Regulation of phosphatidylglycerol and phosphatidylinositol synthesis in alveolar type II cells isolated from adult rat lung

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1. INTRODUCTION

The surfactant, which lines the alveolar surface of the lung for the prevention of alveolar collapse and transudation, consists largely of phospholipids [1]. Dipalmitoylphosphatidylcholine is the major active component [1]. However, there are strong indications that phosphatidylglycerol (PG) is also essential [2].

In the fetal lung the synthesis of surfactant components is turned on in the terminal part of gestation (review [3,4]). The percentage phosphatidylinositol (PI) initially increases concomitantly with that of disaturated phosphatidylcholine (PC), whereas PG appears later [2,5–7]. The appearance of PG is accompanied by a decrease in the percentage of PI [2,5–7]. Three mechanisms have been proposed for the switch-over from PI to PG synthesis:

(1) It was suggested in [7] that the increase of the PG content of the surfactant is at least partly caused by the increase of the microsomal glycerolphosphate phosphatidyltransferase activity.

(2) A decrease in serum inositol at the end of gestation causes the relative decrease of PI and the increase of PG in lung around term. This mechanism was proposed in [8], where rabbit lung microsomal CDP-diacylglycerol–inositol phosphatidyltransferase and glycerolphosphate phosphatidyltransferase compete for CDP-diacylglycerol when the availability of this common substrate is rate limiting, and that inositol enhanced PI synthesis at the expense of PG production.

(3) The proposed [9–12] switch-over from PI to PG synthesis around term is caused by the elevated CMP level [11] resulting from enhanced PC synthesis. This was based on the observation that rabbit lung CDP-diacylglycerol–inositol phosphadityltransferase catalyzes a reversible reaction and on evidence that in lung microsomes CMP stimulates PG synthesis by providing CDP-diacylglycerol via a reverse reaction of CDP-diacylglycerol–inositol phosphatidyltransferase [12].

These proposed mechanisms were all based on experiments with preparations from whole lung tissue. Due to the heterogeneity of lung tissue, which consists of ~40 cell types [13], these studies cannot be extrapolated directly to the producers of surfactant, the alveolar type II epithelial cells [14,15]. Therefore, it was of interest to study whether evidence for these mechanisms could be obtained in experiments with isolated type II cells. These results show that in type II cells isolated from adult rat lung, inositol stimulates PI synthesis and decreases PG synthesis, which supports mechanism (2) proposed in [8]. Although stimulation of PC synthesis by addition of choline was accompanied by an increase in PG synthesis in the type II cells, a rise rather than a decrease in PI synthesis observed under these conditions argues against the occurrence of mechanism (3) proposed in [9–12].

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2. MATERIALS AND METHODS

2.1. *Materials*

CDP-diacylglycerol (derived from egg PC) was obtained from P-L Biochemicals (Milwaukee WI), [1(3)-^{3}H]glycerol (spec. act. 2.5 Ci/mmol) and [U-^{14}C]glucose (spec. act. 270 Ci/mol) from the Radiochemical Centre (Amersham) *sn*-[U-^{14}C]-glycerol-3-phosphate (spec. act. 144 Ci/mol) from New England Nuclear (Boston MA).

2.2. *Isolation of type II cells*

Type II cells were obtained from lungs of male Wistar rats (180–200 g) by trypsinization, density gradient centrifugation and differential adherence in monolayer culture [16–18]. Yield, purity and viability were in the same range as in [19].

2.3. *Measurement of phospholipid synthesis from labelled substrates by intact type II cells*

The type II cells, which were attached to 35 mm tissue culture dishes, were incubated as in [19]. The incubation medium of each culture dish (1.5 ml) contained 125 mM NaCl, 5 mM KCl, 2.5 mM Na_2HPO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 17 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES), 50 μg/ml gentamycin, 0.2 mM palmitate (complexed to bovine serum albumin in a ratio 5.3:1) and other components as specified in the legends to the figures and table. The pH of the incubation medium was 7.4 at room temperature. After the appropriate incubation times the reactions were terminated by transferring cells and incubation medium to chloroform/methanol as in [19]. Extraction of the phospholipids, their separation by two-dimensional thin-layer chromatography and determination of their radioactivity were carried out as in [20].

