

Suppression of neural activity of bronchial irritant receptors by surface-active phospholipid in comparison with topical drugs commonly prescribed for asthma

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Abstract

Background Much indirect evidence has been put forward previously in support of the concept that surface-active phospholipid (SAPL) normally masks irritant receptors in the lungs and upper respiratory tract; but this physical barrier is deficient in asthmatics, imparting hyperresponsiveness of the bronchoconstrictor reflex.

Objective To determine whether exogenous SAPL applied to bronchial mucosa reduces the sensitivity of irritant receptors to a standard challenge used clinically to diagnose asthma and to compare the effects with those of corticosteroids and β -stimulation.

Methods Nerve fibres in the vagi were monitored to record action potentials from irritant receptors identified in the upper airways of rat lungs in response to a methacholine challenge. SAPL in the form of dipalmitoyl phosphatidylcholine (PC) and phosphatidylglycerol (PG) – 7:3 PC:PG – was applied as a fine dry powder to enhance surface activity and, hence, chemisorption to epithelium. Comparison was also made with clinical doses of i.v. hydrocortisone and instilled salbutamol together with liquid or solid controls, as appropriate.

Results Neural activity of irritant receptors was found to be significantly ($P = 0.0018$) decreased by topical SAPL by 35.8% in response to a methacholine challenge in contrast to an increase of 11.2% in response to a solid (lactose) control. Instilled salbutamol and i.v. hydrocortisone also decreased responses to the same challenge by 43.4% and 14.7%, respectively, in contrast to a liquid (saline) control which increased by 24.5%.

Conclusions Surface-active phospholipid has an appreciable effect upon irritant receptors in rat airways, reducing neural response to a methacholine challenge by an amount comparable to that of Salbutamol. These results support the concept of SAPL masking bronchial irritant receptors and warrant placebo-controlled clinical trials of this dry powder as a means of controlling asthma without the side-effects of current medication. Other possible roles discussed for the SAPL epithelial barrier include the exclusion of viruses and allergens.

Keywords: asthma, β_2 -agonists, bronchoconstriction, corticosteroids, epithelial barrier, receptor masking, surface-active phospholipid, surfactant

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Introduction

The scientific fascination with asthma is the particularly wide diversity of agents that can trigger or sensitize the

airways to this disease, ranging from pure mineral surfaces [1] to allergens invoking the most complex molecular immunobiology [2]. In an attempt to reconcile such diverse physical and biological factors, it has been speculated that there is a physical barrier separating any noxious stimulus entering the upper airways of the lung from receptors which elicit the bronchoconstrictor reflex [3]. This barrier could be

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provided by surface-active phospholipid (SAPL) present in appreciable quantities in the upper airways [4] and clearly demonstrated by electron microscopy [5] as an oligolamellar lining bound to the epithelial surface. This is similar to 'surfactant' studied at the alveolar level for its ability to reduce the surface tension of any aqueous lining; although this may not be its only role [6].

The oligolamellar lining of SAPL is adsorbed to bronchial epithelium at sites where receptors or, at least, peripheral nerve endings are likely to be located along the airway wall, thus 'masking' those receptors. 'Unmasking' of receptors is a theory much in vogue in neurophysiology for explaining the sensitization of reflexes in the CNS [7,8] where, normally, only 1% or so are 'uncovered' at any one time. SAPL should be a good 'masking' substance since it has been shown to provide an excellent barrier to noxious agents in the stomach [9] and other organs when adsorbed [6]. Thus the hypothesis which has been proposed [3] is that the normal barrier of adsorbed SAPL is deficient in asthmatics, thus causing hyperresponsiveness of the bronchoconstrictor reflex common to all forms of asthma [2]. Much indirect evidence has been collected to support this approach [10], features ranging from the hydrophobicity of mucus-free pulmonary epithelium [11] to the effects of corticosteroids and β_2 -agonists in promoting surfactant secretion in the lung as a whole [12,13]. Both agents are widely used clinically as effective treatments for asthma and its prevention [2].

