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The pharmacological modulation of [^{3}H]-disaturated phosphatidylcholine overflow from perifused lung slices of adult rats: a new method for the study of lung surfactant secretion

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1 Lung slices from adult rats incubated in [methyl-^{3}H]-choline chloride formed [^{3}H]-disaturated phosphatidylcholine ([^{3}H]-DSPC) which was used as an index of lung surfactant.

2 The slices were perifused after 3 h incubation in [methyl-^{3}H]-choline chloride and the overflow of [^{3}H]-DSPC, as a rate coefficient, was used as a measure of surfactant secretion. The basal overflow of [^{3}H]-DSPC rapidly declined over the first 30 min of perifusion and then declined slowly.

3 Salbutamol induced a prolonged, and sometimes delayed, increase in [^{3}H]-DSPC overflow, which was reduced by (±)-propranolol. Potassium chloride produced an immediate, and usually transient, increase in [^{3}H]-DSPC overflow which was not modified by atropine or (±)-propranolol. Adenosine 5′-triphosphate, but not phenylephrine, also increased [^{3}H]-DSPC overflow.

4 This method can measure the magnitude and time-course of lung surfactant secretion induced by drugs.

Introduction

Lung surfactant is crucial to the maintenance of a low surface tension at the air-liquid interface and so prevents alveolar collapse during expiration. Respiratory distress syndrome of human neonates is thought to be a consequence of a deficiency of lung surfactant (Avery & Fletcher, 1974). The major component of lung surfactant is dipalmitoyl phosphatidylcholine (Goerke, 1974; Van Golde, 1976) and therefore measurement of disaturated phosphatidylcholine (DSPC) may be used for the assay of surfactant. Cell membranes contain mainly mono- and di-unsaturated phosphatidylcholines (Van Golde, 1976).

Lung surfactant appears to be synthesized in Type 2 pneumocytes and stored as discrete intracellular organelles known as osmiophilic lamellar bodies (Goerke, 1974). The synthetic pathway and the secretion of surfactant into alveoli possess many of the features of exocytosis described for exocrine and endocrine glands (Butcher, 1978). These features include electron micrographs showing passage of osmiophilic lamellar bodies into alveoli (Ahmed & Chiswick, 1974) and progression of radioactive label through the cytoplasm, lamellar bodies and finally into alveoli (Chevalier & Collet, 1972).

Until recently the most widely used method to study the pharmacological modulation of lung surfactant secretion has been *in vivo* or *ex vivo* using lung lavage (e.g., Oyarzún & Clements, 1977; Abdellatif & Hollingsworth, 1980). This involves instillation of a physiological salt solution into the lungs via the trachea, withdrawal and analysis of the solution for surfactant content. As this is thought to sample the alveolar pool, treatment of animals *in vivo* with drugs which stimulate surfactant secretion should increase the amount of surfactant recoverable in the lavage. Such experiments have provided evidence that the secretagogues for lung surfactant *in vivo* include muscarinic and β-adrenoceptor agonists (Abdellatif & Hollingsworth, 1980; Smith & Bogues, 1980). However, there are problems in the interpretation of the results obtained using this method, such as the exact origin of the lavage surfactant and whether drugs are solely affecting secretion (Oyarzún & Cle-

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ments, 1977; Gilfillan, 1981). Therefore, *in vitro* techniques have recently been developed. Abdellatif & Hollingsworth (1980) developed an isolated, perfused lung preparation; drugs were infused via the pulmonary artery and their effects measured on the lavage content of surfactant. Brown & Longmore (1981) increased the sensitivity of such a method by labelling the DSPC using a radioactive precursor. Another approach has been to study the drug-induced release of labelled DSPC from either cultures of purified Type 2 pneumocytes (Dobbs & Mason, 1979; Brown & Longmore, 1981) or from lung slices after the injection of animals with [^{3}H]-choline (Delahunty & Johnston, 1979; Marino & Rooney, 1980; 1981). These *in vitro* techniques have many advantages compared to *in vivo* methods for pharmacological studies but have been little used to study the time course of drug-induced surfactant release.

The objective of the present study was to develop a simple and sensitive *in vitro* method to examine some of the characteristics of drug-induced surfactant secretion. Lung slices from adult rats were incubated in [methyl-^{3}H]-choline chloride to label the DSPC, based on the method of Epstein & Farrell (1975). The drug-induced overflow of [^{3}H]-DSPC from the perifused lung slices was then used as a measure of stimulated surfactant secretion. Preliminary results have been published (Gilfillan & Hollingsworth, 1981).

