PHOSPHOLIPID BIOSYNTHESIS IN THE LUNGS IN CHRONIC INFLAMMATORY BRONCOPNEUMONIA

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Data in the literature on the important physiological role of surfactants in the lung have prompted research workers to study more closely the principles governing formation of the surfactant complex in inflammatory diseases of the lungs and changes in their content of the principal lipid component, namely phospholipids (PL). In previous experiments [1] the writers showed a marked fall in the content of individual PL in the lungs during the development of chronic nonspecific lung diseases (CNLD).

To identify the precise location of lesions in the chain of reactions involved in phosphatide production, it was decided to study the state of the glycerokinase and glycolytic pathways of PL formation. For this purpose, activity of glycerokinase (GK), glycerophosphate dehydrogenaee (GPDH), and glycerophosphate acyltransferase (GPAT) activity, the concentration of glycerophosphate (GP) and dihydroxyacetone phosphate (DHAP) in the lung tissue, and also the intensity of incorporation of ^{14}C-glucose *in vivo* into PL of the surfactants were studied under normal conditions and in chronic bronchopneumonia (CBP).

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 2-3 kg. CBP was produced by the method described previously [3]. The animals were killed under thiopental anesthesia 5-6 months after reproduction of the disease. GK, GPDH, and GPAT activity and DHAP and GP concentrations were determined in the supramitochondrial fluid [6, 8-11].

Uniformly labeled ^{14}C-glucose was injected intraperitoneally in a dose of 300 μCi/kg body weight, and the duration of radioactive exposure was 60 min.

The surface-active fraction (surfactant complex) was isolated by differential centrifugation [5]. PL were fractionated by thin-layer chromatography [4] in the writers' modification [2]. Incorporation of radioactivity into the composition of PL was measured on an SL-4221 scintillation spectrometer (Intertechnique, France) in Bray's scintillator [7], after preliminary solubilization of the samples in Protosol (from New England Nuclear Corp., USA). The data were converted into absolute units of radioactivity (CPM) by the external standardization method and were analyzed by computer to obtain the final results.

EXPERIMENTAL RESULTS

Comparison of the reaction velocity of reduction of DHAP into GP and of activation of free glycerol in the lung tissue of intact animals showed significant differences in the intensity of these reactions (Table 1). GPDH activity was about ten times higher than GK activity, and with the appearance of CBP, GPDH activity was greatly inhibited, against the background of the almost total loss of GK activity in the lung tissue (Table 1). In only 4 of 19 cases, incidentally, could a very low level of activity (0.07 ± 0.02 μmole/g) of GK be found. Inhibition of the glycerokinase and glycolytic pathways of formation of GP, the key metabolite of lysogenesis, was accompanied by a sharp decline of its level in the lung tissue and an appreciable increase (by 53.3%) of the DHAP concentration in it. Against this background a marked reduction (by 47.7%) was observed in the activity of GPAT, whose

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TABLE 1. Changes in Activity of Enzyme Systems and Level of Metabolites of Lipogenesis (in μmoles NADH and palmitoyl-CoA respectively) in Lung Tissue of Rabbits (M±m)

Parameter	Control	CBP
GPDH	1,71±0,09 (10)	1,01±0,06 (16)
GK	0,17±0,02 (9)	0 (15)
GPAT	7,38±0,17 (7)	3,86±0,20* (7)
GP	0,16±0,02 (8)	trace (17)
DHAP	1,20±0,05 (11)	1,84±0,07* (15)

Legend. Number of animals in parentheses. *P < 0.001.

