented in this paper.



Scheme 2. Harmane family alkaloids tested on the regulation of ODC activity induced by 0.5 mM ornithine.

Table 4. Short-term effect of harman alkaloids on the induction of ornithine decarboxylase by ornithine in perifused Ehrlich ascites cells

Perifusion conditions	Ornithine decarboxylase activity (nmol CO_2/hr/10^8 cells)	(% inhibition)	N—N Distance (Å)
0.5 mM Orn + 55 μM harmine	4.05 ± 0.07	4	3.57
0.5 mM Orn + 55 μM harmaline	0.29 ± 0.01	96	3.59/5.95*
0.5 mM Orn + 55 μM noreleagnine	4.08 ± 0.21	4	3.67

Experimental conditions as described in Table 1.
* N—N distance if the ring became opened to form 1*H*-indole 2-ethanone-3-ethanamine-6-methoxy.

Table 5. Intracellular concentrations of different polyamines after 3.5 hr of continuous perifusion

Polyamine added to the perifusion medium	Intracellular concentration (mM)	pK_{a1}	pk_{a2}
Putrescine	11.7 ± 0.5	9.35	10.80
Spermidine	11.3 ± 1.1	>9.00	>10.00
Spermine	10.9 ± 3.0	>9.00	>10.00
Histamine	4.7 ± 0.4	6.04	9.75
Serotonin	10.5 ± 2.7	9.80	—
Tryptamine	7.3 ± 0.5	10.20	—
1,4-Benzenediamine	5.5 ± 1.2	2.67	6.16
1,3-Diaminopropane	5.2 ± 0.7	9.03	10.94
1,5-Diaminopentane	6.8 ± 0.2	10.05	10.93

Tumor cells were incubated continuously for 3.5 hr. The perifusion medium was added with 0.5 mM ornithine and indicated polyamine at 55 μM. Then, cells were collected and centrifuged immediately through 1 mL of silicone–oil mixture, as described in Materials and Methods. The amine concentrations were determined in triplicate; values are means ± SEM of 3 different perifusions for each diamine assayed. The cells for each experiment were obtained from different inoculated animals. The pK_a values are from Ref. 39.

The perifusion technique also enables the medium to be changed during the experiment. Figure 1 shows the effect of changing the different perifusion media on ODC activity after 2 hr of perifusion with 0.5 mM ortnithine, when ODC activity reached 2.19 nmol CO_2/hr/10^8 cells. The change to an ornithine-free medium resulted in a decay of the enzyme activity observed after 90 min. This result agrees very well with the known rapid turnover of the ODC protein. Addition to the saline buffer of 55 μM pyridoxal prevented the decay of the enzyme activity; this "*in vivo*" result agrees with the "*in vitro*" observation that pyridoxal phosphate prevents ODC inactivation [24]. However, this effect of pyridoxal was partially abolished in the presence of 55 μM histamine. The addition of histamine to the perifusion medium decreased enzyme activity, even in the presence of the inducer ornithine.

Table 1 shows the effect of several 1,4-diamines on the induction of ODC activity by ornithine in continuously perifused cells. The natural polyamines putrescine, spermidine and spermine at 55 μM strongly depressed the induction of the ODC; agmatine, synthesized via arginine decarboxylase in plants and microorganisms, behaved in the same manner. In a previous work the effect of the 1,4-diamines histamine and serotonin was reported [16]. Different concentrations of histamine and serotonin produced a clear dose–effect response. Tryptamine and pyridoxamine, which are also 1,4-diamines, behaved as inhibitors of the induction process. In the search for different 1,4-diamines as possible inhibitors of ODC induction, other non-structurally related polyamines were tested. The behaviour of the aromatic 1,4-benzenediamine was very striking. It is well-known that putrescine and other diamines are "*in vitro*" competitive inhibitors of the ODC activity [25]. Solano *et al.* [26] reported that 1 mM 1,4-benzenediamine inhibits more than 93% of the enzyme activity. In cell-free extracts of Ehrlich ascites tumor cells in which the ODC activity had been induced previously, 55 μM 1,4-benzenediamine inhibited enzyme activity by 56%. A similar inhibition was obtained when the cell-free extracts were assayed in the presence of putrescine. Nevertheless, 1,4-benzenediamine showed a very poor capacity to suppress the induction caused by ornithine in perifused cells.

Table 2 summarizes the effect of some diamine derivatives on the induction of ODC activity by ornithine. In the presence of N^t-methylhistamine and 5-methyltryptamine the full suppressing capacity was conserved. In contrast, *N*-acetyl derivatives showed less inhibitory capacity, this being very marked in the case of *N*-ω-acetylhistamine. *N*,*N*,*N*′,*N*′-Tetramethyl-1,4-benzenediamine

showed a very weak inhibition of the process. The structurally related diamines 1,3-diaminopropane and 1,3-diamino-2-propanol, and the alkaloid gramine (Table 3) did not inhibit the induction process when used at 55 μM in the perifusion medium (see Scheme 1). In the same way, 1,5-diaminopentane (cadaverine) did not suppress the ODC induction. It is remarkable that the antihistaminic agent, chlorpheniramine, which has a 1,4-diamine structure, strongly suppressed the ODC induction. However, the histamine receptor blockers doxylamine and cimetidine, which have a 1,5-diamine structure, were ineffective in suppressing the ODC activity induced by ornithine.

