Respiration 49: 187–194 (1986)

0025-7931/86/0493-0187 $ 2.75/0

Role of Hypocapnia in the Alveolar Surfactant Increase Induced by Free Fatty Acid Intravenous Infusion in the Rabbit[1, 2]

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Key Words. Free fatty acid · Alveolar surfactant · Disaturated phosphatidylcholine · Respiratory failure · Hyperventilation · Hypocapnia

Abstract. Intravenous infusion of free fatty acid (FFA) produces an increase in the alveolar surfactant pool of the rabbit and pulmonary edema, hyperventilation, hypoxemia and hypocapnia. Previous studies suggested that alveolar PCO_2 would be a regulator of intracellular storages of surfactant. In order to study the role of hypocapnia in the increase of lung surfactant in our experiments we administered 20 mg FFA · kg^{-1} · min^{-1} i.v. to rabbits breathing room air (n = 10) or 5% CO_2, 21% O_2, 74% N_2 (n = 7).

Disaturated phosphatidylcholine (DSPC) was determined in bronchial-alveolar lavage fluid as index of alveolar surfactant content, 5% CO_2 in the inspired air prevented the hypocapnia and blocked the increase in DSPC induced by FFA ($p < 0.01$). Pulmonary edema post-FFA was not changed by 5% CO_2 administration.

We conclude that hypocapnia produced by hyperventilation during FFA infusion would be an important factor in the increase of DSPC observed after FFA infusion.

Intravenous infusion of free fatty acids (FFA) in the rabbit in a lethal dose (20 mg · kg^{-1} · min^{-1}) and in a short term (less than 15 min) increases alveolar surfactant content and induces pulmonary edema, hypoxemia, hyperventilation and a decrease in lung compliance [1].

A depletion of the intracellular storages of surfactant (lamellar bodies) from type II alveolar cells, has been found in dogs subjected to lung injury by pulmonary artery occlusion [2]. Lately, *Baritussio and Clements* [3] demonstrated an increase in the alveolar pool size of disaturated phosphatidylcholine (DSPC) in rabbits during the first 2 h of unilateral pulmonary artery occlusion (UPAO). Interestingly, ventilation with 5–6% CO_2 virtually eliminates the development of alveo-

[1] This study was presented at the 26th Annual Meeting of the Sociedad de Biologia de Chile, Punta de Tralca, Chile, Nov. 1983.

[2] Supported by Grant M-1436 from Departamento de Investigación y Bibliotecas, Universidad de Chile.

lar edema [4] and prevented lamellar body depletion from alveolar type II cells after UPAO [2]. Therefore it was postulated that low levels of alveolar PCO_2 following UPAO are related with the lung injury as well as lamellar body depletion.

Using excised dog lungs to evaluate the effects of alveolar CO_2 tension on the lamellar body density of type II alveolar cells, *Shepard* et al. [5] found that alveolar hypocapnia appears to be the major environmental factor mediating the depletion of lamellar bodies.

In our previous experiments, FFA injected i.v. in the rabbit increased alveolar surfactant pool and produced hypocapnia in arterial blood samples obtained at the end of FFA infusion [1]. Therefore we decided in this study to determine the role of alveolar hypocapnia in both the increase in alveolar surfactant pool and in the pulmonary edema induced by a lethal dose of FFA i.v. in the rabbit. To study this problem we administered 20 mg FFA · kg^{-1} · min^{-1} to rabbits breathing room air or a gas mixture containing 5% CO_2, 21% O_2 and 74% N_2.

Methods

As in our other studies [1] we used male rabbits weighing about 2.5 kg, which where anesthetized with sodium pentobarbital 30 mg · kg^{-1} injected into a marginal vein of one of the ears. Anesthesia was maintained during the experiment by injecting small doses of pentobarbital i.v. We performed tracheostomy and we placed catheters into the femoral artery and vein as well as into the pleural space. A Fleisch pneumotachograph was connected to the tracheostomy cannula, in order to get a continuous record of respiratory rate, airflow and tidal volume in a Gilson polygraph. Intrapleural and arterial blood pressures were also recorded on the polygraph by using appropriate Statham transducers. PO_2, PCO_2 and pH were measured periodically in arterial blood samples by means of an IL-213 blood gas analyzer (Instrumentation Laboratories, Lexington, Mass.). All of the measurements were done while rabbits were breathing spontaneously.