Clearly the hypothesis outlined above would take many studies to prove but one of the most direct evaluations would be to monitor afferent neural feedback to the brainstem with and without the topical application of exogenous SAPL in response to a typical (methacholine) challenge, widely used clinically to diagnose asthma [14]. This study has therefore been designed to compare the firing of irritant receptors in rat airways in response to a methacholine challenge after enhancing masking with exogenous SAPL and, furthermore, to compare these responses with standard topical treatments employing β_2 -agonists or corticosteroids.

Materials and methods

Principle

Nerve fibres in the vagi were monitored to record action potentials from deflationary rapidly adapting stretch receptors identified as irritant receptors in the upper airways of the rat. Such recordings in response to a standard methacholine challenge were then compared with and without topical pretreatment with airborne dry SAPL, salbutamol (a β_2 -agonist) or the steroid hydrocortisone, using liquid or powder controls as appropriate.

Rat model

Male Wistar rats (400–450 g body weight) were anaesthetized with pentobarbitone sodium (initial dose 60 mg/Kg, i.p.) with supplementary doses i.v. as required. Animals were intubated with a tracheal tube near the carina and ventilated with a pressure ventilator (ULCO Medical, Marrickville, NSW, Australia) in the supine position. The right jugular vein was cannulated for supplementary doses of pentobarbitone sodium and the administration of fluids (0.9% sodium chloride, Baxter, Australia, or/and sodium bicarbonate inj. B.P. 8.4% w/v, Astra, Australia). The right carotid artery was also cannulated for sampling of arterial blood and recording systemic arterial blood pressure. Arterial blood P_{O_2} , P_{CO_2} and pH were determined by a blood analyser (ABL330, Radiometer, Copenhagen, Denmark). A three-way tap was placed between the tracheal tube and the ventilator to enable ventilation rates and transpulmonary pressure (P_{TP}) to be monitored using a pressure transducer. Animals were ventilated with oxygen mixed with medical air. Ventilation conditions were set up with a ventilation pressure of 10–14 cm H_2O ; $T_I:T_E$ of 1:2.5, a ventilation rate of 25–50 per min and tidal volume in the region of 2 mL. The ventilation rate was adjusted as needed to keep the P_{aCO_2} within 30–45 mmHg and P_{aO_2} above 150 mmHg. The pH was maintained between 7.30 and 7.45 by i.v. administration of sodium bicarbonate. Body temperature was maintained at 37–38 °C throughout the experiment using a heating pad.

Procedure

The left vagus nerve was isolated and separated from the carotid artery and cut as high in the neck as possible. The distal end was placed in Paraffin liquid to prevent drying of the nerve. The nerve sheath was separated from the distal root of the vagus. The fine filaments were then gently teased from the nerve and placed on a platinum monopolar electrode connected to a high impedance differential amplifier. The signal from the nerve filament was then filtered and monitored using an oscilloscope and an audio amplifier. Data storage and analysis were performed by a MacLab system (AD Instruments, Castle Hill, NSW, Australia).

Pancuronium (1.5 mg/Kg) was used to paralyse the animals. The ventilation rate was then adjusted to ensure that arterial P_{O_2} , P_{CO_2} and pH were maintained within acceptable levels. The lungs were then hyperinflated to three or four times the tidal volume by injecting air into the airways, and deflated by drawing air out through a syringe. Receptors that responded to hyperinflation with brief firing, and responded to deflation with sustained firing, were regarded as rapidly adapting irritant and studied further. The results generated from our preliminary study on identifying these receptors

has been presented elsewhere [15]. When the desired neural activity was detected, the nerve filament was then further subdivided until the activity of a single unit could be clearly discriminated. After each methacholine challenge, firing bursts were recorded during the expiratory phase as they increased to a peak and then returned to baseline levels. The 20 highest firing bursts, occurring either side of the peak, were selected from the whole recorded neural activity and then averaged as the mean neural action potential (NAP). Total neural action potentials discharged during the expiration phase of each respiratory cycle were quantified using a ratemeter to give a response in impulses per second, and a Chart analysis program adapted by the MacLab system.

