Effect of Postganglionic Parasympathetic Blockade on Pulmonary Surfactant[1] (35445)

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The existence of a highly surface-active lining at the air-tissue interface in the lungs of a wide variety of species is well established, even though the exact chemical nature of the substance *in situ* is the subject of controversy (3). Tooley *et al.* (5) and Bolande and Klaus (1) reported that extracts obtained from the lungs of guinea pigs 2–4 hr after bilateral cervical vagotomy exhibited higher minimum surface tensions than similar extracts obtained from control animals, although the latter investigators felt that the altered surface properties of lung extracts obtained postvagotomy were secondary to the development of pulmonary edema.

Pulmonary edema is the inevitable consequence of bilateral cervical vagotomy, and edemagenesis could easily mask any effect which interruption of the parasympathetic nerve supply to the lungs might have on the production and/or release of pulmonary surfactant. It was therefore felt that postganglionic parasympathetic blockade with atropine would aid in revealing whether alterations in pulmonary surfactant following vagotomy were the result of the interruption of parasympathetic nerves, or whether the alterations were secondary to pulmonary edemagenesis.

Materials and Methods. Eighty Duncan-Hartley guinea pigs (320–380 g) were randomly assigned to either a treatment or control group; each group was composed of equal numbers of each sex. The treatment group received atropine, 10 mg/kg, sc (as the SO_4), while the control animals received normal saline (1 ml/kg) by the same route. Injections were given at 6-hr intervals. One-half of the animals of each group were sacrificed 12 hr after the first atropine dose, and the remaining animals were sacrificed at 24 hr, *i.e.*, 12 hr after the second dose of atropine. A similar study was carried out on male albino Sprague-Dawley rats, 170–200 g.

Two additional studies were carried out on rats, as follows:

1. Eighty rats, 190–220 g, were divided into two groups as above. The first group received atropine, 8 mg/kg, sc, and the second group received normal saline, 1 ml/kg, sc. One-half the animals of each group were sacrificed at 4 hr (1 atropine dose), and the remaining animals were sacrificed at 8 hr (2 atropine doses).

2. Fifty rats, 130–160 g, were divided into four treatment and one control groups. Treatment groups received one of the following doses of atropine, 32, 100, 320, or 1000 mg/kg, and control animals were injected with saline. All animals were sacrificed 8 hr postinjection.

At the time of sacrifice, the animals were anesthetized with pentobarbital Na, 30 mg/kg. The trachea was exposed and clamped, and the animal was decapitated and exsanguinated. The lungs were quickly excised, cleared of extraneous tissue, weighed, and examined grossly; any lung showing evidence of gross pathology was discarded. An appropriate sample of lung tissue was then passed through a tissue press (1-mm sieve holes), accurately weighed and suspended in cold normal saline. This suspension was shaken in an ice bath for 30 min, then subjected to an intermittent vacuum to disrupt foam and remove air and surfactant material from

[1] A preliminary report of this work was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Pittsburgh, Aug. 24–28, 1969.

still-inflated alveoli. The suspension was centrifuged at 300*g* at 0° for 15 min; the supernatant was drawn off and stored at 4° for subsequent testing on the surface balance.

The surface balance used is similar to the modified Wilhelmy balance described by Brown *et al.* (2). The surface area of the balance trough was 350 cm^2. Before spreading the lung extract on the surface balance, sufficient *n*-butanol was added to give a final concentration of 8%; the *n*-butanol served to facilitate surface spreading of the surfactant material (J. A. Clements, personal communication). An amount of the lung extract–butanol mixture, sufficient to give an initial surface tension of 40 dynes/cm, was spread dropwise on the normal saline hypophase of the surface balance; the trough area was compressed to 20% of original and then reexpanded, one complete cycle requiring 65 sec. Surface tension vs surface area was recorded continuously on an X-Y recorder.

Control and treatment groups were compared in terms of units of surfactant per gram of minced lung (1 unit = quantity of surfactant required to give initial surface tension of 40 dynes/cm), minimum surface tension upon compression to 20% of original area (γ_{mim}), and lung weight–body weight ratio. The latter measurement serves as a postmortem index of pulmonary edema (6).

Results. The effects of 12 or 24 hr postganglionic parasympathetic blockade with atropine on pulmonary surfactant in the guinea pig and rat are summarized in Table I, (A) and (B), respectively. The surface area–surface tension curves obtained with lung extracts from both treated and control animals were comparable, and this was true for both species. Both male and female guinea pigs were used, but there were no apparent sex-related differences in any of the parameters measured.