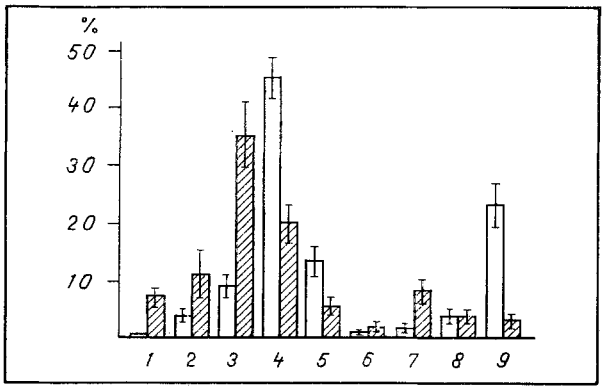

Fig. 1. Incorporation of ^{14}C-glucose *in vitro* into individual PL of surfactant in CBP. Ordinate, intensity of incorporation of label (in per cent of total). 1) lysophosphatidylcholines, 2) phosphoinositol, 3) sphingomyelins, 4) phosphatidylcholines, 5) phosphatidylethanolamines, 6) phosphatidylserines, 7) phosphatidic acid, 8) diphosphoglycerate, 9) phosphatidylglycerols. Unshaded columns — control, shaded — experiment.

regulatory role in lipid metabolism is responsibility for reactions of conversion of GP into phosphatidic acid (PA). The results described above are evidence of inhibition of these particular stages of PL biosynthesis in lung tissue in this pathology.

As the results of subsequent investigations showed (Fig. 1), the development of CBP also was accompanied by marked inhibition of incorporation of ^{14}C-glucose into the composition of the PL-surfactant *in vivo*. The distribution of radioactivity under these circumstances between individual PL was as follows: on the one hand, a decrease in the intensity of incorporation of label was observed into the composition of phosphatidylcholines, phosphatidylglycerols, and phosphatidylethanolamines, on the other hand there was intensification of incorporation of the label into spingomyelins and lysophosphatidylcholines.

The results of this investigation thus show that CBP is characterized by inhibition of synthesis of most phospholipids — the most important components of the surface-active substances of the lungs. This inhibition is due mainly to a decrease in the rate of the glycolytic and glycerokinase pathways of GP formation and delay of its subsequent conversion into PA. The possibility likewise cannot be ruled out that the fall in the PL level is due to a certain degree of intensification of lipolysis in the lungs, which was demonstrated in the writers' previous investigations, in which a marked increase in activity of phospholipase A_2 was observed, with release of large quantities of lysophosphatidylcholines and free glycerol into the lung tissue [1].

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INTERACTION BETWEEN THE THIRD FRACTION OF THYMOSIN AND OPIATE RECEPTORS

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Proof of the existence of opiate receptors of lymphocytes is based both on data showing changes in the state of the immune system in patients with opiate addiction and in persons treated with opiates for a long time [9, 10] and on the results of experiments *in vitro*. It has been shown, in particular, that endogenous and exogenous opiates affect the level of E-rosette formation [14], the cAMP concentration in the lymphocytes [1, 3], and the proliferative response of these cells, stimulated by phytohemagglutinin (PHA) [7].

The functional role of lymphocyte opiate receptors is probably determined by interaction with opioids found in the composition of interferon [5, 6], a bone marrow humoral factor stimulating antibody production [2, 4], and also with opioids synthesized by the adrenals and other endocrine glands [13]. Meanwhile, the possible role of biologically active substances of this group in the action of the thymus on development of the immune response has not hitherto been investigated.

The aim of the present investigation was to test the hypothesis that the unpurified thymosin fraction contains substances which interact with opiate receptors.

EXPERIMENTAL METHOD

The third fraction of thymosin (T_3), isolated from calf thymus by Goldstein's method [8], was used. To analyze the ability of T_3 to interact with opiate receptors of rat brain the method of competitive replacement of ^{3}H-naloxone and ^{3}H-morphine by the T_3 preparation was used.

The membrane fraction was obtained from the brain of male Wistar rats weighing 200-250 g by a modified Simantov's method [12], as described previously [4].

The reaction mixture, in a volume of 1 ml, contained 0.7 ml of a membrane suspension of protein, 4 nM of labeled morphine or naloxone, and 50 µg of bacitracin. To displace the label with the T_3 preparation, it was used in concentrations of 5 µg/ml to 6 mg/ml. The amount of specific binding of the label was determined as the difference in binding of ^{3}H-

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