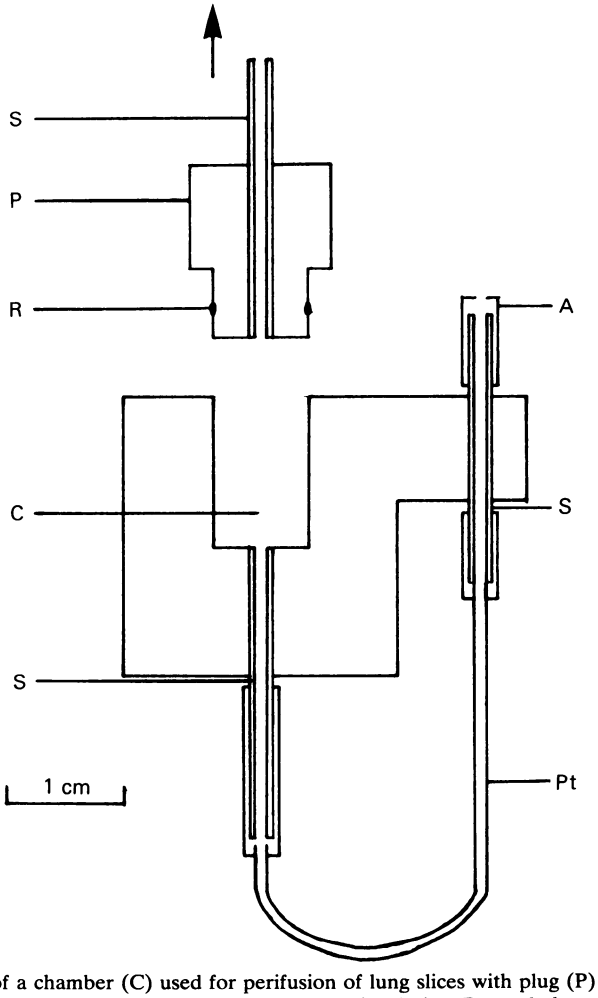

Figure 1 Cross-section of a chamber (C) used for perifusion of lung slices with plug (P) removed. A = point of attachment of tubing from roller pump containing Krebs-Henseleit solution. Pt = polythene tubing (Portex, pp20). R = rubber washer. S = stainless steel tubing. ↑ = to fraction collector.

Methods

Incubation and perifusion

Lung slices (approximately 0.5 × 0.5 × 0.05 cm) were prepared from adult Sprague-Dawley rats (200–350 g) by use of a McIlwain tissue chopper. Three slices, total weight approximately 20 mg, of about equal size and shape and without visible blood vessels or airways were selected. These were preincubated together in 5 ml of Krebs-Henseleit solution (Hollingsworth, 1975) in a perspex pot at 37°C and bubbled with 95% O_2, 5% CO_2 for 15 min in a shaking water bath. [Methyl-^{3}H]-choline chloride (77–84 Ci/mmol) was added (see Results for amount) and slices incubated, generally for a further 3 h. Distilled water (0.29 ml) was added after 30 min and then every hour to replace water lost by evaporation. The slices were transferred to a chamber of the perifusion apparatus (Figure 1). This apparatus is a modification of that described by Dascombe & Foote (1981) for perifusion of brain slices. A perspex block was drilled to produce a series of chambers, each of 0.5 ml volume. Into the top of each chamber fitted a perspex plug with a rubber washer to ensure a watertight fit. Stainless steel tubing (internal diameter 1.2 mm, external diameter 1.6 mm) passed through the plug, the base of the chamber and the perspex block parallel to the chamber. The chamber had already been filled with Krebs-Henseleit solution, bubbled with 95% O_2, 5% CO_2 and the whole assembly was supported in a water bath to maintain the chamber at 37°C. The slices were then perifused at a rate of 0.2 ml/min from a reservoir by means of a Watson-Marlow flow inducer (model MHRE 22) fitted with a delta head. The polythene tubing (Portex pp 20) containing the perifusate led to the collection head of a L.K.B. Minirac 17000 multichannel fraction collector. Use of 4 perifusion chambers enabled 4 replicate experiments to be performed simultaneously from the same lung.

Perifusate was generally collected in 5 min fractions for the first 45 min and then every 10 min for a further 140 min. Each perifusate fraction was collected into a centrifuge tube containing 1 ml tertiary butanol, 1.5 ml chloroform and 20 μg of cold DSPC as carrier (plus 1 ml of distilled water if 5 min fraction). Each perifusate fraction, and the tissue after perifusion, was assayed for [^{3}H]-DSPC content.