Methacholine challenge was performed by instilling 0.1 mL saline solution of methacholine (source) into the airways at a dose rate of 60 $\mu\text{g/Kg}$ body weight. This is half of a human dose on a w/w basis, preliminary trials demonstrating that the same rate (w/w) gave too great a stimulation to be practical. Two methacholine challenges were performed on each rat.

Tests of receptor modification

Having identified an irritant receptor and established viability of the model in each case, experiments were then performed to detect whether various agents 'protected' these receptors as indicated by any modification of their neural responses to the same methacholine challenge. Each agent required its own control, namely saline for salbutamol solution and for i.v. hydrocortisone and topical powdered lactose for SAPL similarly dispersed as a fine powder of comparable particle size. Neural responses were quantified as the NAP levels for the top 20 firing bursts recorded and then averaged.

Saline control

Five rats were used for this study after the successful recording of single-unit firing. Soon after neural responses to the first methacholine challenge were recorded and averaged, conditions were allowed to stabilize for 5–10 mins before 0.1 mL saline was instilled into the airways through a catheter. The second methacholine challenge was performed approximately 90 min after the saline instillation.

Salbutamol treatment

Another five rats were used in this section of the study in which almost identical procedures were followed. Following the recording of neural responses to the first (control) methacholine challenge, 0.1 mL salbutamol solution (0.25 mg/Kg body weight), was substituted for saline

and instilled into the lungs of the animal. The waiting time between the instillation of salbutamol and the second methacholine challenge (and recording of NAP) were similar to those in the saline control.

Hydrocortisone treatment

Another five rats were used in this section of the study. Irritant receptor activity (NAP) was recorded in response to a first (control) methacholine challenge consisting of 1 mL saline administered intravenously. As soon as conditions stabilized, 0.1 mL of saline was instilled into the lung of the animal and at the same time, 1 mL of hydrocortisone solution containing 40 mg/Kg body weight was administered intravenously. The waiting time between the administration of hydrocortisone and the second methacholine challenge (and recording of NAP) were similar to those in the saline control.

Surface-active phospholipid

The form of SAPL selected was a 7 : 3 mixture of L α -DPPC and phosphatidylglycerol (PG) commercially available under the name ALEC from Britannia Pharmaceuticals, Redhill, UK, as a fine dry powder widely prescribed for treating the respiratory distress syndrome (RDS) in neonates [16]. ALEC was selected in preference to alternative sources since it is the only commercially available formulation which is 100% surface-active phospholipid. It is also the only form of SAPL prescribed for RDS which does not contain proteolipids co-extracted from animal sources, nor additives such as hexadecanol or Tyloxapol in Exosurf likely to invoke an irritant response.

An aerodynamic particle sizer (APS Model 3310 A, TSI) was used to measure the size distribution of ALEC, ranging from 0.7 μm to 15 μm with a median value of about 10 μm . The dose of powder (SAPL or lactose control) was standardized by blowing a known amount of propellant (HFA) through a vial of the powder. The liquefied propellant is HFA 134a gas [1,1,1–2-Tetrafluoroethane ($\text{CF}_3\text{CH}_2\text{F}$), ICI Klea, UK] stored in a canister which is housed in an apparatus (Pharmaceutical Technology Group, School of Pharmacy & Pharmacology, University of Bath, UK), designed to release 50 μL of compressed liquefied propellant with each actuation of the release valve. The resulting puff of gaseous propellant passes via a tube (O.D. 2.58 mm, I.D. 2.08 mm, L 160 mm) into the vial containing SAPL, thus dispersing the powder through an outlet tube (O.D. 1.5 mm, I.D. 1.0 mm, L 500 mm). The dispersed powder was delivered to the animal via the outlet tube inserted into the trachea to a point just above the carina. Weights of powder delivered from vials using the system described above were tested before the experiment. Average

weight of SAPL powder in three 'puffs' was 29.6 ± 4.3 mg (mean $\pm$ SEM).