After 1 h of control measurements, the following experimental protocols were performed: (A) *Control vehicle:* rabbits breathing room air (n=9) in which only the vehicle of FFA (ethanol: 1% Na_2CO_3 = 1:9 v/v) was injected through a femoral vein at a rate of 0.6 ml · min^{-1} during 15 min. (B) *Control vehicle breathing 5% CO_2:* the vehicle of FFA was injected at the same infusion rate and for the same period of time as described for series 'A' meanwhile the rabbits (n=6) were breathing a gas mixture containing 5% CO_2, 21% O_2 and 74% N_2. (C) *FFA i.v. breathing room air:* a FFA mixture containing 74.5% of oleic acid and other FFA: 26.5% (gas liquid chromatography analysis) was injected intravenously in a dose of 20 mg · kg^{-1} · min^{-1} at a rate of 0.6 ml · min^{-1}. The mean survival time of this series (n=10) was 10.15 min (± 2.67 SD). (D) *FFA i.v. breathing CO_2:* we administered 20 mg FFA · kg^{-1} · min^{-1} i.v. to rabbits (n=7) breathing a gas mixture containing 5% CO_2, 21% O_2 and 74% N_2. The mean survival time of this series was 8.11 min (± 3.22 SD).

The rabbits were killed by the FFA i.v. infusion or by an overdose of pentobarbital and lungs were removed, degassed and weighed. We then washed them three times through the airways, using 0.9% NaCl at total lung capacity (9 ml · g^{-1} of fresh lung) at room temperature. The three pulmonary lavage fluids were pooled and DSPC was determined [6]. We also determined the protein content [7] and the total number of cells in the pulmonary lavage fluid by using crystal violet and a Spencer hemocytometer chamber. Lung weight/body weight ratio was determined in all the experiments as an index of lung water content. Determinations in lung lavage fluid were expressed per kilogram of body weight because of the presence of pulmonary edema.

A histological study of the lung was carried out in one experiment of each one of the experimental protocols. Lungs were injected with 5% formalin through the airways until total lung capacity and then they were immersed in 20% formalin. Lung sections were stained with hematoxylin and eosin for the light microscopy study.

In the statistical analysis of the results we used

Table I. Arterial blood gases and pH in rabbits injected i.v. with FFA breathing room air and 5% CO_2 and 21% O_2

	$PaCO_2$, mm Hg			PaO_2, mm Hg			pH		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
Vehicle of FFA[a]									
Room air	29		28	74		76	7.508		7.491
(n = 9)	(9)		(8)	(22)		(20)	(0.053)		(0.146)
5% CO_2, 21% O_2, 74% N_2[b]	31	36[c]	32[c]	70	83[c]	82[c]	7.451	7.397[c]	7.418
(n = 6)	(5)	(8)	(9)	(4)	(5)	(7)	(0.035)	(0.070)	(0.061)
FFA 20 mg·kg^{-1}·min^{-1}[a]									
Room air	24		18[c]	72		51[c]	7.491		7.524
(n = 8)	(10)		(9)	(11)		(14)	(0.105)		(0.108)
5% CO_2, 21% O_2, 74% N_2[b]	37	41	37	73	97[c]	74[d]	7.451	7.401[c]	7.388[c]
(n = 6)	(4)	(3)	(5)	(18)	(19)	(20)	(0.020)	(0.038)	(0.066)

Each value represent the mean, in parentheses its standard deviation. S1 = basal level; S2 = after 5 min breathing 5% CO_2; S3 = at the end of FFA or vehicle infusion.

[a] Paired Student's t test.

[b] Analysis of variance for repeated samples.

[c] Significant as compared to S1.