Separation and assay of [^{3}H]-disaturated phosphatidylcholine

This was a modification of the method of Mason, Nellenbogen & Clements (1976). The contents of each tube were vortexed, centrifuged and the aqueous layer discarded. The organic layer, containing lipids, was evaporated at 60°C under N_2. The sides of the tubes were washed down with 0.5 ml carbon tetrachloride, 10 μl of osmium tetroxide solution (50 mg/ml in carbon tetrachloride) added and left for 15 min at room temperature. The carbon tetrachloride was evaporated at 60°C under N_2. The residues were spotted onto thin-layer chromatography plates (Merck F_{254}) and the phospholipids separated using chloroform/methanol/ammonia solution (65/35/5, v/v/v) as solvent and located using dichlorofluorescein (0.3 mg/ml in 95% ethanol). The spots corresponding to those of the DSPC standard were scraped into low potassium glass scintillation vials. Scintillant (Packard Scintillator 299 TM; 10 ml) was added and the ^{3}H content counted by means of a Packard liquid scintillation spectrometer (model 3320) using the automatic external standard channels ratio method.

Lung slices were homogenized, using a Tri-R Stir-R homogenizer (model 63C), in 2 ml of chloroform/methanol (3/1, v/v) for 2 min, filtered and the tissue debris homogenized in a further 2 ml of chloroform/methanol (3/1, v/v). To duplicate 40 μl aliquots of the combined filtered homogenates were added 2 ml of water plus 1 ml of tertiary butanol and 1.5 ml chloroform, vortexed and treated as for perifusate fractions to assay [^{3}H]-DSPC content.

The overflow of [^{3}H]-DSPC was expressed at a rate coefficient, in units of %/min, and calculated using a Data General Nova computer.

$$\text{Rate coefficient} = \frac{\text{Content of [}^3\text{H]-DSPC in perifusate fraction (nCi)}}{\text{Tissue content of [}^3\text{H]-DSPC calculated to be present at the mid-point of the fraction (nCi)} \times \text{Duration of fraction (min)}} \times 100$$

Experimental design for drug modification of [^{3}H]-DSPC overflow and calculation of results

Lung slices were incubated in [methyl-^{3}H]-choline chloride (1 μCi/ml) for 3 h and then perfused for 3 h. During an efflux challenge experiment the first 6 perifusate fractions (0–30 min) were discarded. When the tissues were challenged with an agonist, a separate length of polythene tubing from the drug solution (in Krebs-Henseleit solution bubbled with 95% O_2, 5% CO_2 and warmed to 37°C) was attached to the inlet of the perifusion chamber (A in Figure 1). Agonist drugs were perifused as a 10 min pulse from 45 to 55 min after the start of perifusion. This time period was chosen as basal release was falling much more slowly and regularly than earlier (Figure 3). The tubing containing drug solution was replaced to continue perifusion with normal Krebs-Henseleit solution. Allowance was made for the dead space between A and the perifusion chamber. For some experiments the 'normal' Krebs-Henseleit solution

contained an antagonist throughout the perifusion period. The effect of an agonist on the overflow of [^{3}H]-DSPC was measured as the maximum rate coefficient after drug addition minus the mean of the rate coefficients of the last 3 pre-drug perifusate fractions. Two qualifications were added. First, the rate coefficients of control release of [^{3}H]-DSPC for the 30–35 and 35–40 min fractions were only used for the calculation of mean pre-drug rate coefficients if they were not greater than 1.5 times the value for the 40–45 min fraction. Second, a factor was incorporated into the programme to correct for the slow, linear decline that occurred in basal overflow from control tissues over the period 30 min to 3 h after the start of perifusion (Figure 3).

Assay of cold disaturated phosphatidylcholine

The lung tissue content of unlabelled DSPC was either determined by the method of Gilfillan, Harkes & Hollingsworth (1980) or DSPC separated by the osmium tetroxide method described above for [^{3}H]-DSPC followed by the phosphorus assay used by Gilfillan *et al.* (1980). A portion of lung was heated at 150°C for 48 h to obtain dry lung weight.

Assay of lactic acid dehydrogenase

Lung slices were prepared, incubated (but without [methyl-^{3}H]-choline chloride) and perifused as described above. Fractions were collected every 30 min over the 3 h period. Each fraction and the tissue after perifusion were assayed for their content of lactic dehydrogenase by the method of Wroblewski & La Due (1955).

