Changes in L-ornithine decarboxylase activity in regenerating lung lobes

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Growth of regenerating lung lobes is preceded by an increase of the activity of L-ornithine decarboxylase (ODC). Mechanical factors play a role in determining both the growth rate and enhancement of activity of ODC in regenerating lung lobes. Hormonal regulation also appears of importance as shown by the increased ODC activity in a transplanted lobe.

Regeneration *Transplantation* *Lung lobe* *Ornithine decarboxylase*

1. INTRODUCTION

During the last several years significant progress has been made concerning the regulatory role of L-ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17 [ODC]) in the biosynthesis of polyamines. Increased activity of the enzyme was observed in regenerating liver [1–4], compensatory growing kidney [5,6] and hypertrophic cardiac muscle [7,8]. On the other hand, compensatory growth of the remaining lung after pneumonectomy is a well-known phenomenon, but the mechanism of this process remains unelucidated. The growth is primarily due to the proliferation of type 2 pneumocytes which differentiate into type 1 pneumocytes [9]. During this process various biochemical changes could be observed [9–11]. Mechanical factors (e.g., increased distension caused by the absence of the neighbouring lobe) [12] or humoral factors [13–15] may play a role during the induction of the mitosis of type 2 pneumocytes. Here, changes in ODC activity of the remaining lobes have been investigated during regeneration.

2. MATERIALS AND METHODS

2.1. *Surgical procedure*

Studies were carried out in inbred BALB/c female mice weighing 18–20 g, kept in conventional laboratory environment, fed on a semisynthetic diet (Lati, Gödöllö, Hungary) and tap water ad libitum. For lobectomy and sham-operation the animals were anesthetized with an injection of 30 mg/kg Nembutal (Abbot, Saint Remy), and cannulated into the trachea through the mouth. The cannula was connected to a rodent respirator (Kutesz, Budapest) set to deliver a tidal volume of 0.5 ml at a rate of 50 breaths per min. After alcohol cleansing of the skin, the left chest was opened through a lateral incision in the fifth intercostal space. The upper lobe of the left lung was isolated, tied at the hilum and removed. Only after the thorax had been closed by careful suture of muscles and skin were the animals extubated. The sham-operation consisted of a thoracotomy with collapse and ventilation of the lungs without resection.

To determine the enzyme activity, the lungs were perfused with physiological saline in situ, removed, weighed, homogenized, centrifuged (20000 × *g*, 30 min, 5°C) and the supernatant was assayed for ODC activity.

2.2. *Chemicals*

Pyridoxal 5-phosphate and dithiothreitol were purchased from Serva (Heidelberg). DL-[1-^{14}C]Or-

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Table 1

Lung lobe weights in BALB/c female mice

Experimental group	Body weight (g)	Right upper lobe	Right middle lobe	Right low lobe	Left upper lobe	Left low lobe	Total lung weight
Intact mice (6)	21.5 ± 0.2	20.7 ± 0.4	16.3 ± 0.7	29.4 ± 0.6	42.4 ± 1.0	11.6 ± 0.3	120.2 ± 2.1
Sham-operated mice (5)	21.1 ± 0.7	22.5 ± 1.1	16.3 ± 0.9	30.4 ± 0.5	43.8 ± 1.5	11.3 ± 0.5	124.3 ± 1.3
Lobectomized mice, 14 days after surgical procedure (5)	19.7 ± 0.5	30.4[a] ± 0.6	24.6[a] ± 0.4	41.6[a] ± 0.4	–	24.7[a] ± 1.2	122.6 ± 2.1

[a] Compared to sham-operated animals $p < 0.01$

Lobe weights are expressed in mg ± SE wet weight

nithine·HCl (spec. act. 1.57 mCi/mmol) was obtained from the Isotope Institute of Hungarian Academy of Sciences (Budapest). All other compounds were Reanal (Budapest) products of reagent grade.

2.3. *Ornithine decarboxylase assay*

The method in [2] was applied with the following modifications. Samples were homogenized with a Potter-type Teflon homogenizer in 4 vols ice-cold 100 mM phosphate buffer (pH 7.2) containing 5 mM dithiothreitol and 5 mM pyridoxal 5-phosphate, then centrifuged at 20000 × *g* for 30 min at 5°C and aliquots of the clear supernatant were assayed. The assay mixtures without substrate were preheated to 37°C under constant shaking. After adding the substrate the vials were sealed air tight with rubber stoppers from which a strip of filter paper impregnated with 50 μl (1.0 M) hyamine hydroxide was suspended. The reaction proceeded for 30 min at 37°C, and was stopped by injecting 0.2 ml of 50% (w/v) trichloroacetic acid. Shaking was continued for 60 min to make the absorption of $^{14}CO_2$ complete. Paper strips were placed into scintillation vials containing 5 ml Tritosol liquid scintillation solution. Radioactivity was determined by an ISOCAP/300 Nuclear Chicago (USA) scintillation spectrometer. The protein content of the supernatant was determined as in [16] and the activity of ODC expressed as pmol $^{14}CO_2$/h per mg protein.