[d] Significant as compared to S2.

paired and unpaired Student's t test, χ^2 test, analysis of variance, Newman-Keuls test and analysis of variance for repeated samples according to the case [8]. A p value less than 0.05 was considered significant.

Results

Administration of 5% CO_2 in the gas mixture for breathing was able to prevent the hypocapnia observed after FFA i.v. infusion in rabbits breathing room air (table I). In regard to PaO_2, administration of 5% CO_2 was able to keep the initial value at the end of FFA infusion (table I). In general, pH values in arterial blood presented small but significant changes; 5% CO_2 in the inspired air increased ventilation in series treated with FFA as well as in series receiving the vehicle of FFA, although 5% CO_2 in the inspired air did not change the significant decrease in lung compliance observed after FFA infusion in rabbits breathing room air (table II).

There was a significant increase in protein content in lung lavage fluid in the series injected with FFA (fig. 1). We found significant differences in lung weight/body weight ratios in animals injected with FFA breathing room air as compared to controls (receiving the vehicle of FFA). Administration of 5% CO_2 in the inspired air did not significantly change lung

Table II. Ventilation and lung compliance of rabbit injected i.v. with FFA breathing room air and 5% CO_2 and 21% O_2

	Ventilation, $ml \cdot kg^{-1} \cdot min^{-1}$		Compliance, $ml \cdot kg^{-1} \cdot cm\ H_2O^{-1}$	
	initial	final	initial	final
Control vehicle				
Room air	419	449	1.6	1.5
(n = 9)	(183)	(123)	(0.8)	(0.5)
5% CO_2, 21% O_2, 74% N_2	521	699*	1.4	1.3
(n = 5)	(63)	(73)	(0.4)	(0.5)
FFA 20 $mg \cdot kg^{-1} \cdot min^{-1}$				
Room air	404	613*	1.6	0.9*
(n = 7)	(130)	(197)	(0.4)	(0.5)
5% CO_2, 21% O_2, 74% N_2	456	561	1.6	0.7*
(n = 7)	(133)	(95)	(0.5)	(0.3)

Each value represents the mean, in parentheses its standard deviation. Initial = basal level; final = at the end of FFA or vehicle infusion. * $p < 0.05$ paired Student's t test.

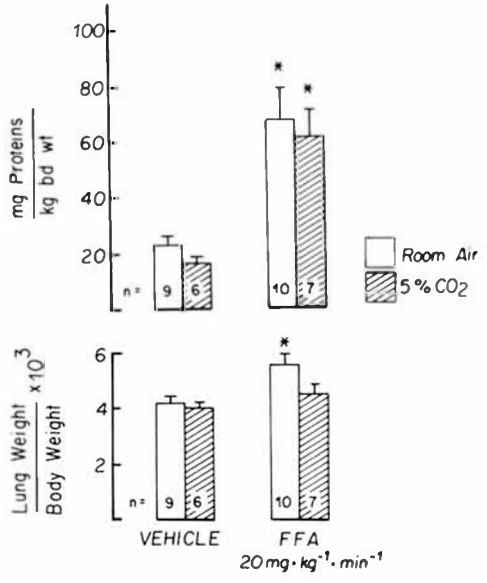

Fig. 1. Protein content in lung lavage fluid and lung weight/body weight ratio in rabbits injected intravenously with FFA breathing room air or 5% CO_2, 21% O_2, 74% N_2; p calculated by analysis of variance and Newman-Keuls test; *$p < 0.05$ as compared to control vehicle. Vertical lines = + 1 SE.

weight/body weight ratio in animals treated with FFA as compared to controls (fig. 1), although intraalveolar as well as perivascular edema was observed in the histological studies of the lung from rabbits injected with FFA breathing room air or the gas mixture containing 5% CO_2 (fig. 2).