Light and electron microscopy

For light microscopy, sections were fixed in formaldehyde solution and paraffin sections stained with haematoxylin and eosin. Tissues for electron microscopy were fixed in 2.5% glutaraldehyde, rinsed in sodium cacodylate buffer and post-fixed in osmium tetroxide in cacodylate buffer. Tissues were dehydrated in a series of ethanol solutions and propylene oxide and embedded in resin. Sections were stained with uranyl acetate followed by lead citrate and examined with an AE1 6B electron microscope.

Drugs and materials

The following were used: adenosine 5′-triphosphate (grade 1, Sigma); atropine sulphate (BDH); dichlorofluorescein (BDH); DL-α-dipalmitoyl phosphatidylcholine (Sigma); [methyl-^{3}H]-choline chloride (Radiochemical Centre, Amersham); osmium tetroxide (Sigma); (−)-phenylephrine hydrochloride (Sigma); potassium chloride (Analar, BDH); (±)-propranolol hydrochloride (I.C.I. Pharmaceuticals); salbutamol hydrochloride (Glaxo Ltd).

Statistical methods

Values are means ± s.e.mean. Statistical analysis was by the Mann Whitney U-test (Siegel, 1956); $2P < 0.05$ was taken as statistically significant.

Results

Incorporation of [methyl-^{3}H]-choline chloride

Location of [^{3}H]-DSPC in lung slices: Lung slices were incubated in Krebs-Henseleit solution plus [methyl-^{3}H]-choline chloride (1 μCi/ml) for 3 h. The slices were briefly washed in cold Krebs-Henseleit solution, homogenized, the filtrate extracted and the residue separated by thin-layer chromatography. A radiochromatogram scan (Packard Scanner, model 7201) of the thin-layer plate of the nonoxidized extract showed two peaks, the major peak with an R_F value corresponding to phosphatidylcholine. The phosphatidycholine (PC) spots were scraped from the plates, as was the rest of the channel, and their ^{3}H contents measured by scintillation counting. This demonstrated that 96 ± 0.2% ($n = 4$) of the extracted ^{3}H was found in the PC spot. This experiment was repeated but after oxidizing the extract with osmium tetroxide. Now, as well as a peak corresponding to phosphatidylcholine, there was a broad peak just above the origin. The former was due to DSPC, 57.6 ± 1.3% of the total [^{3}H]-phosphatidylcholine ($n = 4$), while the latter was due to oxidized products of unsaturated phosphatidylcholine.

Incorporation of ^{3}H into disaturated phosphatidylcholine as a function of time and concentration: Lung slices were incubated in Kreb-Henseleit solution plus [methyl-^{3}H]-choline chloride for between 0.5 and 6.0 h and in concentrations from 0.125 to 8 μCi/ml. Slices were then briefly washed in fresh, cold Krebs-Henseleit solution and their [^{3}H]-DSPC content measured. Dry weights were measured on other non-incubated sections of the same lungs. There was an increase in [^{3}H]-DSPC content up to 3 h of incubation but then no further change (Figure 2a). There was a linear relationship between the concentration of [methyl-^{3}H]-choline chloride and its incorporation into [^{3}H]-DSPC (Figure 2b).

Assessment of changes in content of unlabelled disaturated phosphatidylcholine during incubation: Lung slices were incubated for 6 h, twice the standard incubation time, in normal Krebs-Henseleit solution.

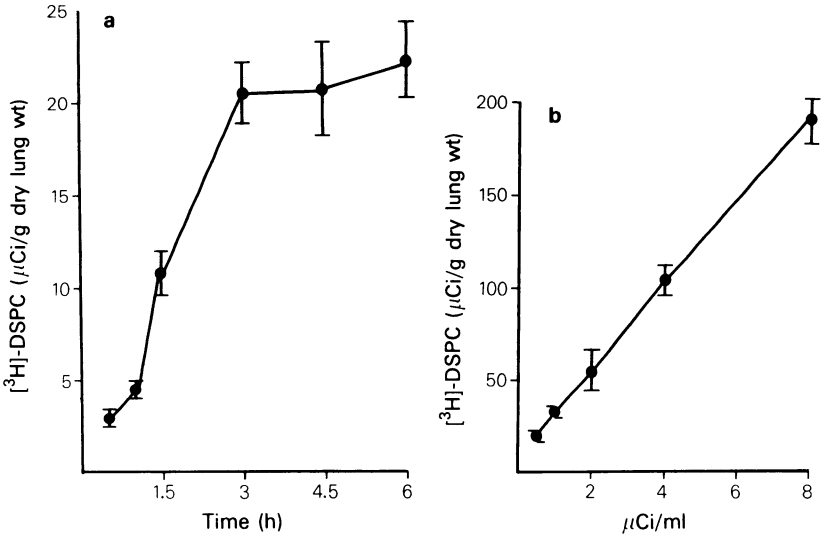

Figure 2 Incorporation of [methyl-^{3}H]-choline chloride into disaturated phosphatidylcholine (DSPC). Lung slices were either incubated in 1 μCi/ml for various times (a) or for 3 h in various concentrations (b). The values are means of n =6 for (a) and n = 3 for (b); s.e.mean shown by vertical lines.

