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Histamine and serotonin inhibit induction of ornithine decarboxylase by ornithine in perifused Ehrlich ascites tumour cells

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Ornithine induced more than 36-fold the ornithine decarboxylase activity in confined Ehrlich ascites tumour cells after 3.5 h of continuous perifusion with 0.5 mM ornithine; arginine and glutamine also induced the activity 3- and 4-fold, respectively. The addition of cycloheximide or actinomycin D antibiotics to the perifusion medium confirmed that the regulation of the enzyme synthesis takes place at the level of translation. Perifusion in the presence of 0.5. mM ornithine and 55, 25, and 10 μM histamine suppressed the induction by 91, 53, and 35%, respectively. Similar results were obtained in the presence of serotonin. Histidine also showed inhibitory effect but 5 mM histidine was required to produce 21% inhibition; other basic amino acids were ineffective.

Ornithine decarboxylase; Histamine; Serotonin; Perifusion

1. INTRODUCTION

Ornithine decarboxylase (EC 4.1.1.17) is described as the prototype of inducible enzyme [1]. It is the first key regulatory enzyme in the polyamine biosynthesis induced in target tissues by a variety of trophic agents, hormones, pharmaceuticals, amino acids, and cell growth stimuli [2]. The protein has a very rapid turnover [3]. Enzyme production is controlled at the translational level [4] by putrescine and spermidine in regenerating liver, hepatoma cells [5] and Ehrlich ascites tumour cells [6]. In addition, in rat hepatomas a non-competitive protein inhibitor or antienzyme seems to be induced by the presence of putrescine and other polyamines [7,8]. In Ehrlich ascites cell cultures 1,3-diaminopropane and some of its derivatives inhibit the ornithine decarboxylase induction and prevent the polyamine accumulation; the mechanism of action of aminopropanol involves the synthesis of the antienzyme protein [9]. The voluminous literature on the subject has been extensively reviewed by Tabor and Tabor [2], and Pegg [10,11].

In developing HTC hepatomas, the induction of histidine decarboxylase (EC 4.1.1.22) is parallel to the induction of the ornithine decarboxylase; both monofluoromethylhistamine [12] and the H_2 receptor antagonist, cimetidine [13] significantly reduce tumour formation in mice, slowing metastatic development and prolonging survival [14]. Very recently, Burtin et al. [15] reported the inhibition of tumour growth by the injection of histamine in both experimental tumours and cancer patients [16]. The present work was undertaken to clarify the role of histamine and serotonin in mimicking the action of polyamine on ornithine decarboxylase induction. A very useful perifusion system [17], that allows the study of enzyme induction under steady-state conditions, was employed; low concentrations of histamine and serotonin strongly inhibit the induction of ornithine decarboxylase activity by ornithine.

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2. MATERIALS AND METHODS

2.1. *Ehrlich ascites cells*

A hyperdiploid Lettré strain was maintained as previously reported [18]. Mice were inoculated with 5×10^6 tumour cells from different infested animals; cells were harvested on the 10th–11th day after tumour transplantation at the beginning of the stationary growth phase [19]. Animals were killed by cervical dislocation immediately before the ascitic tumour samples were taken. The cells were washed once with 0.9% NaCl, and twice with phosphate saline buffer consisting of 6.16 mM KCl, 154 mM NaCl, 1.65 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, pH 7.4, centrifuged at $500 \times g$ for 5 min. The final cell suspension was 4.2×10^8 cell/ml.

2.2. *Perifusion system*

The perifusion system first described by Van der Meer [20] and improved by Groen et al. [21] was used with the following modifications [17]: the 30 ml cylindrical perifusion chamber was fitted with a 13 mm diameter filter of 8 μm pore size (Sartorius SM M 301) placed at the top of the chamber. A magnetic stirrer at the bottom prevented cell deposition and clogging of the filter for at least 3.5 h of continuous perifusion. The temperature of the chamber was maintained at 37 ± 1°C using an external heater. The perifusion system was previously filled with phosphate saline buffer, to which the appropriate substrates and inhibitors had been added. Buffer temperature was maintained at 37°C by a thermostatically controlled water bath. Tumour cells were quickly introduced into the chamber through the inlet tube to give a final cell concentration of 14×10^6 cell/ml. The flow rate was kept at 0.3 ml/min by means of a Microperpex roller pump (Pharmacia, Sweden). After 3.5 h of continuous perifusion, the erythrosine test revealed that cell integrity was more than 80%.

