CHANGES IN THE LUNG SURFACTANT OF RABBITS DURING ENDOTRACHEAL HALOTHANE ANESTHESIA

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UDC 615.212.7.015.4:612.215.3

Endotracheal halothane anesthesia without any attendant operation in rabbits for 3.5 h causes changes in the surface-active properties of the surfactant, with an increase in the surface tension of lung washings as measured on Wilhelmy scales. A study of phospholipid fractions in lung washings by thin-layer chromatography on silica gel showed a decrease in phosphatidylcholine, one of the principal structures of the surface-active lipoprotein, and a simultaneous increase in the lysolecithin fraction. Determination of the content of free fatty acids in lung washings after halothane anesthesia showed a significant increase over the control. The possible mechanism of the harmful action of halothane anesthesia on the lung surfactant is discussed.

KEY WORDS: *lung; halothane anesthesia; surfactant; phosphatidylcholine.*

Experiments on human volunteers and animals have shown that various inhalation anesthetics, in the absence of any operation, can cause the development of foci of atelectasis in the lungs [1]. The alveolar surface of the lungs in mammals is covered with a film of surface-active material (surfactant), which consists mainly of lipids and certain proteins. This film is important for stabilization of the alveoli. In its absence the alveoli collapse [11, 12].

The object of this investigation was to study the state of the lung surfactant during inhalational anesthesia with halothane, without any attendant operation, and to assess the possible mechanisms of destruction of this surfactant.

EXPERIMENTAL METHOD

In rabbits of both sexes weighing 2.5-3.0 kg the trachea was intubated under intravenous pentobarbital anesthesia (40 mg/kg). In a state of apnea (tubocurarine, 200 μg/kg intravenously) the control animals were killed whereas the experimental animals were switched to endotracheal halothane anesthesia (100 ml halothane, air flow 6 ml/min) with artificial ventilation of the lungs (2.5 breaths/min, pressure at the height of inspiration 7-8 mm Hg). The depth of anesthesia was monitored by the state of the ocular reflexes. The animals were decapitated after 3.5 h and the lungs removed and weighed, and placed in a vacuum (—0.8 atm). After extirpation of the lung all procedures were carried out at 0-5°C. Washings were obtained through the trachea by injecting physiological saline 3 times into the lungs in a volume equivalent to 1/10 of the weight of the organ, 85-90% of the quantity injected being returned. The surface tension of the washings was measured on Wilhelmy scales by determining the force required to detach a glass plate (24 × 24 × 1 mm) from the surface of the subphase at 37°C, expressed in dynes/cm. Compression of the surface film by the moving barrier from 375 to 7.5 cm^2 took 108 sec. The essential property of the surfactant is its ability to reduce the surface tension of the subphase below 12 dynes/cm [3]. Lipids were extracted by the method in [7] and phospholipids were fractionated by thin-layer chromatography on silica gel using a system of chloroform:methanol:water (65:25:4). Total phospholipids and the phospholipid fractions were determined from their phosphorus content [5], protein by the biuret method, and the free fatty acids by conductometric titration [2].

Central Research Laboratory, Department of Normal Physiology, Tadjik Medical Institute, Dushanbe. (Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 10, pp. 423-425, October, 1977. Original article submitted April 15, 1977.

0007-4888/77/8410-1419$07.50

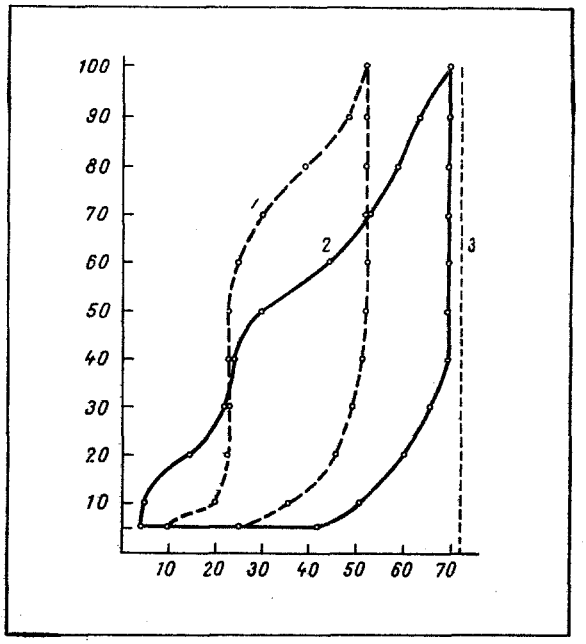

Fig. 1. Surface tension as a function of surface area of film of lung washings from control and experimental rabbits. Abscissa, surface tension (in dynes/cm); ordinate, surface area of film (in %); 1) experiment; 2) control; 3) distilled water.