The DSPC content of these slices was 21.3 ± 1.7 mg/g dry lung (n =6) which was not significantly different from slices assayed without incubation (23.2 ± 1.6 mg/g dry lung, n =6).

Basal overflow of ^3H

Location of ^3H in perifusate Four sets of lung slices were incubated for 3 h in [methyl-^{3}H]-choline chloride (1 μCi/ml) and then perifused for 3 h. From each tissue six 30 min fractions were collected and each assayed for total ^{3}H, ^{3}H extractable into organic solvent, [^{3}H]-phosphatidylcholine and [^{3}H]-DSPC contents. Tissues at the end of perifusion were assayed in the same manner. These experiments demonstrated that there were significant quantities of ^{3}H-labelled compounds, other than DSPC, in perifusate fractions and in tissues at the end of perifusion.

Basal overflow of [^{3}H]-disaturated phosphatidylcholine Lung slices were incubated for 3 h in [methyl-^{3}H]-choline chloride (1 μCi/ml) and then perifused for 3 h. Fractions were collected at 5 min intervals over the first 45 min and then every 10 min. These and the tissues after perifusion were assayed for their [^{3}H]-DSPC contents. The overflow was expressed as a rate coefficient.

The basal overflow declined rapidly over the first 30 min and then decreased slowly between 30 and 180 min after the start of perifusion (Figure 3). Least squares regression analysis showed that the second phase had a linear slope significantly ($2P<0.01$) different from zero; the mean slope was 7.3×10^{-6}% (n = 7). Basal overflow of [^{3}H]-DSPC during this second phase was about 60 d/min for each 10 min fraction which was 2 to 3 times background count rate. This expressed as a rate coefficient was of the order of 2×10^{-3}%/min. The total basal overflow of [^{3}H]-DSPC over 3 h was 0.62 ± 0.10% of the tissue content.

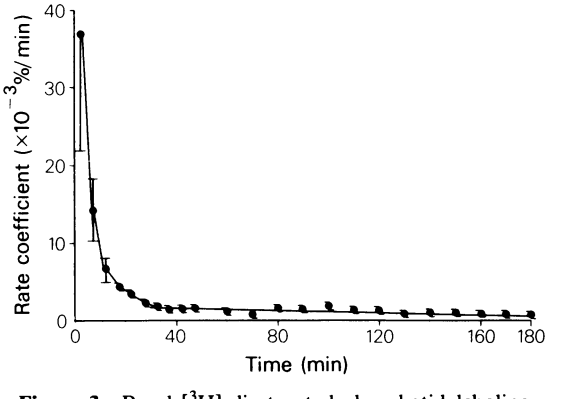

Figure 3 Basal [^{3}H]-disaturated phosphatidylcholine ([^{3}H]-DSPC) overflow from lung slices after incubation in [methyl-^{3}H]-choline chloride (1 μCi/ml) for 3 h. Values are means of 4 observations and are plotted at the midpoint of the fraction intervals; s.e.means shown by vertical lines.

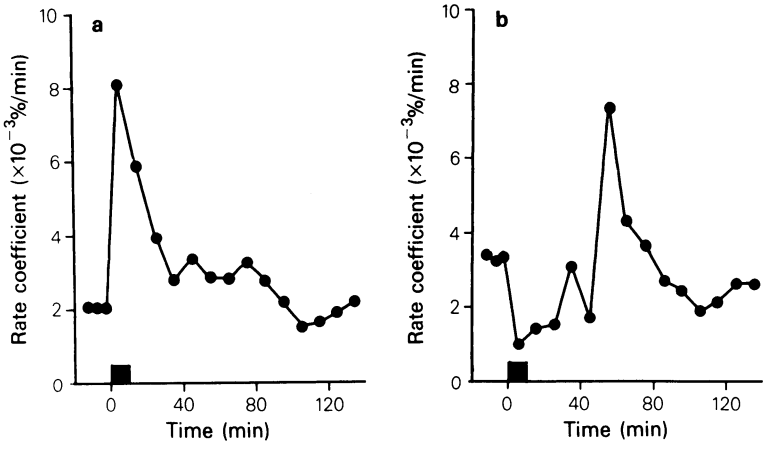

Figure 4 The effect of salbutamol (10^{-6} mol/l) (a) and (10^{-5} mol/l) (b) on the overflow of [^{3}H]-disaturated phosphatidylcholine ([^{3}H]-DSPC) from lung slices. Slices had been incubated in [methyl-^{3}H]-choline chloride (1 μCi/ml) for 3 h. Salbutamol was administered for 10 min (■), 45 min after the start of perifusion. The time scale is time after the start of drug administration.