2.3. *Ornithine decarboxylase assay*

After the required perifusion time, cells were extracted from the chamber, centrifuged at $3000 \times g$ for 120 s, immediately frozen, and stored at −20°C until use. For the ornithine decarboxylase assays frozen cells were resuspended in 1.0 ml of buffer, consisting of 50 mM Hepes, 1 mM EDTA, 10 mM dithiothreitol, 50 μM pyridoxal 5′-phosphate, pH 7.2. The suspension was sonicated (two strokes of 20 s at high frequency ultrasonic oscillations) and then centrifuged at $115\,000 \times g$ for 40 min; all the operations were carried out at 4°C. The supernatants were collected and ornithine decarboxylase activity was determined as described by Mitchell et al. [22]. The final concentration of ornithine was 0.6 mM, including the 18.5 kBq of L-[1-^{14}C]ornithine (spec. act. 2.22 GBq/mmol) added before each assay. The reactions were conducted in 7 ml conical flasks sealed with a rubber stopper from which hung a perforated 0.3 ml Eppendorf tube containing a paper strip (35×2 mm) moistened with 1 M hyamine hydroxide in methanol. The reaction was stopped by the addition of 0.5 ml of 2 M citric acid. After 30 min the tubes containing the hyamine were transferred to scintillation vials for 20 min to allow the hyamine to diffuse into the scintillation liquid. Finally the samples were counted in a LKB Rack Beta (Sweden) scintillation counter. All assays were carried out in duplicate. Proteins were determined by the method of Bradford [23].

3. RESULTS

Fig.1 shows the short-term induction of ornithine decarboxylase activity in perifused Ehrlich ascites cells at different intervals during 210 min of continuous perifusion in the presence of 0.5 mM ornithine. The 3.5 h perifusion time was chosen for all reported experiments. The addition of 0.5 mM ornithine induced more than 36-fold the ornithine decarboxylase activity, as compared with the cells perifused with only saline buffer in the absence of external source of energy or nitrogen. Under these conditions, glucose is avidly consumed by Ehrlich ascites cells [17], but it did not induce the enzyme activity (table 1). The addition of 0.5 mM arginine and 0.5 mM glutamine, the cellular precursors of ornithine, induced the ornithine decarboxylase activity 3- and 4-fold, respectively. The addition of 5 mM glucose to the perifusion medium containing 0.5 mM glutamine significantly decreased glutamine enzyme induction. This result could be explained, by the fact that the production of ornithine by cells incubated in the presence of glutamine was decreased following the addition of glucose (results not shown). In all cases, the presence of cycloheximide suppressed the synthesis of ornithine decarboxylase. Nevertheless, actinomycin D was ineffective.

The addition of near physiological concentra-

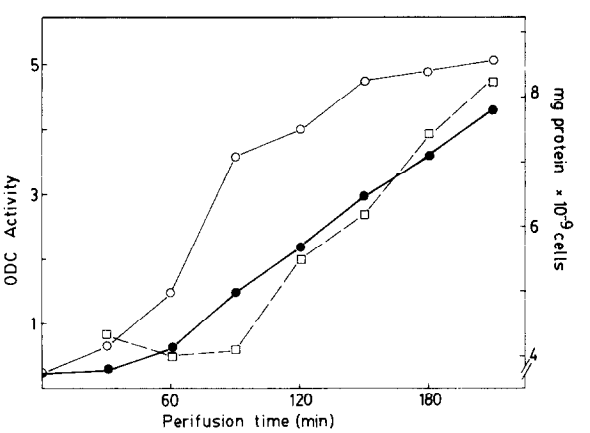

Fig.1. Short-term induction of ornithine decarboxylase by ornithine in perifused Ehrlich ascites tumour cells. Each point represents the means of two different perifusions. Ornithine decarboxylase activity (●) expressed as nmol CO_2/h per 10^8 cells; activity (○) expressed as nmol CO_2/h per mg protein; (□) total protein in the cell-free extract.

Table 1

Induction of ornithine decarboxylase by amino acids in perifused Ehrlich ascites tumour cells

Perifusion conditions	Ornithine decarboxylase activity (nmol CO_2/h per 10^8 cells)			
		Induction (fold)	Actinomycin D (10 μg/ml)	Cycloheximide (250 μg/ml)
Saline buffer	0.12 ± 0.01 (3)	-	N.D.	N.D.
Glucose (5 mM)	0.14 ± 0.01 (3)	-	N.D.	N.D.
Glutamine (0.5 mM)	0.52 ± 0.01 (4)	4.3	0.50 (2)	0.12 ± 0.01 (3)
Glucose (5 mM) + glutamine (0.5 mM)	0.27 ± 0.02 (4)	2.3*	N.D.	N.D.
Arginine (0.5 mM)	0.36 ± 0.03 (3)	3.0	0.39 (2)	0.13 ± 0.03 (3)
Ornithine (0.5 mM)	4.32 ± 0.25 (3)	36.0	4.22 ± 0.11 (3)	0.15 ± 0.01 (3)

N.D., not determined. Control is the value of ornithine decarboxylase activity induced by perifusion with only saline buffer; in parentheses the number of different perifusions of cells obtained from different inoculated animals. The U-Mann Witney no parametric significance was used: * $p < 0.01$ versus the value of ornithine decarboxylase activity by perifusion with only 0.5 mM glutamine

Table 2

Effect of histamine and serotonin on the induction by ornithine of ornithine decarboxylase activity in perifused Ehrlich ascites tumour cells