Lactose control

Lactose was selected as a dry powder control of $10\ \mu\text{m}$ median particle size commercially available for this purpose from Astra Pharmaceuticals, Lund, Sweden. The amount of lactose delivered in three 'puffs' by the same mechanism was 32.8 ± 2.1 mg (mean $\pm$ SEM) which is very close to the quantity of SAPL delivered. A total of eight rats were used for this study after the successful recording of single-unit firing. Soon after the first methacholine challenge neural responses were recorded and averaged. The first two puffs of lactose powder were given once the experimental conditions had stabilized from the first methacholine challenge. Positive end-expiratory pressure (PEEP) ($4\text{cmH}_2\text{O}$) was maintained for 3 min to facilitate the distribution of powder inside the lungs. The third puff was given 5 min after the first two and PEEP ($4\text{cmH}_2\text{O}$) was again introduced and maintained for another 3 min. Approximately 60 min after the last puff of lactose powder, the second methacholine challenge was performed. Neural responses were recorded and NAP values measured and averaged for the top 20 firing bursts.

SAPL treatment

Another eight rats were used in this study in which almost identical procedures were followed. After recording NAP values for the first methacholine challenge, two puffs of ALEC, instead of lactose, were delivered into the lung. The waiting time between the third ALEC puff and the second methacholine challenge, and the recording of NAP were similar to those of the lactose control.

Results

Salbutamol and hydrocortisone treatments

Typical responses for each treatment and control are shown in Fig. 1. Both salbutamol treatment and i.v. hydrocortisone treatment were found to decrease neural activity of irritant receptors in response to a standard methacholine challenge. Mean ($\pm$ SEM) values of NAP recorded for the top 20 firing bursts decreased in response to the second (test) methacholine challenge by $43.4 \pm 8.6\%$ for salbutamol and by $14.7 \pm 4.3\%$ for i.v. hydrocortisone. In contrast, saline controls increased by $24.5 \pm 10.9\%$. The results for individual rats are given in Table 1 with mean values depicted in Fig. 2.

SAPL treatment

Treatment with topical SAPL was also found to decrease neural activity of irritant receptors in response to a standard methacholine challenge. Mean ($\pm$ SEM) values of NAP recorded decreased in response to the second (test) challenge by $35.8 \pm 6.7\%$. By contrast, lactose controls increased NAP_t by $11.2 \pm 12.6\%$. The results for individual rats are given in Table 2 with mean values depicted in Fig. 2.

Statistical analysis

Statistical analyses were performed using the *U*-test and one-way ANOVA. Comparison of NAP values post-treatment and pretreatment demonstrated highly significant changes of $P=0.008$ for salbutamol and i.v. hydrocortisone and reaching $P=0.0018$ for SAPL powder. We also used the Kruskal–Wallis test to determine whether salbutamol and