The total number of cells in lung lavage fluid was not statistically different among the different series (analysis of variance). Five percent CO_2 in the inspired air was able to prevent the increase of surfactant (DSPC) in lung lavage fluid induced by FFA i.v. infusion (analysis of variance and Newman-Keuls' test) (fig. 3). However, administration of 5% CO_2 in the inspired air did not significantly modify the survival rate with 20 mg FFA $\cdot$ kg^{-1} $\cdot$ min^{-1} i.v. (χ^2 test) (fig. 4). The mean survival time of the series treated with FFA breathing room air was not statistically different

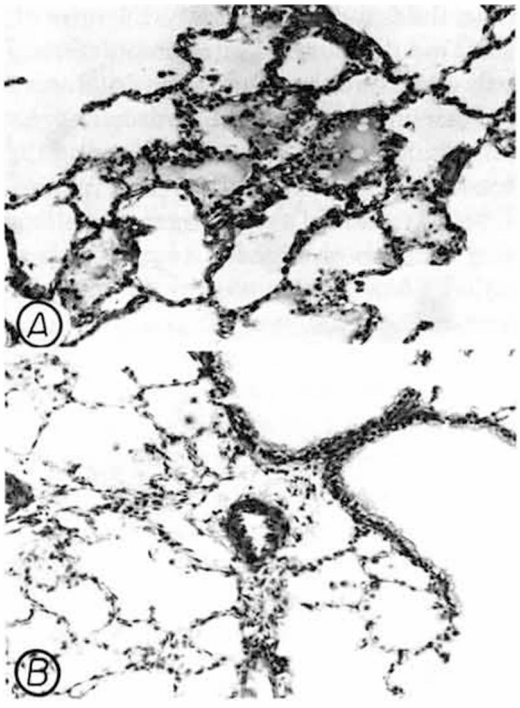

Fig. 2. Lung histology of a rabbit injected with FFA (20 mg · kg^{-1} · min^{-1}) breathing room air **(A)** or a gas mixture containing 5% CO_2, 21% O_2 and 74% N_2 **(B)**. Intraalveolar and perivascular edema was present in both conditions. HE. ×100.

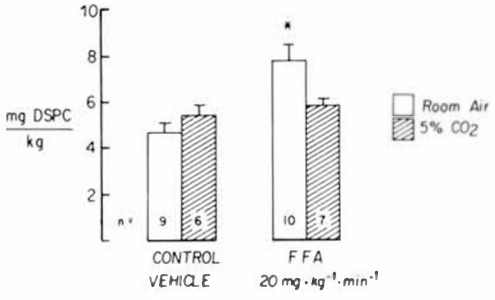

Fig. 3. DSPC content in lung lavage fluid in rabbits injected intravenously with FFA breathing room air or 5% CO_2, 21% O_2 and 74% N_2; p calculated by analysis of variance and Newman-Keuls test; * $p < 0.05$ as compared to all of the other series. Symbols as in figure 1.

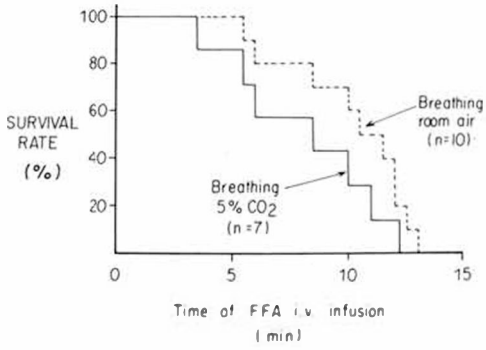

Fig. 4. Survival rate of rabbits injected with FFA (20 mg · kg^{-1} · min^{-1}) breathing room air or 5% CO_2, 21% O_2 and 74% N_2.

from the series breathing 5% CO_2 in the inspired air and treated with FFA (unpaired Student's t test).

Discussion

Our results are in agreement with those previous studies [2, 5, 9], which indicated that alveolar PCO_2 plays an important role in regulating extracellular surfactant pool. In this regard in our work we have found that the increase in alveolar surfactant pool induced by FFA i.v. infusion is prevented if the hypocapnia associated to the hyperventilation induced by FFA is avoided by administering 5% CO_2 in the inspired air.

Spontaneous ventilation with 5% CO_2 in anesthetized control rabbits promptly increased $PaCO_2$, however after 15 min, $PaCO_2$ went back to the basal level (table I), because of the increase in ventilation (table II). This increase in ventilation was accounted by both increase in respiratory rate and increase in tidal volume. The

rise in ventilation following 5% CO_2 administration also explains the significant increase in PaO_2 observed in controls.