3. RESULTS AND DISCUSSION

Fig.1 shows that the incorporation by intact isolated type II cells of labelled glycerol into PI is increased while the incorporation of glycerol into PG is decreased by inclusion of *myo*-inositol in the incubation medium. The effect of inositol is observed immediately after the start of the incubation. The incorporation of glycerol into PC is unaffected.

The effect of inositol on the formation of PG and PI from labelled glycerol is apparent at ≥ 100 μM (fig.2). These concentrations are considerably higher than those (≥ 0.5 μM) at which a decreased rate of glycerolphosphate phosphatidyltransferase reaction and an increased rate of CDP-diacylglycerol–inositol phosphatidyltransferase reaction was observed in microsomes from whole adult rabbit lung [8].

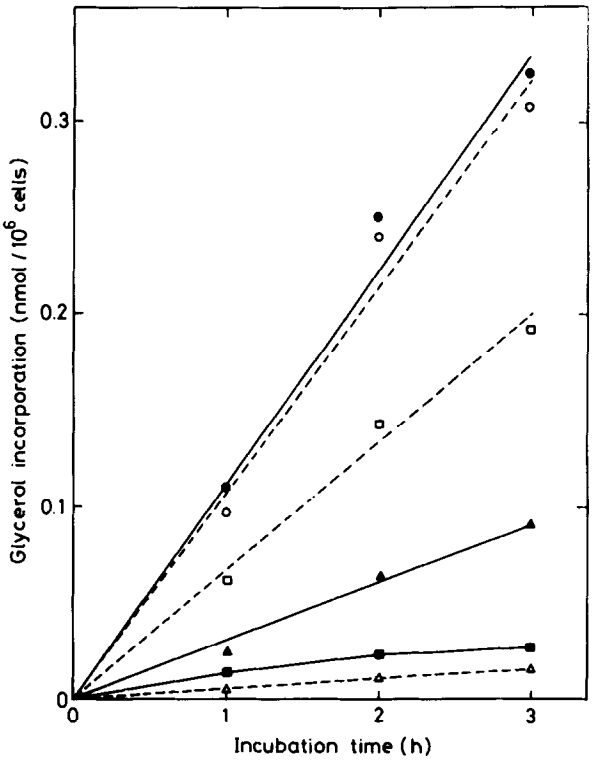

Fig.1. The incorporation of glycerol into PC, PG and PI by intact type II cells as a function of time in the presence and absence of inositol. Beside the standard components the incubation medium contained 0.25 mM [1(3)-^{3}H]glycerol (spec. act. 2×10^5 dpm/nmol), 0.2 mM choline and, where indicated, 5 mM *myo*-inositol: (●,○) PC; (■,□) PG; (▲,△) PI; open symbols, dashed line, without inositol; closed symbols, solid line, with inositol. The data are representative of 2 expt each carried out in duplicate.

Table 1 shows the effects of inositol on the synthesis of PC, PG and PI when [U-^{14}C]glucose is used as the labelled precursor. Again, inositol decreases the synthesis of PG and increases the formation of PI, but has no effect on the synthesis of PC.