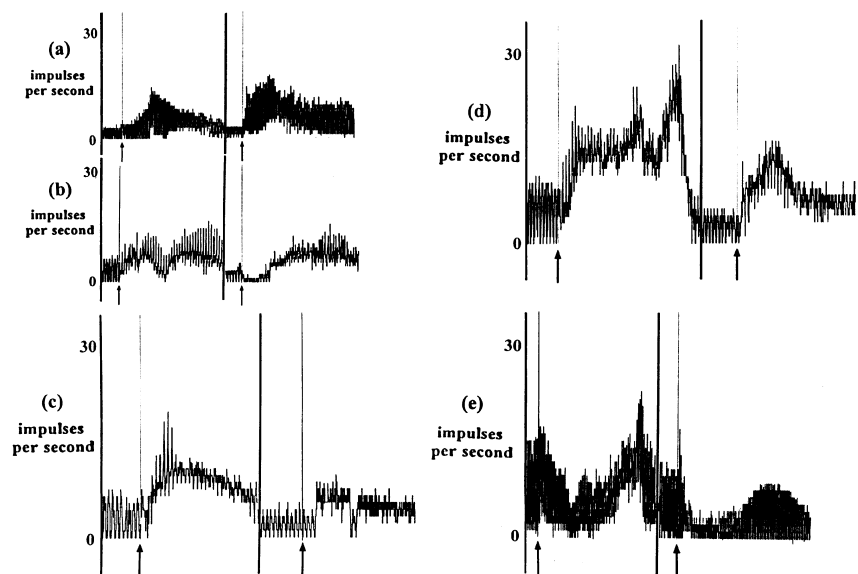


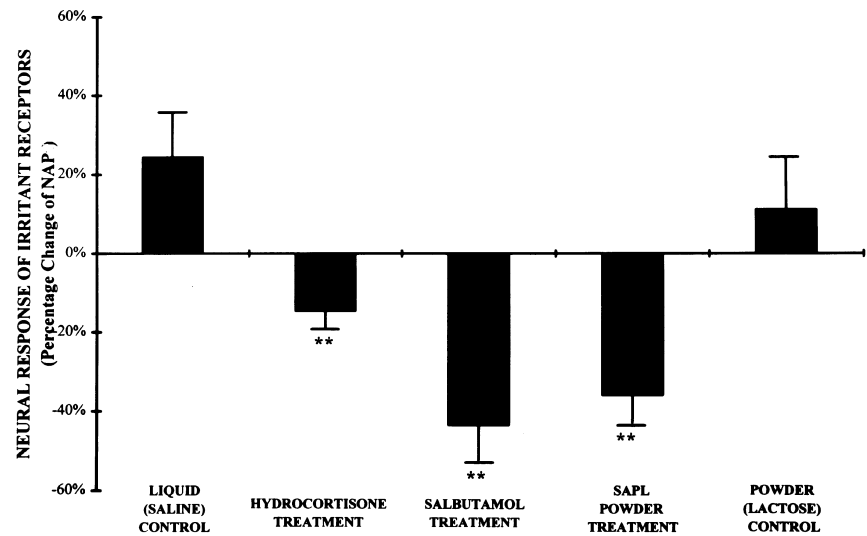
Fig. 1. Displaying typical responses of bronchial irritant receptors to two methacholine challenges (arrowed) (a) before and after saline instillation as a control for c and d (b) before and after administration of powder (lactose) as a control for e (c) before and after i.v. hydrocortisone (d) before and after instillation of salbutamol and (e) before and after administration of SAPL powder.

Table 1. Comparison of neural responses* (NAP) induced by methacholine challenges after salbutamol or i.v. hydrocortisone treatments vs saline control

	Rat										Mean $\pm$ SEM	P-value
	I		II		III		IV		V			
<i>Saline control</i>												
Methacholine challenge	Before saline	After saline	Before saline	After saline	Before saline	After saline	Before saline	After saline	Before saline	After saline		
Mean $\pm$ SEM ($n = 20$)	30.6 $\pm$ 2.2	49.5 $\pm$ 1.9	24.4 $\pm$ 1.1	31.9 $\pm$ 0.9	45.3 $\pm$ 1.8	48.2 $\pm$ 1.3	38.0 $\pm$ 2.3	37.8 $\pm$ 1.2	63.1 $\pm$ 2.4	78.0 $\pm$ 2.4		
Percentage change (%)	+61.9		+30.6		+6.5		-0.004		+23.6		+24.5 $\pm$ 10.9	
<i>Salbutamol treatment</i>												
Methacholine challenge	Before saline	After saline	Before saline	After saline	Before saline	After saline	Before saline	After saline	Before saline	After saline		
Mean $\pm$ SEM ($n = 20$)	41.2 $\pm$ 1.8	27.9 $\pm$ 1.4	80.5 $\pm$ 5.4	29.6 $\pm$ 2.7	88.4 $\pm$ 5.9	72.1 $\pm$ 2.6	69.0 $\pm$ 1.1	26.3 $\pm$ 1.2	61.3 $\pm$ 1.6	36.3 $\pm$ 1.6		
Percentage change (%)	-32.5		-63.3		-18.4		-61.9		-40.8		-43.4 $\pm$ 8.6	†0.008
<i>i.v. hydrocortisone treatment</i>												
Methacholine challenge	Before saline	After saline	Before saline	After saline	Before saline	After saline	Before saline	After saline	Before saline	After saline		
Mean $\pm$ SEM ($n = 20$)	76.7 $\pm$ 1.0	70.4 $\pm$ 2.5	59.4 $\pm$ 2.5	53.5 $\pm$ 1.0	91.6 $\pm$ 4.1	86.3 $\pm$ 2.1	93.6 $\pm$ 2.4	67.7 $\pm$ 3.1	101.9 $\pm$ 2.2	79.3 $\pm$ 1.6		
Percentage change (%)	-8.2		-9.8		-5.7		-27.7		-22.1		-14.7 $\pm$ 4.3	†0.008

