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Pulmonary surfactant and its components inhibit secretion of phosphatidylcholine from cultured rat alveolar type II cells

(lung/secretion/surfactant proteins)

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ABSTRACT **Pulmonary surfactant is synthesized and secreted by alveolar type II cells. Radioactive phosphatidylcholine has been used as a marker for surfactant secretion. We report findings that suggest that surfactant inhibits secretion of ^{3}H-labeled phosphatidylcholine by cultured rat type II cells. The lipid components and the surfactant protein group of M_r 26,000–36,000 (SP 26–36) inhibit secretion to different extents. Surfactant lipids do not completely inhibit release; in concentrations of 100 μg/ml, lipids inhibit stimulated secretion by 40%. SP 26–36 inhibits release with an EC_{50} of 0.1 μg/ml. At concentrations of 1.0 μg/ml, SP 26–36 inhibits basal secretion and reduces to basal levels secretion stimulated by terbutaline, phorbol 12-myristate 13-acetate, and the ionophore A23187. The inhibitory effect of SP 26–36 can be blocked by washing type II cells after adding SP 26–36, by heating the proteins to 100°C for 10 min, by adding antiserum specific to SP 26–36, or by incubating cells in the presence of 0.2 mM EGTA. SP 26–36 isolated from canine and human sources also inhibits phosphatidylcholine release from rat type II cells. Neither type I collagen nor serum apolipoprotein A-1 inhibits secretion. These findings are compatible with the hypothesis that surfactant secretion is under feedback regulatory control.**

Pulmonary surfactant, a complex mixture of lipids and proteins, is synthesized by the alveolar type II cell, where it is stored intracellularly in lamellar bodies (1). These, when secreted into the alveoli, may form tubular myelin (2), which is felt to be the principal precursor of the surface monomolecular film that lowers surface tension. Although both lamellar bodies (3, 4) and tubular myelin contain lipid and protein (5) components of surfactant, the compressed surface film is thought to consist essentially of dipalmitoyl phosphatidylcholine (for review, see ref. 6). The fate of the surfactant lipids and proteins that leave the film upon compression is unknown, but presumably many surfactant constituents reenter the alveolar subphase. Because the apical surface of the type II cell lies in proximity to these various forms of surfactant, we thought that surfactant might play a role in regulating its own secretion.

In primary cultures of type II cells, various pharmacologic agents stimulate secretion of phosphatidylcholine (PtdCho), a marker for surfactant. Secretagogues that are active *in vitro* include those that cause an increase in cellular cAMP (7–9) or in protein kinase C (10). In addition, calcium ionophores (11) and agents that affect cytoskeletal elements (9, 12) modulate secretion in cultured type II cells.

We have found that surfactant, when added to type II cells, inhibits secretion of PtdCho. Both the surfactant protein group of M_r 26,000–36,000 (SP 26–36) and surfactant lipids (at higher concentrations and to a lesser extent than the proteins) inhibit secretion. Our findings support the concept that surfactant secretion is under feedback inhibitory control. We have previously reported this information in preliminary form (13).

MATERIALS AND METHODS

Type II Cell Isolation, Culture, and Measurement of Secretion. We isolated type II cells from rat lungs by elastase digestion and panning cells on IgG-coated plates (14). Type II cells were cultured at a density of 5×10^5 cells per 35-mm culture dish for 21 hr with ^{3}H-labeled choline (1 μCi/ml; 1 Ci = 37 GBq) in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. We washed the cells and added fresh serum-free medium without radioactivity and solutions containing test substances. We used ^{3}H-labeled PtdCho as a marker for surfactant secretion (11). Secretion is expressed either as (*i*) % secretion equals radioactivity in medium per radioactivity in medium plus radioactivity in cells, or as (*ii*) % secretion induced by 10^{-8} M phorbol 12-myristate 13-acetate (PMA) equals % secretion per % secretion induced by 10^{-8} M PMA. Each condition was done in duplicate or triplicate tissue culture dishes; *n* refers to the number of different type II cell isolations that were studied.