EXPERIMENTAL RESULTS

During titration of samples of lung washings from the control and experimental rabbits with the aid of Wilhelmy scales a significant change was found in the surface-active properties of the surfactant. Starting from the volume of the sample and the area it occupied with the barrier in different positions, the minimal quantity of surfactant, expressed in micrograms total phospholipids per square centimeter of surface, which caused a reduction of surface tension to below 12 dynes/cm in the control and experimental animals was calculated. The results of the measurements show that to reduce the surface tension below 12 dynes/cm in the six experimental animals 2.1 times more of the test substance was required than in the same number of control animals: 5.04 ± 0.28 and 2.48 ± 0.38 μg total phospholipids/cm^2 respectively ($P < 0.01$).

The graph of surface tension as a function of surface area of the film in samples from the experimental rabbits shows flattening of the hysteresis loop, characteristic of substances with lower surface-active properties (Fig. 1).

The results indicate that halothane anesthesia causes changes in the surface-active properties of the surfactant, with a consequent increase in the surface tension of the surfactant of the alveolar structures. A change in its surface-active properties and an increase in surface tension under experimental conditions were described previously during inhalation of pure oxygen [6], prolonged inhalation of nitrous oxide [4], and hyperventilation with large respiratory volumes [8]. All the above procedures led to the development of atelectatic changes in the lung tissue of the animals.

The main structure with which the surface-active properties of the mammalian lung surfactant are connected is dipalmitol-phosphatidylcholine [11]. The surface-active lipoprotein isolated from the lung is very rich in it [10]. Phosphatidylcholine accounts for up to 80% of the lipids present in the lipoprotein, and 60% of the phosphatidylcholine is saturated dipalmitol-phosphatidylcholine, whereas the phosphatidylcholine of the parenchymatous tissue of the lung is a mixture of various structures [14].

Lysolecithin, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, and unidentified fractions, described as X fractions, were determined in the lung washings. Comparison of the quantities of total phospholipids in the control and experimental animals showed no significant differences (11.68 ± 1.81 and 11.73 ± 0.66 μg/ml washings respectively). Com-

TABLE 1. Fractions of Phospholipids (in % of total phospholipids) in Washings from Rabbits' Lungs (M ± m)

Group	Lysolecithin	Sphingomyelin	Phosphatidylcholine	Phosphatidylethanolamine	X-fractions
Control (n = 6)	2,3±0,16	4,7±0,69	82,5±1,97	9,8±1,81	1,3±2,11
Experimental (n = 6)	4,8±0,54	6,7±0,52	77,5±1,06	10,4±0,94	1,0±0,63
P	<0,01	<0,05	<0,05	>0,05	>0,05

parison with respect to fractions, however, showed a significant decrease in the phosphatidylcholine level and a simultaneous increase in lysolecithin (Table 1) in the experimental group.

The mechanism whereby the surfactant is inactivated is not clear. With the aid of radioactive labels for palmitic acid and glucose, the half-life of phosphatidylcholine has been found to be approximately 14 h [10]. The view has been expressed that phospholipase A_2, an enzyme widely distributed in cellular and subcellular membranes, including those of alveolar macrophages, plays a role in the destruction of the phosphatidylcholine of the surfactant [9]. The activity of the lung phospholipase A_2 is much higher than that of the enzyme from liver tissue [13]. Phospholipase A_2 is known to rupture the ester bonds at the 2nd carbon atom of the phosphatide group with the formation of lysophosphatidylcholine and free fatty acids. The quantity of lysophosphatidylcholine and the content of free fatty acids are thus indirect indicators of phospholipase A_2 activity.

Measurement of the concentration of free fatty acids in washings from the rabbits' lungs after halothane anesthesia showed a significant increase (control 6.45 ± 0.32, experiments 8.42 ± 0.14 meq/ml washings; $P < 0.01$). It can tentatively be suggested that the reduction in the phosphatidylcholine content with a simultaneous increase in the lysolecithin fraction and in the level of free fatty acids in the lung washings is due to increased phospholipase A_2 activity.

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