* Neural responses recorded as the mean number of action potentials for the top 20 firing bursts. †Significant difference ($P < 0.01$) in percentage changes of mean NAP values between saline control and salbutamol or i.v. hydrocortisone treatments (U -test).

Fig. 2. Average responses of irritant receptors for three treatments, viz. hydrocortisone, salbutamol and SAPL relative to liquid (saline) and powder (lactose) controls. Each treatment displayed a significant decrease in neural activity in response to a methacholine challenge, while the difference in responses between salbutamol and SAPL was not significant.



hydrocortisone treatments attenuated the methacholine-induced increase in neural activity, reaching the same conclusion ($P < 0.01$).

Discussion

The preliminary tests performed to characterize the responses to lung inflation/deflation and the magnitude of the response to a methacholine challenge leave no doubt that we were recording from irritant receptors in the upper airways of the lung. Moreover the appreciable decrease in the responses after treatment with salbutamol and hydrocortisone are in keeping with the general theory that afferent neural feedback from these receptors via the vagi elicits the bronchoconstrictor reflex. Both β_2 -agonists and corticosteroids are effective treatments for asthma [2], indicating that the rat offers a convenient small-animal model for studying this disease.

Since β -stimulation and steroids are known to promote surfactant secretion in the upper airways of the lung [13], this is consistent with earlier speculation [3] that, if that surface-active phospholipid (SAPL) were adsorbed to pulmonary epithelium, it could also coat and, hence, further mask irritant receptors located on that surface. It is just possible that SAPL might be a mediator for the action of β_2 -agonists and steroids in the lung. However, it can be argued on a more conventional basis that β -agonists may decrease afferent neural activity either directly [17] or secondary to functional antagonism of bronchoconstriction. Differences between clinical experience and the pharmacokinetics of steroids [18], possibly attributable to 'receptor defects' [19], might be attributed to masking of the receptors at which those drugs are known to act. However, steroids are generally recognized as functioning through their anti-inflammatory properties, raising the issue why adverse effects are often

induced in asthmatics by nonsteroidal anti-inflammatory drugs (NSAIDs) which, incidentally, happen to be a barrier-breaker for SAPL in the stomach, at least [9].

There is ample morphological evidence to support a coating adjacent to airway receptors, starting with the finding that bronchial mucosa rinsed free of mucus is appreciably hydrophobic and is enhanced by SAPL adsorption *ex vivo* [11]. Assuming that the pulmonary membrane has the standard lipid-bilayer structure with polar groups orientated outwards, it should be perfectly wettable — which it is not. In the physical sciences, the standard method of transforming a hydrophilic to a hydrophobic surface is the chemisorption of a surfactant [6]. Moreover, if an excess of any surfactant is applied *in vitro*, it forms oligolamellar layers [20] closely resembling those of SAPL described on pulmonary epithelium [5,21]. The reason why many studies using the electron microscope do not display that oligolamellar coating is simply that, in order to visualize a *lipid* structure, it is necessary to employ a *lipid* fixative, viz. tannic acid. Although tannic acid can cause phospholipid migration [22], the use of hydrophobic probes in epifluorescence microscopy avoids this possible objection, studies by Ueda *et al.* [21] and ourselves in ongoing studies [23] confirming the oligolamellar coating of SAPL. Similar coatings of SAPL adjacent to surface taste receptors have been reported on the reptile tongue [24].