3. RESULTS AND DISCUSSION

To examine the kinetics of lung regeneration, the left upper lobe was removed, and on the 14th

Table 2

ODC activities in lungs intervals of BALB/c female mice at various intervals

Experimental group	Time (h)						
	0	4	8	12	24	48	72
Intact mice (6)	5.4 ± 1.5	5.4 ± 1.4	4.8 ± 0.8	4.9 ± 0.7	7.0 ± 0.9	7.6 ± 1.1	4.0 ± 0.9
Sham-operated mice (5)	5.4 ± 1.5	5.7 ± 1.6	6.7 ± 1.8	3.4 ± 0.6	8.9 ± 2.1	4.6 ± 0.9	6.6 ± 1.1
Lobectomized mice (6)	5.4 ± 1.5	24.5[a] ± 5.4	14.0[a] ± 3.2	11.8[a] ± 2.6	9.3 1.9	12.8[a] ± 2.7	7.8 ± 1.8

[a] Compared to sham-operated animals $p < 0.01$

ODC activities are expressed in pmol·h^{-1}·mg $protein^{-1}$ ± SE

Table 3

ODC activities in lung lobes of BALB/c female mice

Experimental group	Right upper lobe	Right middle lobe	Right low lobe	Left upper lobe	Left low lobe
Intact mice (5)	8.3 ± 0.2	5.2 ± 2.4	7.4 ± 0.6	6.8 ± 1.6	8.8 ± 1.5
Sham-operated mice (5)	10.2 ± 0.2	6.9 ± 1.8	6.9 ± 1.6	7.5 ± 1.5	8.6 ± 1.5
Lobectomized mice, 4 h after surgical procedure (5)	25.8[a] ± 8.6	18.9[a] ± 4.1	14.6[a] ± 4.9	–	37.1 ± 4.3

[a] Compared to sham-operated animals $p < 0.01$

ODC activities are expressed in pmol·h^{-1}·mg protein^{-1} ± SE

day following operation the weight of the remaining 4 lung lobes was measured and compared to that of intact, and sham-operated mice of the same age (table 1). On the 14th day after removing the left upper lobe, the remaining lobes reached the original weight of the whole lung before operation. The participation of the 4 lobes in the compensatory growth was disproportionate, however. Each lobe of the right lung showed a 30–50% increase in weight, while the most distended left lower lobe doubled in weight compared to the original.

These results indicate that mechanical factors play a role in determining the growth of regenerating lung lobes.

The results in table 2 indicate that the enzyme activity increased rapidly and levelled at the 4th hour after lobectomy, then dropped near to the normal value by the 72nd hour thereafter. The enhancement of ODC activity seems to be characteristic of all growing organs, including the remaining lung lobes (table 2).

The ODC activity of all remaining lobes was determined separately at the 4th hour after lobectomy. The participation of the 4 lobes in the enhancement of ODC activity was disproportionate. Whereas each lobe of the right lung showed a 30–50% increase of the original ODC activity, the enzyme activity of the most distended left lower lobe was enhanced 5-fold compared to its original level. These results indicate that a correlation exists between growth rate and ODC activity of individual pulmonary lobes (table 3).

In another set of experiments left lower lobe fragments obtained from sister BALB/c mice were transplanted under the renal capsule of BALB/c

Table 4

ODC activities in its own and in the transplanted left lower lung lobes of BALB/c female mice

Experimental group	Left lower lobe in situ	Transplanted left lower lobe
Sham-operated mice (5)	4.9 ± 0.9	4.5 ± 0.1
Lobectomized mice, 6 h after surgical procedure (5)	33.4[a] ± 4.9	13.7[a] ± 2.8

[a] Compared to sham-operated animals $p < 0.01$

ODC activities are expressed in pmol·h^{-1}·mg protein^{-1} ± SE

host animals. Two weeks after the successful transplantation, the left upper lobe of the host animal was removed and ODC activity of the in situ left lower lobe and of the graft was determined, 6 h after lobectomy. An enhancement of ODC activity was observed in both lung lobes. Thus, it can be supposed that humoral factor(s) released from the distended lobes may also have an effect on the ODC synthesis (table 4).

Our results are consistent with the idea that (i) the regeneration of lung lobes is associated with an increased ODC activity; (ii) both mechanical and humoral factors can elicit the growth of the remaining lobes.

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