Preparation of Surfactant, Surfactant Proteins, and Surfactant Lipids. Surfactant was isolated from rat lung lavage as described previously (15, 16) and was homogenized ten times in a Dounce homogenizer before use. Two different preparations of surfactant were used in these experiments.

Surfactant lipids and proteins were prepared by sequential extractions of surfactant with 1-butanol and 20 mM octyl β-D-glucopyranoside (octyl glucoside) (16). Both the octyl glucoside-insoluble proteins (which contain SP 26–36) and the octyl glucoside soluble proteins (which are similar to serum proteins) (16) were resuspended in 5 mM Tris (pH 7.4) and dialyzed exhaustively to remove octyl glucoside. After dialysis, the fraction containing SP 26–36 was centrifuged at 100,000 × *g* for 1 hr to remove insoluble material; the resulting supernatant, which contained the SP 26–36, was frozen in aliquots containing 30 μg to 1.5 mg of SP 26–36 per ml. Purity was assessed by $NaDodSO_4$/PAGE (16, 17). Protein was measured by the method of Lowry *et al.* (18), as modified by Dulley and Grieve (19). We used four different preparations of rat SP 26–36 (yields ranged from 5 to 20 μg of SP 26–36 per rat lung) in these experiments. Before use, SP 26–36 was diluted in 5 mM Tris (pH 7.4) and added in 50-μl aliquots to the culture dishes.

Surfactant lipids were isolated from the 1-butanol fraction by evaporating the solutions to dryness, resuspending the residue in chloroform/methanol (2:1), and partitioning the

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Abbreviations: SP 26–36, surfactant protein group of M_r 26,000–36,000; PtdCho, phosphatidylcholine; LDH, lactic acid dehydrogenase; PMA, phorbol 12-myristate 13-acetate.

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lipids by the method of Folch *et al.* (20). Residual protein was removed by chromatography over a Sephadex LH-20 column (Pharmacia) (21). The resulting lipids contained no detectable proteins by direct measurement (19) or by $NaDodSO_4$/PAGE.

Antibody to SP 26–36. Polyclonal antiserum against SP 26–36 was prepared from a rabbit immunized with partially purified SP 26–36. Antibodies to serum proteins were removed by chromatography over Sepharose 4B-conjugated rat serum proteins. Control serum was obtained from a rabbit that had not been immunized.

Measurement of Lactic Acid Dehydrogenase. Lactic acid dehydrogenase (LDH) was measured in samples of media and cells by the method of Fanestil and Barrows (22).

Distribution of Radioactivity in Lipids. Lipids were extracted from type II cells and from media by the method of Folch *et al.* (20) using carrier lipids prepared from dog lung. Lipids were separated by two-dimensional thin-layer chromatography (23). Spots were identified by brief exposure to iodine vapor and were scraped into scintillation vials. Radioactivity was measured in a Beckman scintillation counter (model LS 7500).

Liposome Preparation. We prepared unilamellar liposomes (24) using either lipids extracted from surfactant, termed "surfactant lipids," or 55% dipalmitoyl PtdCho/27% egg PtdCho/10% phosphatidylglycerol prepared from egg PtdCho/8% cholesterol (all wt/wt), termed "synthetic lipids."

Materials. Tissue culture medium and fetal bovine serum were obtained from the University of California Cell Culture Facility. We purchased terbutaline from Merrill-Dow (Cincinnati, OH), A23187 from Calbiochem, PMA from Consolidated Midland (Brewster, NY), apolipoprotein A-1 from Sigma, and ^{3}H-labeled choline from Amersham. Lipids were purchased from Avanti Polar Lipids; purity was confirmed by two-dimensional thin-layer chromatography (23). We prepared type I collagen from rat tails (25).

Statistical Analysis. Results were evaluated by a one-way analysis of variance and a Newman–Keul's test, unless stated otherwise.

RESULTS

Inhibitory Effects of Surfactant. Surfactant inhibited PMA-induced release of ^{3}H-labeled PtdCho from rat type II cells (Fig. 1) in a concentration-dependent fashion; the EC_{50} was approximately 8 μg of protein per ml.