Salbutamol-induced overflow of [^{3}H]-disaturated phosphatidylcholine

Salbutamol (10^{-9}–10^{-5} mol/l) induced an increase in the overflow of [^{3}H]-DSPC. The peak effect was usually seen in the first fraction (Figure 4a), although this did occur up to 60 min after the start of salbutamol perifusion. The increase in overflow was often prolonged, lasting for up to 70 min. With salbutamol (10^{-5} mol/l) an initial decrease in overflow was observed before a rise (Figure 4b). The maximum increase in rate coefficient minus pre-drug rate coefficient was used to calculate a concentration-effect curve (Figure 6). The concentration to produce 50% of the maximal effect (EC_{50}) for salbutamol was 1.25×10^{-7} mol/l. (±)-Propranolol (10^{-7} mol/l), in the Krebs-Henseleit solution throughout the 3 h of perifusion, had no effect on basal [^{3}H]-DSPC overflow but significantly reduced ($2P < 0.01$) the increase due to salbutamol (10^{-6} mol/l) from $3.1 \pm 0.7 \times 10^{-3}$%/min to $1.0 \pm 0.4 \times 10^{-3}$%/min ($n = 5$).

Perifusion with salbutamol (10^{-6} mol/l) for 10 min did not alter the [^{3}H]-DSPC content of the tissue. This was 29.6 ± 1.9 μCi/g dry weight ($n = 6$) after incubation only, 28.3 ± 2.9 μCi/g ($n = 6$) after incubation and perifusion and 26.3 ± 2.5 μCi/g ($n = 6$) after incubation and salbutamol challenge during perifusion.

Potassium chloride-induced overflow of [^{3}H]-disaturated phosphatidylcholine

The potassium chloride concentration of Krebs-Henseleit solution was raised with osmotic pressure kept constant, as assessed by means of an Advanced Wide Range Osmometer Model 3W (Fisons Ltd), by removing an osmotically equivalent amount of sodium chloride. Potassium chloride (2.5×10^{-2} to 10^{-1} mol/l) produced an increase in the overflow of [^{3}H]-DSPC. The peak effect was usually seen in the first fraction after the start of potassium chloride perifusion and was often more transient than that due to salbutamol (Figure 5). Although the effect was concentration-related (Figure 6), and EC_{50} could not be calculated as it was not known whether 10^{-1} mol/l had produced a maximal effect. It was observed in pilot experiments that perifusion with Krebs-

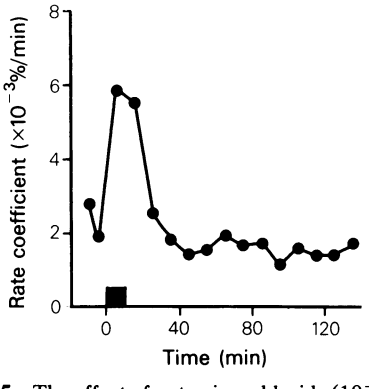

Figure 5 The effect of potassium chloride (10^{-1} mol/l) on the overflow of [^{3}H]-disaturated phosphatidylcholine ([^{3}H]-DSPC) from lung slices. Slices had been incubated in [methyl-^{3}H]-choline chloride (1 μCi/ml) for 3 h. Potassium chloride was administered for 10 min (■), 45 min after the start of perifusion. The time scale represents time after the start of drug administration.

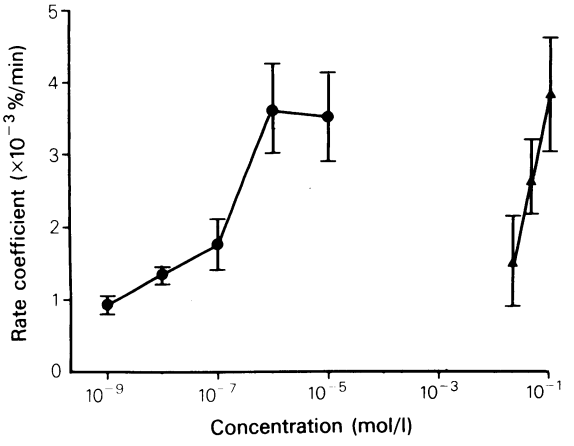

Figure 6 Concentration-effect relationship of salbutamol (●) and potassium chloride (▲) on maximum increase in rate coefficient of [^{3}H]-disaturated phosphatidylcholine ([^{3}H]-DSPC) overflow minus pre-drug rate coefficient. Values are means of $n = 4$ to 8; s.e.means shown by vertical lines.