The next question is whether this oligolamellar SAPL lining is actually masking irritant receptors in the upper airways of the lung. The results in Table 2 certainly support this mechanism. We selected a fine dry-powder form of SAPL because it is so much more surface-active in this state than in 'wet' forms, such as those commonly prescribed for (RDS), the physical chemistry being well expounded by Morley *et al.* [16].

One important property required for a coating to effect

Table 2. Comparison of neural responses* (NAP) induced by methacholine challenges after SAPL treatment vs lactose control.

Rat #	I		II		III		IV		V		VI		VII		VIII			
	LACTOSE CONTROL																Mean $\pm$ SEM	
Methacholine challenge	Before Lactose	After Lactose	Before Lactose	After Lactose	Before Lactose	After Lactose	Before Lactose	After Lactose	Before Lactose	After Lactose	Before Lactose	After Lactose	Before Lactose	After Lactose	Before Lactose	After Lactose	(<i>n</i> = 8)	<i>P</i>
Mean $\pm$ SEM (<i>n</i> = 20)	85.5 $\pm$ 0.8	153.4 $\pm$ 3.9	66.6 $\pm$ 0.7	64.1 $\pm$ 1.9	83.6 $\pm$ 1.3	78.8 $\pm$ 1.2	109.9 $\pm$ 4.7	110.0 $\pm$ 5.7	52.5 $\pm$ 1.8	65.6 $\pm$ 3.3	96.2 $\pm$ 2.8	73.1 $\pm$ 3.8	69.3 $\pm$ 0.9	82.7 $\pm$ 2.1	53.1 $\pm$ 0.9	61.9 $\pm$ 0.9		
Percentage change (%)	*79.4		−3.7		−5.7		*0.001		*24.8		−24.0		*19.3		*16.6		*11.2 $\pm$ 12.6	
	SAPL TREATMENT																	
Methacholine challenge	Before SAPL	After SAPL	Before SAPL	After SAPL	Before SAPL	After SAPL	Before SAPL	After SAPL	Before SAPL	After SAPL	Before SAPL	After SAPL	Before SAPL	After SAPL	Before SAPL	After SAPL		
Mean $\pm$ SEM (<i>n</i> = 20)	40.1 $\pm$ 1.3	23.5 $\pm$ 0.6	74.5 $\pm$ 2.6	51.5 $\pm$ 1.4	82.3 $\pm$ 2.5	62.5 $\pm$ 2.5	82.6 $\pm$ 6.5	38.1 $\pm$ 0.5	82.3 $\pm$ 5.2	33.2 $\pm$ 1.3	102.3 $\pm$ 2.8	100.7 $\pm$ 4.0	143.2 $\pm$ 3.7	84.7 $\pm$ 5.4	97.4 $\pm$ 6.7	57.7 $\pm$ 0.7		
Percentage change (%)	−41.4		−30.9		−16.8		−53.9		−59.7		−1.6		−40.9		−40.8		−35.8 $\pm$ 6.7	*0.0018

Neural responses recorded as the mean number of action potentials for the top 20 firing bursts. *Significant difference ($P < 0.002$) in percentage changes of mean NAP values between lactose control and SAPL treatment (*U*-test).