Perifusion conditions	Ornithine decarboxylase activity (nmol CO_2/h per 10^8 cells)	
		Inhibition (%)
Ornithine (0.5 mM)	4.32 ± 0.25	-
Ornithine (0.5 mM) + histamine (55 μM)	0.49 ± 0.03	91
Ornithine (0.5 mM) + histamine (25 μM)	2.06 ± 0.08	53
Ornithine (0.5 mM) + histamine (10 μM)	2.80 ± 0.10	35
Ornithine (0.5 mM) + serotonin (55 μM)	0.47 ± 0.01	91
Ornithine (0.5 mM) + serotonin (10 μM)	3.25 ± 0.12	24

Control is the value of ornithine decarboxylase activity induced by perifusion with only 0.5 mM ornithine. Values are means of three different perifusions of cells obtained from different inoculated animals

Table 3

Effect of basic amino acids on the induction by ornithine of ornithine decarboxylase activity in perifused Ehrlich ascites tumour cells

Perifusion conditions	Ornithine decarboxylase activity (nmol/h per 10^8 cells)		
		% Increase of control	% Decrease of control
Ornithine (0.5 mM) + lysine (0.5 mM)	4.34 (2)	-	-
Ornithine (0.5 mM) + tryptophane (0.5 mM)	4.58 (2)	8	-
Ornithine (0.5 mM) + citrulline (0.5 mM)	4.44 (2)	5	-
Ornithine (0.5 mM) + arginine (0.5 mM)	4.73 (2)	10	-
Ornithine (0.5 mM) + histidine (0.5 mM)	3.70 ± 0.05 (3)	-	13*
Ornithine (0.5 mM) + histidine (5 mM)	3.39 ± 0.07 (3)	-	21**

Control is the value of ornithine decarboxylase activity induced by perifusion with only 0.5 mM ornithine; in parentheses the number of different perifusions of cells obtained from different inoculated animals. The U-Mann Witney no parametric significance was used: * $p < 0.05$ versus control; ** $p < 0.01$ versus control

tions of histamine [24] suppressed the induction caused by ornithine, when this was present in the perifusion medium in concentrations at least 10-times those of histamine (table 2). Different concentrations of histamine produced a clear dose-response effect. Similar results were obtained in the perifusion experiments carried out in the presence of serotonin. Histidine, the cellular precursor of histamine also inhibited the induction of ornithine decarboxylase activity, but higher concentrations of this amino acid were needed (table 3). Indeed, 5 mM histidine were required to produce 21% inhibition. On the other hand, basic amino acids such as arginine, lysine, tryptophan and citrulline were ineffective, even when present in concentrations ten times those of ornithine. Moreover, in the presence of arginine, an additive effect was observed, because arginine alone (table 1) induced the ornithine decarboxylase activity.

4. DISCUSSION

The perifusion technique has several advantages over that of batch incubation in that it allows the continuous solution changes and also the continuous removal of the reaction products. In those cases in which product inhibition occurs, batch incubation may give misleading results. Using the perifusion technique, the study of enzyme induction may be carried out under steady-state conditions. Kanamato et al. [25] recently reported that asparagine is the most effective amino acid in eliciting the ornithine decarboxylase activity in primary cultures of hepatocytes. The degree of induction depends on the concentrations of asparagine; however, 10 mM asparagine are needed to produce a 20-fold increase of activity. On the other hand, using the perifusion technique, 0.5 mM ornithine added to the saline buffer induced ornithine decarboxylase activity 36-fold in Ehrlich ascites cells. This concentration of ornithine is similar to the concentration of ornithine found in tumour cells (unpublished results).

The complete suppression of the induction caused by cycloheximide clearly indicates that the enzyme protein was synthesises 'de novo' after 3.5 h of continuous perifusion in the presence or ornithine; the fact that actinomycin D had no effect confirms once more that the ornithine decarboxylase induction is regulated at the translation level [4,11,25,26]. Many biological and synthetic amines are described as having a feedback control effect on the ornithine decarboxylase synthesis [6,7,27]; the histamine and serotonin effect is observed at micromolar concentrations in the presence of relatively high concentrations of the ornithine inductor. The inhibitory effect of the amino acid histidine is probably caused via histamine, because cancer cells show an inducible histidine decarboxylase activity [28]. This effect of histidine on the ornithine decarboxylase induction might explain the inhibition of cell growth caused in cultured Ehrlich ascites cells by histidine [29].

The present results appear to explain the experimental results of Burtin et al. [30] who have reported that tumour growth decreases and survival rate increases following injections of histamine or serotonin in mice methylcholanthrene-induced fibrosarcomas. Bartholeyns and Fozard [31] have postulated that histamine could play a similar role to the biogenic amines in rapidly growing tissues. The present results confirm this hypothesis and show that histamine and serotonin control the induction of the ornithine decarboxylase activity and, in consequence, cell growth.

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