Henseleit solution plus extra potassium chloride, without correction of osmotic pressure, produced a substantially larger overflow of [^{3}H]-DSPC. Neither (±)-propranolol (10^{-7} mol/l) nor atropine (10^{-7} mol/l) affected potassium chloride (10^{-1} mol/l)-induced increase in [^{3}H]-DSPC overflow.

Adenosine 5′-triphosphate, phenylephrine and overflow of [^{3}H]-disaturated phosphatidylcholine

Adenosine 5′-triphosphate (10^{-3} mol/l) produced an increase in rate coefficient of $3.4 \pm 0.7 \times 10^{-3}$%/min ($n = 8$). The effect was immediate and often prolonged. Phenylephrine (10^{-7} or 10^{-4} ml/l) did not increase the overflow of [^{3}H]-DSPC.

Lactate dehydrogenase

In controls, a very small proportion (2.3 ± 0.5%, $n = 6$) of tissue lactate dehydrogenase was released into the medium in total during 3 h of perifusion. This was not increased by addition of salbutamol (10^{-5} mol/l; 2.6 ± 1.4%; $n = 6$) or potassium chloride (10^{-1} mol/l; 2.4 ± 0.1%; $n = 5$) between 45 and 55 min after the start of perifusion. Measurement of lactate dehydrogenase in separate 30 min fractions demonstrated no increase related to drug administration. Perifusion with distilled water for 3 h induced the release of 46.8% of the tissue content of lactate dehydrogenase (mean of 2) demonstrating that tissue damage could be detected by this method.

Light and electron microscopy

There were no observable differences between sections of lung tissue fixed fresh on removal from rats or after 3 h of incubation followed by 3 h of perifusion when examined by light or electron microscopy. Both sets of tissues had intact alveoli, Type 2 pneumocytes containing distinct nuclei and osmiophilic lamellar bodies and an absence of pulmonary oedema.

Discussion

The present study demonstrates that [methyl-^{3}H]-choline chloride is incorporated into DSPC of adult rat lung slices and the subsequent overflow of [^{3}H]-DSPC can be enhanced by salbutamol, potassium chloride and adenosine 5′-triphosphate.

As DSPC is a major component of lung surfactant (Goerke, 1974; Van Golde, 1976) its release may be used as a measure of lung surfactant secretion. It was necessary to use extraction, oxidation and thin-layer chromatography to assay specifically [^{3}H]-DSPC due to the presence of an excess of [methyl-^{3}H]-choline chloride and its incorporation into other lipids, especially mono- and di-unsaturated phosphatidylcholine. Using a concentration of 1 μCi/ml, the incorporation of [methyl-^{3}H]-choline chloride into DSPC had reached a maximum by 3 h. These incubation conditions produced labelling of the DSPC such that basal overflow of [^{3}H]-DSPC was 2 to 3 times background count rate.

The previously described methods of studing surfactant secretion *in vitro* (see Introduction) involve analysis of the incubation medium for [^{3}H]-DSPC contents at fixed and relatively long points in time after drug administration and therefore measure the accumulation of [^{3}H]-DSPC. The perifusion system enabled the magnitude and time course of spontaneous and drug-induced overflow of [^{3}H]-DSPC to be measured. To reduce between-slice variability, the overflow of [^{3}H]-DSPC was expressed as a rate coefficient rather than as d/min. The overflow of [^{3}H]-DSPC will be equal to that released, less any losses due to processes such as metabolism and/or reuptake. These latter mechanisms cannot currently be readily assessed but are likely to be less with a perifusion compared to an incubation system. There was negligible adsorption of [^{3}H]-DSPC onto the walls of the perifusion apparatus or tubing (Gilfillan, 1981). Perifusion systems are usually designed so that the kinetics of the appearance of a secretory product in an overflow fraction are solely dependent on the characteristics of secretion of the tissue. Experiments have shown that any delay in attainment of fixed drug concentrations in the slice chamber or efflux of [^{3}H]-DSPC into the collected perifusate

introduced by the apparatus are small relative to the kinetics of secretion (Hollingsworth & Kent, unpublished).