masking of receptors is the ability to form an effective barrier in the adsorbed state. The basic principles in the very common use of synthetic surfactants as protective barriers in industry, e.g. as corrosion inhibitors [20], is strong adsorption to the subphase and strong cohesion inducing close-packing of those adsorbed molecules with their neighbours [6]. One common example *in vivo* is believed to be the gastric mucosal barrier for which much evidence [9] supports the concept that SAPL provides this barrier to the back-diffusion of ions as small as hydrogen ions [25]. The molecular features of the major component (DPPC) are ideal for both strong adsorption and strong cohesion as described in detail elsewhere [9]. It is also pointed out how the choline group of the methacholine molecule would compete for adsorption sites with the terminal choline group in the DPPC molecule to dislocate the protective mosaic of close-packed fatty-acid chains [3]. This raises another reason for selecting methacholine in this study ahead of other standard challenges employed to diagnose asthma, possibly explaining why, in high doses or in children [26], this challenge can compromise pulmonary function in nonasthmatics, including experimental rats. However, a more conventional alternative to this physical approach is that methacholine could be inducing an increase in afferent activity secondary to bronchospasm, not that one mechanism excludes the other. Implying SAPL deficiency as the common link, it is interesting that patients with peptic ulcer often display compromised pulmonary function [27].

Another aspect of adsorbed layers of SAPL is how they span cell junctions [28], offering a barrier to the exudation of serous fluid in one direction but also a barrier to pathogens or allergens invading from the air spaces. Current morphological studies in this laboratory have even demonstrated these layers in extrathoracic airways including the sinuses. This is consistent with the close association between asthma and virus infection of the upper respiratory tract [28] consistent with a common sinonasal mucosa extending to the bronchi [29]. This implies a common barrier to pathogens which, experimentally, often cannot be made to invade the URT unless the mucosa is first treated with polidocanol (PDOC). Interestingly, the instillation of PDOC or some similar adjuvant into the URT is necessary to effect efficient viral vector entry into epithelial cells [30]. A brief test in the laboratory shows that PDOC is a solvent for SAPL, i.e. a barrier breaker.

While a substantial case can be made for SAPL masking neuroreceptors on the airway walls [3,10], it could also mask any receptors on smooth-muscle fibres, consistent with its smooth-muscle relaxant effect [31] and hence, restructuring of the airway wall which is now recognized as a key feature in chronic asthma [2,32]. At a fundamental level, a deficiency of surfactant in the airway wall of asthmatics could also exacerbate restructuring by elevating

surface energy causing cell surfaces to attempt to reduce their surface areas compatible with the morphological changes observed.

There is also the possibility that SAPL might be masking receptors in general—a ubiquitous masking agent. To test such a hypothesis one would envisage an experiment in which a known antigen (viz. mitogen) is added to a popular cell culture (viz. lymphocytes) to initiate a cascade of events in which a known marker (viz. immunoglobulin) is secreted into the medium where it can be quantified by analysis. We were preparing such a study, when it was found that Bartmann *et al.* [33] had already performed exactly that experiment for totally different reasons, never mentioning masking. The results were most interesting, demonstrating how only 5 mg/mL SAPL per 10^6 mononuclear cells could appreciably reduce the formation of immunoglobulin. Hence the possibility that SAPL is a potential masking agent for various antigen–receptor interactions would seem to warrant further investigation.

In conclusion, whether the masking theory is valid or not, the results of this study seem to warrant placebo-controlled clinical trials of dry-powdered SAPL in its currently available commercial form, viz. ALECTM, or as a finer powder with greater access to bronchial epithelium. Previous clinical trials of surfactant in asthma have differed in results, depending on the biological source of SAPL. Kurashima *et al.* [34] showed a marginal improvement in vital capacity and FEV₁ in asthmatics using a solvent extract of minced lung in the ‘wet’ (nebulized) state, while Oetomo *et al.* [35] found no effect, both using ‘wet’ surfactant extracted from *rinsings* of bovine lungs. One possible explanation for the different results is that the latter method would fail to extract SAPL adsorbed to airway epithelium which is the vital masking component according to the theory expounded above. As a synthetic dry powder used in this study ALECTM should be more surface active and offer no risk of transmitting pathogens or pyrogens inherent in the use of any medication of biological origin. It has the exciting possibility of offering the same advantages of β_2 -agonists or steroids without the side effects.

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