Some harman family indol alkaloids (Scheme 2) which have a 1,4-diamine structure, were also tested (Table 4). Harmine and noreleagnine at 55 μM did not show any inhibitory effect. In contrast, 55 μM harmaline showed the strongest effect of all 1,4-diamines tested on the inhibition of ODC induction by ornithine.

In order to test whether the differences in capacity to depress ODC induction could be due to differences in diamine uptake by the cells, the intracellular concentrations of some representative diamines were determined after 3.5 hr of continuous perifusion with 0.5 mM ornithine and 55 μM amine. The results shown in Table 5 indicate that all the diamines tested were transported and stored by Ehrlich ascites cells very efficiently. Under the experimental conditions the intracellular diamine concentrations ranged between 5 and 10 mM. It is noteworthy that 1,4-benzenediamine, 1,3-diaminopropane and 1,5-diaminopentane, which failed to depress ODC induction, were accumulated similarly to histamine, which strongly inhibited ODC induction. The polyamine uptake is an energy-dependent and saturable system [27] resembling the uptake system of amino acids in mammalian cells. These transport characteristics could explain why the assayed diamines can be stored by perifused cells at a concentration more than 100-fold that in the perifusion medium.

DISCUSSION

The feedback inhibition of ODC induction by natural or structurally related polyamines seems to be well established [1, 4, 6, 12]; the elevation of intracellular levels of polyamine resulted in both a reduction of enzymatic activity and ODC protein level. The following mechanisms have been proposed to explain the polyamine effect: (a) inhibition of the ODC translation process [4, 11, 12, 28], (b) changes in protein stability [1, 6, 29] and (c) hindering of the ODC subunit dimerization [30]. The exact mechanism still remains under discussion.

The data presented here take advantage of the simplicity of the perifusion technique which allows experimentation with whole cells in the presence of the inducer ornithine only and different diamines. The fact that all the tested diamines are easily transported, either depressing or not depressing ODC induction, rules out the possibility that the differences in inhibition are due to differences in diamine uptake by the cells.

The N—C—C—C—C—N structure of amines with a N—N distance of about 6 Å seems to be very critical for the regulatory capacity of the different compounds assayed. In fact, the structurally related polyamines with a shorter N—N distance (4.92 Å), 1,3-diaminopropane and 1,3-diamino-2-propranol, showed a very weak effect (6 and 5% inhibition, respectively) as compared with putrescine. Similarly, 1,5-diaminopentane (N—N distance, 7.40 Å) did not exhibit an inhibitory capacity on ODC induction by ornithine. Interestingly, 1-amino-oxo-3-aminopropane, which prevented an increase in ODC activity when Ehrlich ascites tumor cells were reseeded in fresh media [7], was shown to have a N—N distance of 5.94 Å. The results reported here could appear to be inconsistent with many references to the effect of 1,3-diaminopropane or its derivatives on inhibition of ODC induction in both cell cultures and whole animals [31–33]. However, in all cases the effect of 1,3-diaminopropane was observed at concentrations higher than those used in this paper.

The uncharged *N*-acetylpolyamine derivatives are less effective in inhibiting ODC induction. In contrast, *N*-methyl derivatives conserved the inhibitory capacity. One charged amino group of the polyamines seems to be critical in depressing the induction of ODC in the presence of ornithine being very marked in the case of *N*-acetylhistamine, which inhibits 80% less than histamine at the same concentration. According to Porter and Bergeron [34], retention of the charge was consistently found to be an essential feature for molecular function. Moreover, all diamines which showed a down-regulation effect have at least one pK_a value higher than 9.75. In contrast, the pK_a values of 1,4-benzenediamine are 2.67 and 6.16, and it is almost deproteinized at experimental pH 7.4. These findings could explain why the catabolic pathway of natural polyamines is accomplished via terminal *N*-acetyl derivatives [35] which are less active compounds than the biogenic polyamines in the complex feedback regulation of ODC.

The behavior of the histamine receptor blockers chlorpheniramine, doxylamine and cimetidine is also worth mentioning. Only chlorpheniramine, having a 1,4-diamine structure, showed an inhibitory capacity at the used concentration. These data, together with the effect of histamine and serotonin discussed elsewhere [16], confirm that several polyamines could play a similar role to that of putrescine in rapidly dividing tissues [36]. The results concerning the harman family alkaloids harmine and noreleagnine, which conserved the 1,4-diamine structure, indicate that the 1,4-diamine structure is a necessary but not the only condition required for an inhibitory effect on ODC activity in the whole cell. These cyclic diamines showed a rather restricted spatial conformation of the N—C—C—C—C—N carbon skeleton. In the case of harmaline, which showed a very strong effect, it is probable that the Schiff base structure of the C ring opened after protonation at the perifusion pH.

Many efforts have been made in the search for ODC inhibitors in living systems in order to decrease cell growth and proliferation [37, 38]. The results presented here confirm that many natural and

synthetic diamines are able to inhibit strongly the ODC induction at very low concentrations, even in the presence of physiological concentrations of the inducer ornithine. They also suggest some chemical features necessary for achieving the optimal inhibition of ODC induction: 1,4-diamine structure, retention of the charge on one amino group and flexibility of the N—C—C—C—C—N skeleton.

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