In animals treated with FFA, 5% CO_2 in the inspired air prevented the decrease in $PaCO_2$ induced by FFA, and a prompt increase in PaO_2 after CO_2 administration was observed. This effect on PaO_2 was likely due to the change in the pattern of breathing induced by CO_2, which in turn probably improved ventilation/perfusion ratios in the lung. After FFA administration there was a significant decrease in PaO_2 because of the development of pulmonary edema, although the final level reached by PaO_2 was smaller in the rabbits breathing room air than in the rabbits receiving 5% CO_2 ($p < 0.05$ unpaired Student's t test). We think that in both cases there was an impairment of the pulmonary gas exchange and that CO_2 administration only improved the initial level of PaO_2.

The ventilatory indices of anesthetized rabbits usually have wide individual variations [10] which may account for the different levels of ventilation observed in our series before injecting FFA or the vehicle solution.

In our experiments, supplementing inspired air with 5% CO_2 did not protect against pulmonary edema, because lung histology showed similar changes (perivascular and intraalveolar edema) in the rabbits treated with FFA breathing room air or 5% CO_2; besides, there were no significant differences in protein content in lung lavage fluid nor in lung weight/body weight ratio between the series receiving FFA (fig. 1). Pulmonary edema was likely caused by an increased vascular permeability, because protein content in lung lavage fluid was significantly augmented. Since we did not measure the pulmonary artery pressure, we cannot rule out that an increase in hydrostatic intravascular pressure could also be present facilitating the production of pulmonary edema. We believe that most of the changes in pulmonary function observed in animals treated with FFA are accounted by pulmonary edema. These results are different as compared to those obtained by *Edmunds and Holm* [4]. In their study, inhalation of 5–6% CO_2 for 2, 5 or 10 days decreased alveolar hemorrhage and edema due to unilateral pulmonary arterial ligation in dogs. This protective effect was related to the time of exposure of CO_2 being maximal at 5 and 10 days. In our experiments, inhalation of 5% CO_2 did not modify pulmonary edema, not only because differences in the kind of lung injury and species, but also because of the short time of CO_2 exposure (only 15 min) as compared to the longer periods of CO_2 exposure used by *Edmunds and Holm* [4]. Pulmonary edema is able to inactivate alveolar surfactant [11] promoting alveolar collapse by increasing surface tension of the alveolus. This increase in alveolar surface tension may enhance alveolar edema, which could lead to further inactivation of surfactant, so that a vicious circle might be set up [12]. Hypocapnia-induced increase of surfactant may be a beneficial effect, because it might counterbalance the inactivation of surfactant function induced by pulmonary edema.

On the basis of our results we conclude that hypocapnia plays an important role in the increase of alveolar surfactant induced by FFA i.v. infusion in the rabbit in high doses and in a short term (less than

15 min). The mechanisms by which hypocapnia would regulate alveolar surfactant pool is unknown. *Shepard* et al. [5] have suggested that the intracellular PCO_2 reduction could produce depletion of intracellular surfactant by decreasing the intracellular concentrations of bicarbonate or hydrogenions. Their experiments were carried out in excised dog lung, which would discard a neurogenic mechanism of action for hypocapnia.

In a previous study [13] we found that cyclooxygenase inhibitors as well as thromboxane synthetase inhibitors administered before FFA ($20\,mg \cdot kg^{-1} \cdot min^{-1}$ i.v.) were able to block the increase in DSPC induced by FFA, suggesting that thromboxane could be involved in the surfactant increase FFA-induced. In those experiments [13] we observed hypocapnia post-FFA only in the series treated previously with indomethacin, all the other inhibitors used (meclofenamate, imidazole or dazoxiben) prevented the hypocapnia post-FFA. At present we do not know the relationship between hypocapnia and thromboxane synthesis, anyway it seems intriguing that in three out of four series of experiments, in which thromboxane synthesis was likely inhibited, hypocapnia following FFA infusion was prevented.