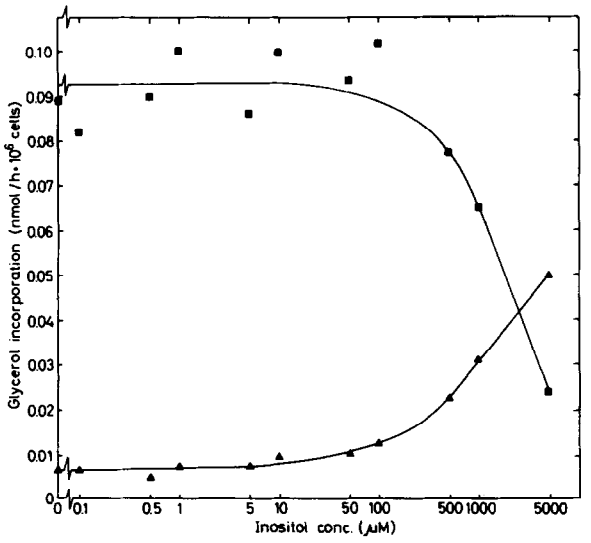

Fig.2. The incorporation of glycerol into PG and PI by intact type II cells as a function of the inositol concentration. Beside the standard components the incubation medium contained 0.25 mM [1(3)^3-H]glycerol (spec. act. 2×10^5 dpm/nmol), 0.2 mM choline and a variable concentration of *myo*-inositol. Incubation was carried out for 2.5 h: (■) PG; (▲) PI. The data are representative of 2 expt each carried out in duplicate.

In an effort to find evidence for mechanism (3), proposed in [9–12], the rate of PC synthesis in isolated type II cells was stimulated by addition of choline (table 1). Upon stimulation of PC synthesis by addition of choline there is, indeed, a small but significant increase in the rate of PG formation, both in the absence and presence of inositol. However, there is no concomitant depression of the PI synthesis, but rather an increase. Therefore, it is unlikely that the rise in the rate of PG synthesis, which accompanies the rise in PC synthesis, is brought about via an inhibition of CDP-diacylglycerol–inositol phosphatidyltransferase by an increased CMP level. This argues against the occurrence of mechanism (3) proposed in [9–12].

The experiments with intact type II cells shown in fig.1,2 and table 1 indicate that in these cells glycercolphosphate phosphatidyl-transferase and CDP-diacylglycerol–inositol phosphatidyltransferase compete for the same pool of CDP-diacylglycerol. This means that in type II cells the switch-over from PI to PG synthesis during development may indeed be effected via a competition for CDP-diacylglycerol and a decrease in the serum inositol concentration, as suggested by studies with whole lung preparations [8].

Table 1

Effects of inositol and choline on the incorporation of [U-^{14}C]glucose into PC, PG and PI by intact alveolar type II cells isolated from adult rat lung

Conditions	Additions to medium	Incorp. cond. A, B, C or D / Incorp. cond. A			Incorp. cond. D / Incorp. cond. C		
		PC	PG	PI	PC	PG	PI
A	None	1	1	1			
B	Choline	1.80 ± 0.22^b	1.16 ± 0.09^a	1.35 ± 0.21^a			
C	Inositol	0.96 ± 0.06	0.28 ± 0.07^d	7.74 ± 1.29^c			
D	Inositol + choline	1.81 ± 0.14^d	0.33 ± 0.09^d	8.63 ± 2.82^b	1.89 ± 0.16^a	1.19 ± 0.11^a	1.10 ± 0.21

Statistical significance was determined using Student's *t*-test: $^ap<0.05$; $^bp<0.025$; $^cp<0.01$; $^dp<0.005$

The cells were incubated for 2.5 h in the standard incubation medium supplemented with 5.6 mM [U-^{14}C]glucose (spec. act. 9×10^3 dpm/nmol) and, where indicated, 5 mM inositol and/or 0.1 mM choline. The absolute rates in nmol/h. 10^6 cells (means ± SD, $N=3$) of glucose incorporation under condition A were 0.47 ± 0.10 for PC, 0.25 ± 0.05 for PG and 0.017 ± 0.007 for PI. The values in the table represent the ratio of the rate under a certain condition over the rate in the control incubation (A or C). The data given are the means ± SD of ratios observed in 3 expt with 3 different cell preparations.

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