Effects of Surfactant Proteins on Secretion. SP 26–36 inhibited release of ^{3}H-labeled PtdCho with an EC_{50} of ≈0.1 μg/ml (Fig. 2). At concentrations of 1.0 μg/ml, SP 26–36 inhibited basal secretion (Table 1) and markedly reduced the amount of stimulated secretion (Table 1 and Fig. 2). In concentrations greater than 5 μg/ml, the inhibitory effect of SP 26–36 was consistently less than in concentrations of 0.5–3 μg/ml.

Proteins extracted from surfactant with 20 mM octyl glucoside did not inhibit secretion (Fig. 2).

Effects of Surfactant Lipids on Secretion. Surfactant lipids also inhibited ^{3}H-labeled PtdCho secretion, although not as completely as either surfactant or SP 26–36 (Fig. 3). In concentrations greater than 100 μg/ml, surfactant lipids increased cellular release of LDH, suggesting that cells were damaged; at this concentration, lipids inhibited PMA-induced secretion by ≈40%.

Inhibitory Effects of SP 26–36 on Basal Secretion and Secretion Stimulated by Different Secretagogues. SP 26–36 inhibited basal secretion and secretion stimulated by three different types of secretagogues—PMA, terbutaline, and the ionophore A23187 (Table 1).

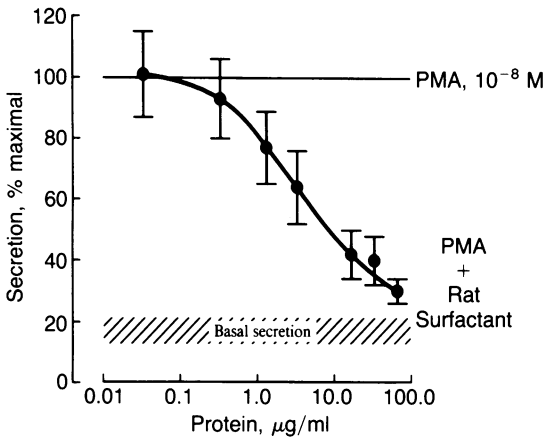

FIG. 1. Inhibition of ^{3}H-labeled PtdCho secretion by rat surfactant. Type II cells were cultured with ^{3}H-labeled choline for 21 hr. Control solutions, PMA (10^{-8} M), or PMA plus various concentrations of rat surfactant were added to the cells, and secretion was measured after 3 hr. Results are expressed as % maximal secretion stimulated by PMA. Basal secretion represents secretion in control solutions. Values are the mean ± SD of four experiments. Secretion induced by 10^{-8} M PMA ranged from 8.2 to 11.1% in these experiments.

The Apparent Inhibitory Effect of SP 26–36 Is Not Due Solely to Precipitation or Bulk Reuptake of PtdCho. To test whether the SP 26–36 effect on secretion of ^{3}H-labeled PtdCho could be attributed either to lipid aggregation and precipitation or to stimulated reuptake of ^{3}H-labeled PtdCho, we transferred radioactive secretions obtained from type II cells stimulated with 10^{-8} M PMA to fresh culture dishes, keeping both the amount of secreted material and the volume of medium per dish constant. Some of these dishes contained type II cells that had been incubated for 24 hr without radioactivity; other dishes contained no cells but had been incubated with medium for 24 hr. We then added SP 26–36 (1 μg/ml) to some dishes. Plates were incubated for 3 hr, media were removed, and media and plates (either containing cells or without cells) were processed as for secretion. The percentage of radioactivity on plates (with the remainder of radioactivity in the medium) under the different conditions was as follows: plates without cells, 7 ± 1%; plates without cells plus SP 26–36, 8 ± 1%; plates with type II cells, 9 ± 1%; plates with type II

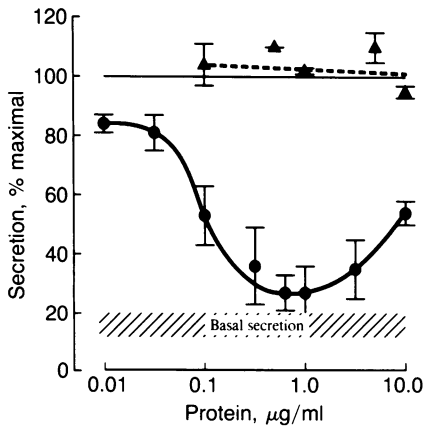

FIG. 2. Effect of various surfactant proteins on secretion. Secretion was measured as described for Fig. 1. Values represent the mean ± SD of four experiments for SP 26–36 and two experiments for octyl glucoside–proteins. PMA (10^{-8} M)-induced secretion (—) varied from 7.8 to 18.4% in these experiments. ●, Secretion in PMA plus SP 26–36; and ▲, secretion in PMA plus octyl glucoside–proteins.