The effects of 4- and 8-hr postganglionic parasympathetic blockade with atropine in the rat are summarized in Table II, and the effects of increasing doses of atropine on surfactant are presented in Table III. As was noted in the previous studies, surface area–surface tension plots for treated and control

TABLE I. Effect of Atropine (10 mg/kg, sc) Every 6 hr on Pulmonary Surfactant in the Guinea Pig (A) and Rat (B).

Group	No. of animals[a]	Surfactant (units/g of minced lung[b]; mean ± SE)	Minimum surface tension (dynes/cm[c]; mean ± SE)	Lung wt/body wt × 10^3 (mean ± SE)
		(A) Guinea pigs		
12 hr (2 atropine doses)				
Control	18	14.5 ± 1.9	9.1 ± 1.3	9.9 ± 1.8
Treated	19	15.4 ± 1.6	8.4 ± 1.2	9.3 ± 0.4
24 hr (4 atropine doses)				
Control	18	12.9 ± 1.9	8.6 ± 1.5	8.6 ± 0.3
Treated	17	13.5 ± 0.7	5.5 ± 0.5	8.9 ± 0.5
		(B) Rats		
12 hr (2 atropine doses)				
Control	17	20.5 ± 1.8	10.9 ± 0.4	5.5 ± 0.3
Treated	18	19.1 ± 1.7	9.9 ± 0.8	5.6 ± 0.1
24 hr (4 atropine doses)				
Control	16	21.7 ± 2.4	9.6 ± 0.4	5.6 ± 0.2
Treated	20	23.4 ± 3.0	9.1 ± 0.3	5.7 ± 0.1

[a] Numbers less than 20 indicate lungs discarded due to pathology.

[b] Unit of surfactant = quantity of surfactant extract required to give initial surface tension of 40 dynes/cm.

[c] Upon compression of surface film to 20% of original area.

TABLE II. Effect of Atropine (8 mg/kg, sc) Every 4 hr on Pulmonary Surfactant in the Rat.

Group	No. of animals	Surfactant (units/g of minced lung[a]; mean ± SE)	Minimum surface tension (dynes/cm[b]; mean ± SE)	Lung wt/body wt × 10^3 (mean ± SE)
4 hr (1 atropine dose)				
Control	20	37.0 ± 3.3	9.2 ± 0.7	5.7 ± 0.1
Treated	20	36.0 ± 2.4	9.4 ± 0.9	5.5 ± 0.1
8 hr (2 atropine doses)				
Control	20	39.9 ± 2.6[c]	8.9 ± 0.8[c]	5.6 ± 0.1
Treated	20	36.5 ± 2.3[d]	9.4 ± 0.7[d]	5.7 ± 0.1

[a] Unit of surfactant = quantity of surfactant extract required to give an initial surface tension of 40 dynes/cm.
[b] Upon compression of surface film to 20% of original area.
[c] Based on 18 samples.
[d] Based on 19 samples.

were again comparable, all exhibiting similar compression and expansion curves which described wide hysteresis loops.

Discussion. The results of these studies indicate that postganglionic parasympathetic blockade causes no alteration in pulmonary surfactant in rats and guinea pigs, even when the block is maintained for as long as 24 hr. The apparent loss of pulmonary surfactant following bilateral cervical vagotomy in guinea pigs (1, 5) is probably secondary to the development of acute pulmonary edema, as suggested by Bolande and Klaus (1). Said *et al.* (4) have demonstrated that dextran-induced pulmonary edema in dogs resulted in marked alterations in the surface properties of lung extracts, although these changes were regional and were most marked in the airless regions of the edematous lung. In the same study, extracts from relatively normal appearing areas of the lungs often exhibited normal surface properties. The results of the present study, plus the data from the above-mentioned vagotomy and pulmonary edema studies, argue against a direct involvement of the parasympathetic nervous system in the synthesis and/or release of pulmonary surfactant.

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TABLE III. Effect of Single High Doses of Atropine on Pulmonary Surfactant 8 hr Postinjection in the Rat.

Dose of atropine (mg/kg, sc)	No. of animals	Surfactant (units/g of minced lung[a]; mean ± SE)	Minimum surface tension (dynes/cm[b]; mean ± SE)	Lung wt/body wt × 10^3 (mean ± SE)
Control	10	28.8 ± 2.0	12.3 ± 1.0	8.5 ± 0.2
32	10	28.5 ± 3.6	9.9 ± 0.8	8.2 ± 0.2
100	10	26.6 ± 2.3	12.4 ± 0.8	8.2 ± 0.2
320	10	31.5 ± 4.1	10.5 ± 1.1	8.4 ± 0.2
1000	10	34.4 ± 4.3	10.9 ± 1.2	8.1 ± 0.2

[a] Unit of surfactant = quantity of surfactant extract required to give an initial surface tension of 40 dynes/cm.
[b] Upon compression of surface film to 20% of original area.

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