It was necessary to assess the extent of tissue damage due to slicing and the prolonged period of incubation and perifusion. The low spontaneous overflow of lactate dehydrogenase (a marker for cytoplasmic enzymes) and of [^{3}H]-DSPC, the lack of a marked change in structure, as assessed by light and electron microscopy, and the ability to respond to drugs by increased overflow of [^{3}H]-DSPC all suggest that viability was well maintained. The larger initial overflow of [^{3}H]-DSPC at the start of perifusion could be due to localized tissue damage associated with the transfer of the slices to the perifusion chambers and/or the release of [^{3}H]-DSPC adhering to the tissue from the incubation medium.

The present results demonstrate that salbutamol, an agonist at β-adrenoceptors with some selectivity for β_2-adrenoceptors (Jack, 1971), was able to increase the overflow of [^{3}H]-DSPC. As the maximum effect was often seen in the first 10 min fraction after the start of perifusion and salbutamol did not increase the [^{3}H]-DSPC content of the tissue by the end of perifusion, it is concluded that salbutamol increased secretion of [^{3}H]-DSPC rather than having a direct effect on synthesis. The increased overflow of [^{3}H]-DSPC after salbutamol without an increased overflow of lactate dehydrogenase suggests a specific effect on surfactant secretion rather than simple tissue damage. The increase in [^{3}H]-DSPC overflow by salbutamol and the antagonism of this effect by (±)-propranolol suggest the presence of β-adrenoceptors mediating surfactant secretion. This confirms similar *in vitro* observations by Dobbs & Mason (1979), Brown & Longmore (1981) and Marino & Rooney (1981) using other β-adrenoceptor agonists. The lack of effect of phenylephrine indicates that if α-adrenoceptors are present they do not mediate increases in secretion. Adenosine 5′-triphosphate markedly stimulated [^{3}H]-DSPC overflow providing direct evidence for an effect on secretion and should be compared with the increased release of [^{14}C]-DSPC from cultures of type 2 pneumocytes produced by 8-bromoadenosine-3′,5′-monophosphate (Dobbs & Mason, 1979).

It has been shown for the first time that potassium chloride produced an immediate and transient increase in [^{3}H]-DSPC overflow. [^{3}H]-DSPC overflow induced by potassium chloride was unaffected by atropine or (±)-propranolol suggesting that this effect was not indirect by the release of neurotransmitters which in turn acted via muscarinic receptors or β-adrenoceptors, as for example, has been proposed for potassium chloride-induced secretion from the pancreatic acinar cell (Case, 1978). While it is possible that potassium chloride is acting indirectly by the release of other humoral substances, it is presumed that it is inducing secretion by depolarization of Type 2 pneumocytes. Depolarization and secretion are not necessarily linked in all secretory tissues (Williams, 1981) and therefore require confirmation by electrophysiological techniques. The larger [^{3}H]-DSPC overflow that occurred after potassium chloride, without correction of osmotic pressure, suggest that secretion is susceptible to raised osmotic pressure.

The secretagogues generally produced their peak effects within the first fraction after the start of their perifusion, that is within 10 min. This was more rapid than the shortest time interval that has been investigated *in vivo*, 30 min (Abdellatif & Hollingsworth, 1980), and more rapid than the time intervals studied by most other groups *in vitro*, 30 min to 3 h (Dobbs & Mason, 1979; Marino & Rooney, 1980; 1981). The prolonged duration of the response (see Figures 4 and 5), despite removal of the agonists, was surprising and can only to a small extent be accounted for by the kinetics of the perifusion apparatus. The maximal increase in rate coefficient after a drug was about 4 times basal release rate but even so only a small proportion of total tissue content of [^{3}H]-DSPC was released, perhaps due to the short duration of drug application, or to the small proportion of tissue [^{3}H]-DSPC that is in a releasable form.

The study therefore suggests that the surfactant secretory sysem shares many characteristics seen with other tissues where substances are stored in a packaged form before secretion (Lacy, 1975; Butcher, 1978; Case, 1978). There was a low basal overflow of [^{3}H]-DSPC, 0.6% over 3 h, somewhat lower than the 2–4% reported by Delahunty & Johnston (1979), Dobbs & Mason (1979), Marino & Rooney (1980, 1981) and Brown & Longmore (1981). Second, low concentrations of certain drugs were able to produce increases in the overflow of [^{3}H]-DSPC. Finally, the onset of response to secretagogues was rapid. This technique should enable further studies of the pharmacology of surfactant secretion.

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