Table 1. Inhibitory effect of SP 26–36 on basal and stimulated secretion of ^{3}H-labeled PtdCho

	^{3}H-labeled PtdCho, % secreted in 3 hr	*n*	*P*
Control	2.0 ± 0.6	4	
SP 26–36 (1 μg/ml)	1.3 ± 0.4	4	0.02
PMA (10^{-8} M)	11.4 ± 3.1	13	
PMA + SP 26–36	2.4 ± 1.0	13	<0.001
Terbutaline (10^{-5} M)	5.6 ± 1.4	4	
Terbutaline + SP 26–36	1.9 ± 0.8	4	<0.005
A23187 (10^{-7} M)	5.6 ± 1.4	4	
A23187 + SP 26–36	1.9 ± 0.8	4	<0.005

Cells were incubated with ^{3}H-labeled choline for 21 hr and washed as described. SP 26–36 (1 μg/ml) was added immediately before adding secretagogues, and secretion was measured after 3 hr. Values are reported as mean ± SD. Statistics were done by paired Student's *t* test comparing the effect of SP 26–36 with the appropriate SP 26–36-deficient solution.

cells plus SP 26–36, 12 ± 3% (values are reported as mean ± SD, $n = 3$).

SP 26–36 Does Not Affect the Distribution of Radioactivity in Phospholipids. SP 26–36 did not alter the distribution of radioactivity in phospholipid classes in either the media or cells. In the media, 95 ± 1% of the radioactivity was in PtdCho when SP 26–36 was absent, and 97 ± 2% of the radioactivity was in PtdCho when SP 26–36 (1 μg/ml) was present. The radioactivity in the untreated cells was distributed as follows: PtdCho, 91 ± 1%; lyso-PtdCho, 3 ± 1%; sphingomyelin, 5 ± 1%. Distribution of radioactivity in cells treated with SP 26–36 was as follows: PtdCho 93 ± 1%; lyso-PtdCho, 2 ± 1%; sphingomyelin 5 ± 0% (values are reported as mean ± SD, $n = 3$).

Effects of Other Proteins on Secretion. We tested whether other proteins inhibit secretion from type II cells. We chose collagen because of the similarity in amino acid sequence of portions of SP 26–36 and collagen (26) and apolipoprotein A-1 because of its affinity for lipids. Neither collagen nor apolipoprotein A-1 in concentrations of 0.1–20 μg/ml inhibited secretion. Results for PMA (10^{-8} M) plus collagen were as follows (in μg of collagen per ml, %PMA-induced secretion): 0.1, 105%; 1.0, 102%; 5.0, 104%; 10.0, 112%; 20.0, 111%. Results for PMA plus apolipoprotein A-1 were as follows (in μg of apolipoprotein A-1 per ml, %PMA-induced secretion): 0.1, 99%; 1.0, 95%; 5.0, 106%; 10.0, 103%; 20.0, 99%.

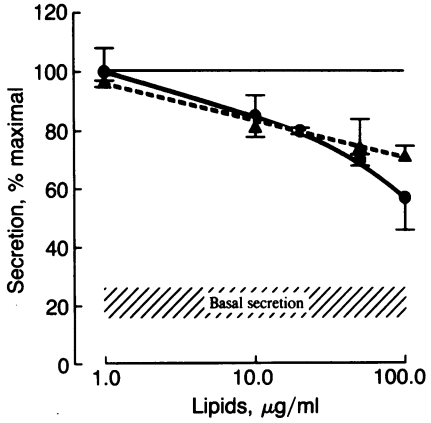

FIG. 3. Effects of lipids on secretion. Secretion was measured as described in Fig. 1. Values represent the mean ± difference of two experiments for PMA plus synthetic lipids (▲) and mean ± SD ($n = 3$) for PMA plus surfactant lipids (●). PMA-induced (10^{-8} M) secretion (—) ranged from 10.1 to 11.7%.