The amount of surfactant in the alveolar space results from a complex process including secretion, recycle between lamellar bodies and alveolar spaces and removal [14, 15]. Therefore the increase of alveolar surfactant pool induced by i.v. infusion of FFA and the prevention of this effect (by inhibitors of thromboxane synthesis or by breathing 5% CO_2) may result from a change or an interference in any or in a particular combination of these steps.

Acknowledgments

The authors thank Mr. *J. Riquelme* for his excellent technical assistance, Ms. *F. Guzmán* for performing the histological preparations and Ms. *A. Vicente* for typing the manuscript.

References

1 Oyarzún, M.J.; Cabezas, E.; Donoso, P.; Quijada, D.: Effects of free fatty acid infusion on rabbit pulmonary surfactant. Influence of corticosteroids. Bull. eur. Physio-path. resp. *20:* 105–111 (1984).

2 Shepard, J.W., Jr.; Hauer, D.; Miyai, K.; Moser, K.M.: Lamellar body depletion in dogs undergoing pulmonary artery occlusion. J. clin. Invest. *66:* 36–42 (1980).

3 Baritussio, A.; Clements, J.A.: Acute effects of pulmonary artery occlusion on the pool of alveolar surfactant. Resp. Physiol. *45:* 323–331 (1981).

4 Edmunds, L.H., Jr.; Holm, J.C.: Effect of inhaled CO_2 on hemorrhagic consolidation due to unilateral pulmonary arterial ligation. J. appl. Physiol. *26:* 710–715 (1969).

5 Shepard, J.W., Jr.; Dolan, G.E.; Yu, S.Y.: Factors regulating lamellar body volume density of type II pneumocytes in excised dog lungs. J. appl. Physiol. *53:* 555–562 (1982).

6 Mason, R.J.; Nellenbogen, J.; Clements, J.A.: Isolation of disaturated phosphatidylcholine with osmium tetroxide. J. Lipid Res. *17:* 281–284 (1976).

7 Lowry, O.H.; Rosebrough, N.J.; Farr A.L.; Randall, R.J.: Protein measurement with the Folin phenol reagent. J. biol. Chem *193:* 265–275 (1951).

8 Zar, J.H.: Biostatistical analysis (Prentice Hall, Englewood Cliffs 1974).

9 Saunders, B.S.; Shepard, J.W., Jr.; Jobe, A.; Miyah, K.; Moser, K.M.; Gluck, L.: Alveolar CO_2 tension as a mediator of lamellar body release in experimental left pulmonary artery occlusion (Abstract). Chest *72:* 411 (1977).

10 Crosfill, M.L.; Widdicombe, J.G.: Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. J. Physiol., Lond. *158:* 1–14 (1961).

11 Said, S.I.; Avery, M.E.; Davis, R.K.; Banerjee, C.M.; El-Gohary, M.: Pulmonary surface activity in induced pulmonary edema. J. clin. Invest. *44:* 458–464 (1965).

12 Petty, T.L.: Adult respiratory distress syndrome; in Intensive and rehabilitative respiratory care, pp. 189–206 (Lea & Febiger, Philadelphia 1982).

13 Oyarzún, M.J.; Donoso, P.; Arias, M.; Quijada, D.: Thromboxane mediates the increase in alveolar surfactant pool induced by free fatty acid infusion in the rabbit. Respiration *46:* 231–240 (1984).

14 Oyarzún, M.J.; Clements, J.A.; Baritussio, A.: Ventilation enhances pulmonary alveolar clearance of radioactive dipalmitoyl-phosphatidylcholine in liposomes. Am. Rev. resp. Dis. *121:* 709–721 (1980).

15 Hallman, M.; Epstein, B.L.; Gluck, L.: Analysis of labeling and clearance of lung surfactant phospholipid in rabbit. Evidence of bi-directional surfactant flux between lamellar bodies and alveolar lavage. J. clin. Invest. *68:* 742–751 (1981).

Received: April 9, 1985
Accepted after revision: July 15, 1985

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