Table 2. Time course: Inhibitory effect of SP 26–36 on secretion

Time, min	^{3}H-labeled PtdCho, % secreted: PMA (10^{-8} M)	PMA (10^{-8} M) with SP 26–36
0	1.1 ± 0.1	1.0 ± 0
15	1.9 ± 0.2	2.6 ± 0.7
30	3.0 ± 0.3	3.1 ± 1.3
60	4.9 ± 0	4.4 ± 0
120	6.9 ± 0.2	7.1 ± 0.4
180	9.9 ± 1.8	9.3 ± 0.5

Cells were incubated with ^{3}H-labeled choline for 21 hr and washed as described. PMA (10^{-8} M) was added. For the PMA control, medium was removed and cells were harvested at each time point. For the SP 26–36 data, 1 μg of that substance per ml was added at each time point, and the media and cells were harvested at 180 min.

Effects of Lipids on Secretion. Although we could not detect protein in preparations of surfactant lipids by either direct measurement or by $NaDodSO_4$/PAGE (data not shown), we could not exclude the possibility that small amounts of protein bound to lipids might escape detection. For this reason, we tested the effect on secretion of liposomes made from a mixture of "synthetic" lipids. We found that these liposomes inhibited surfactant secretion in a pattern very similar to "surfactant" lipids (Fig. 3), although the inhibitory effect of the synthetic lipids was somewhat less.

Effects of Liposomes Associated with SP 26–36. We prepared liposomes from the mixture of synthetic lipids as described and allowed SP 26–36 (1 μg/ml) to react with the liposomes for 15 min at room temperature before adding it to the cells. We used a concentration of liposomes (10 μg/ml) that would have little effect on secretion (Fig. 3). Results expressed as percent of the secretion induced by PMA (10^{-8} M) were as follows: PMA plus liposomes, 87 ± 10%; PMA plus liposomes plus SP 26–36, 40 ± 11% (mean ± SD, $n = 3$).

Time Course of SP 26–36 Inhibition of Secretion. We compared the effect on secretion of adding SP 26–36 at different times to terminating secretion at these times by removing the medium and harvesting the cells. We found (Table 2) that the results of adding SP 26–36 were strikingly similar to harvesting cells at each time, suggesting that SP 26–36 inhibited secretion from the time it was added.

Inhibition of SP 26–36 Effects. The inhibitory effect of SP 26–36 could be blocked by several different methods. Washing cells after exposure to SP 26–36 largely reversed the inhibitory effect (Table 3). Boiling SP 26–36 for 10 min, specific antiserum, and EGTA each blocked most of the inhibitory effect of SP 26–36 (Table 4).

Effects of Canine and Human SP 26–36. Both canine SP

Table 3. Effects of incubating cells with SP 26–36 and of washing cells after exposure to SP 26–36

	Secretion, % of PMA-induced: $t_{-3\ hr}$	$t_{-2\ hr}$	$t_{-1\ hr}$	t_0
Cells washed before adding SP 26–36	25 ± 1	27 ± 1	24 ± 2	28 ± 2
Cells washed before adding SP 26–36 and again at t_0	84 ± 14	85 ± 12	92 ± 12	18 ± 8*

Cells were incubated for 21 hr with ^{14}C-labeled choline and washed before adding SP 26–36 (1 μg/ml) at the indicated time. (SP 26–36 was added to cultures at −3, at −2, or at −1 hr.) Immediately before addition of PMA (10^{-8} M) at t_0, half the culture dishes were washed. Data represent the mean ± difference of two experiments. Basal secretion was 17 ± 5%; PMA-induced secretion was 100%.
*SP 26–36 was added after cells were washed at t_0.

Table 4. Blocking the inhibitory effects of SP 26–36

	Secretion, % PMA-induced	*n*
Basal secretion	18 ± 5	8
PMA (10^{-8} M)	100	8
PMA + SP 26–36 (1 μg/ml)	20 ± 6	8
SP 26–36 heated to 100°C, 10 min	15 ± 5	3
PMA + heated SP 26–36	100 ± 2	3*
PMA, Ca-free medium + 0.2 mM EGTA	88 ± 10	4
PMA, Ca-free medium + 0.2 mM EGTA + SP 26–36	84 ± 9	4*
PMA + antiserum	104 ± 6	3
PMA + control serum	111 ± 4	3
PMA + SP 26–36 + antiserum	86 ± 12	3*
PMA + SP 26–36 + control serum	29 ± 8	3

PMA-induced secretion ranged from 8.3–15.6% in these experiments. Data represent the mean ± SD of *n* experiments.
*Different from PMA + SP 26–36, $P < 0.001$.

26–36 and SP 26–36 prepared from the lavage of a patient with alveolar proteinosis inhibited surfactant secretion (Table 5).

Release of LDH. LDH in media and cells was measured in all experiments. Release of LDH was no different from control conditions except when cells were incubated with liposomes in concentrations greater than 100 μg/ml.

DISCUSSION

The control of surfactant secretion is incompletely understood. Various stimuli, including increased tidal volume (27) and factors altering cellular cAMP (7–9), protein kinase C (10), calcium fluxes (11), and cytoskeletal elements (9, 28, 29), have been shown to modulate secretion.

In this communication we present findings that suggest that surfactant inhibits secretion of ^{3}H-labeled PtdCho (Fig. 1) in primary cultures of type II cells. Although both SP 26–36 (Fig. 2) and surfactant lipids (Fig. 3) inhibit secretion, SP 26–36 is a far more potent and complete inhibitor of secretion than are lipids (Figs. 2 and 3). We have not examined the inhibitory effect of surfactant lipids in detail. Surfactant lipids, rather than proteins, inhibit the mitogenic responses of lymphocytes (30) by an unknown mechanism.

The EC_{50} of rat lung surfactant is 8 μg of protein per ml. SP 26–36 constitutes ≈42% of the protein (31). If one disregards the inhibitory effect of surfactant lipids, the calculated EC_{50} for surfactant-associated SP 26–36 is 3.4 μg per ml. The EC_{50} of isolated SP 26–36 is considerably lower, ≈0.1 μg/ml. The disparity between these two numbers is probably due to the fact that surfactant is multilamellar and aggregated in aqueous medium, such that only a small fraction of the total extractable SP 26–36 may be available to the type II cell surfaces. Alternatively, surfactant might contain substances that either stimulate secretion or block the inhibitory effect of SP 26–36. Although we have not been able to demonstrate such an effect with surfactant fractions, we cannot rule out the possibility that the biologic activity of surfactant is altered by our method of preparation.

Table 5. Effects of heterologous SP 26–36

	Secretion, % cellular ^{3}H-labeled PtdCho in 3 hr, mean ± SD	*n*
Control	1.8 ± 0.6*	5
PMA (10^{-8} M)	11.9 ± 2.8	5
PMA + canine SP 26–36 (1 μg/ml)	2.0 ± 1.3*	3
PMA + human SP 26–36 (1 μg/ml)	1.3 ± 0.4*	4

*$P < 0.001$ (compared with PMA).

At concentrations greater than 3–5 μg/ml, the inhibitory effect of SP 26–36 decreases (Fig. 2). One likely explanation for this observation is that SP 26–36 forms aggregates at higher concentrations. Another possibility is that, at higher protein concentrations, membrane fluidity is changed (32), altering cellular functions.

If SP 26–36 caused secreted material to precipitate or be taken up by type II cells, our conclusion that it inhibited secretion might be incorrect. Although 8–12% of the radioactive secreted material associated with plate surfaces, SP 26–36 did not markedly increase the percentage of radioactivity associated with the surfaces of plates that did not contain cells. Although SP 26–36 stimulates uptake by cells of radioactive unilamellar liposomes (33) or type II cell secretions (see *Results*), it seems unlikely that bulk reuptake of lipid alone can account for the large inhibitory effects of SP 26–36 on secretion. In the present series of experiments, the amount of cell-associated radioactivity represents only a small percentage of the total added radioactivity. Furthermore, the time course of the SP 26–36 effect (Table 2) could be completely explained by reuptake only if SP 26–36 caused virtually all the radioactive secretions to be taken up by the cells.

Although SP 26–36 suppresses secretion from the time it is added to type II cells (Table 2), the inhibitory effects of SP 26–36 can be blocked or reversed. Washing the cells (Table 3) reverses the SP 26–36 effect without permanently altering cellular responsiveness to SP 26–36, a conclusion reached because adding SP 26–36 to previously washed cells inhibits further secretion (Table 3). Incubation with EGTA blocks the SP 26–36 effect. Proteins can be removed from cell surfaces with EGTA (34, 35). We have not proven that EGTA prevents association of SP 26–36 with type II cells, but such a mechanism seems possible. The inhibitory effects of SP 26–36 can also be blocked by adding antiserum raised against rat SP 26–36. Heating SP 26–36 to 100°C for 10 min abolishes its inhibitory activity (Table 4), suggesting that the tertiary structure of the protein may be important to its inhibitory effects.

SP 26–36 sequences isolated from different species have a high degree of amino acid sequence homology (26, 36–38). Not surprisingly, SP 26–36 from these various sources all inhibit secretion by rat type II cells (Table 5).

The mechanisms by which SP 26–36 inhibits secretion are unknown. Although it has been suggested that SP 26–36 may exist as a trimer (36), the actual organization of the protein *in vivo* has not been established. Based on an apparent monomeric M_r of 26,000–36,000, SP 26–36 inhibits surfactant secretion in concentrations of 1–100 nM. The activity of SP 26–36 at these low concentrations and the lack of activity of other surfactant and nonsurfactant proteins suggest that a specific cell interaction may be involved in the SP 26–36 effect. Although type II cells have been shown to internalize and sort lectins that bind to the apical plasma membrane (39), endocytosis of SP 26–36 has not been proven. SP 26–36 inhibits basal secretion and secretion stimulated by PMA, terbutaline, and A23187 (Table 1). The factors that control basal secretion are not known. Beta adrenergic agonists cause an increase in cellular cAMP, PMA stimulates cellular protein kinase C (10) without altering cAMP (8), and A23187, although not affecting cAMP, probably acts by altering cytoplasmic calcium concentrations (11). Because SP 26–36 inhibits secretion stimulated by different types of agents, SP 26–36 may act distally in the secretory process. A similar effect has been suggested to explain the inhibitory action of botulinum toxin D on catecholamine secretion (10, 40). Alternatively, SP 26–36 may act proximally at a step heretofore unrecognized but common to these three types of secretagogues.

The effects of SP 26–36 on surfactant secretion may be an example of feedback regulation. Miles and coworkers (41) suggested that feedback inhibition plays a role in surfactant synthesis when they showed that pulmonary lavage fluid inhibited the incorporation of palmitate into PtdCho. Recently, Bleasdale and coworkers (42) reported that surfactant apoprotein decreased the incorporation of radioactive precursors into phospholipids in type II cells with an EC_{50} of 0.5 μg of protein per ml. The apoproteins were not identified, but the reported EC_{50} is in the same range as that for the inhibitory effect of SP 26–36 on secretion. If surfactant components inhibit surfactant synthesis and secretion *in vivo* as well as *in vitro*, surfactant metabolism could be regulated according to local needs by this mechanism. Morphometric observations showing that type II cells within the same alveolus may respond variably to secretagogues (28) support the concept that local factors may be important in regulating secretion.

Although feedback inhibition is a fundamental phenomenon in many biological systems, its role in the regulation of exocytosis has not been fully accepted. Some evidence suggests that feedback inhibition limits the secretion of norepinephrine by presynaptic noradrenergic neurons (44, 46), but the relevance of these observations to functions in whole animals remains controversial (43, 45). Additional work will be needed to delineate the mechanism of action of surfactant components on secretion and the biological importance of these phenomena *in vivo*.

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